

**PREVALENCE OF GASTRO-INTESTINAL  
PARASITES OF GOATS AND COMPARATIVE  
THERAPEUTIC EFFICACY OF PHYTO-  
ANTHELMINTICS**

**बकरियों के उदर - आंत्र परजीवी की व्यापकता और पादप  
कृमिनाशक की तुलनात्मक चिकित्सीय प्रभावकारिता**

*Thesis*

submitted to the



**Sardar Vallabhbhai Patel University of Agriculture & Technology,  
Meerut-250110 (U.P.), India**

**By  
RAMAKANT  
ID. No.-PG/V-5906/21**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF**

**Doctor of Philosophy  
(Veterinary Medicine)**

**July, 2024**

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**Place: Meerut  
July, 2024**

**(Ramakant)  
Author**



**Dedicated**

**To**

**My Beloved**

**Family**

Certificate-I

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**CERTIFICATE**

This is to certify that the thesis entitled **PREVALENCE OF GASTRO-INTESTINAL PARASITES OF GOATS AND COMPARATIVE THERAPEUTIC EFFICACY OF PHYTO-ANTHELMINTICS** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** with major in **VETERINARY MEDICINE** of the College of Post Graduate Studies, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, is a record of bonafide research carried out by Mr. **RAMAKANT**, ID. No. **PG/V-5906/21** under my supervision and no part of thesis has been submitted for any other degree or diploma.

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**Meerut**  
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### List of abbreviations/Symbols

<b>Abbreviation</b>	<b>Extended/full form</b>
AMT	Adult Mortality Test
AWMA	Adult Worm Mortality Assay
AMIA	Adult Motility Inhibition Assay
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ANOVA	Analysis of variance
AR	Anthelmintic resistance
A:G	Albumin: Globulin
bp	Base pair
BC	Before christ
B.O.D. incubator	Biochemical Oxygen Demand incubator
BUN	Blood Urea Nitrogen
b. wt.	Body Weight
CAT	Catalase
CT	Condensed tannins
CC50	50% cytotoxic concentration
CAE	Crude Aqueous Extract
CME	Crude Methanolic Extract
CP	Crude Powder
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle Medium
DLC	Differential leukocyte count
EDTA	Ethylene diamine tetra acetic acid
EHA	Egg Hatch Assay
EHT	Egg Hatch Test
EPG	Egg per gram of faeces

ED50	Median effective dose
EC50	Median effective concentration
Fig.	Figure
FEC	Faecal egg count
FECR	Faecal Egg Count Reduction
FECRT	Fecal Egg Count Reduction Test
FRAP	Ferric Reducing Ability of Plasma
gm	Gram
GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GIPs	Gastrointestinal parasites
GSH	Glutathione
GDP	Gross domestic product
GGT	Gamma-glutamyl transferase
h	Hour
Hb	Haemoglobin
IC50	Half maximal inhibitory concentration
i.e.	That is
ITS-2	Internal transcribed spacer region-2
kg	Kilogram
L3	Third-stage larvae
LMA	Larval Mortality Assay
LMT	Larval Mortality Test
LD50	Median Lethal Dose
LC50	Median Lethal Concentration
LDA	Larval Development Assay
LDT	Larval Development Test
LDT	Larval Development Test
LMIT	Larval Migration Inhibition Assay

LFIT	Larval Feeding Inhibition Test
LEA	Larva Exsheathment Assay
LPA	Larval Paralysis Assay
MDR	Multidrug resistance
mM	Mill molar
mg	Milligram
min	Minute (s)
ml	Milliliter
MZN	Modified Ziehl-Neelsen
MDA	Malonaldehyde
NFW	Nuclease Free Water
nm	Nanometer
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PCV	Packed cell volume
pH	Log hydrogen ion concentration
PVPP	Polyvinylpolypyrrolidone
ppm	Parts Per Million
rpm	Revolutions per minute
ROS	Reactive Oxygen Species
RPMI	Roswell Park Memorial Institute
rRNA	Ribosomal Ribonucleic acid
rDNA	Ribosomal DNA
s	Second
SEM	Standard error of mean
SOD	Superoxide dismutase
SH	Sulfhydryl
SPSS	Statistical Product and Service Solutions

SRBA	Sulforhodamine B Assay
TEC	Total erythrocyte counts
TLC	Total leukocyte count
TP	Total protein
TWCR	Total worm count reduction
TCA	Tri-chloroacetic acid
TAC	Total antioxidant activity
TSP	Total Serum Protein
USD	United States Dollar
viz.	Namely
V	Volts
v/v	Volume/Volume
WAAVP	World Association for Advancement of Veterinary Parasitology
w/v	Weight/Volume
°C	Degree Celsius
μl	Microliter
μg	Microgram
@	At the rate of
%	Percentage
×	Multiplication
<	Less than
>	Greater than
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
NaCl	Sodium chloride
HCl	Hydrochloric acids

Goat, commonly called as poor man's cow, has presently getting a symbol of prosperity in the rural India. Goat has found to be the best substitute for supplementary income and milk for the rural people who are incapable to rear cattle and buffalo (Tripathi *et al.*, 2020). Goat farming has huge potential in creation of employment and reduction in poverty in small, marginal and landless farmers in rural areas (Kochewad *et al.*, 2023). Goat with its distinctive characteristic of provides maximum returns with minimum care and expenditure has made it the most popular livestock in the rural India (Brahma *et al.*, 2015). Goats have been a nourishment source for people from many years, since their expansion throughout the world (Sevane *et al.*, 2018). Rearing of domestic goats plays a key role in supporting rural household's nutritional, social and financial needs (Monau *et al.*, 2020).

India, rank to first in the world for production of goat milk and second in production of goat meat and skin. Moreover 75% goats are reared by the marginal and landless households, having less than 2.0 hectare of land (Tripathi *et al.*, 2020). Goats contribute more than 52% of household's total income towards assuring food nutrition security of families of goat keepers (Choudhary *et al.*, 2018). The goat sector contributes 8.4% to the India's livestock GDP i.e. 38590 crores through meat (Rs. 22625crore), milk (Rs. 9,564 crores), skin (Rs.1491 crores), manure (Rs.1535 crores) and others 3,360 crores. The goat husbandry also creates about 4.2% rural employments to the small, marginal and landless labors (Ali, 2020). Thirty seven (37) breeds of goats have been characterized in India (NBAGR, 2022). As per the 20<sup>th</sup> livestock census 2019, the goat population in India is 148.88 million, 5<sup>th</sup> highest population in the world, which is a 10.14% growth compared to the 19<sup>th</sup> livestock census-2012. Goats contribute 27.73% to the entire livestock. Among the different states, Rajasthan has highest goat population (20.84 million) followed by West Bengal and Uttar Pradesh, respectively (Rajeev *et al.*, 2022).

Parasitic worms are one of the most common diseases that infect majority of the organism (Mares *et al.*, 2024). Parasitism in goats is a major cause of decreased resistance, production loss and even mortality (Mohamed *et al.*, 2023). The parasite is present all over the tropical and subtropical regions of the world where it is major limitation for advantageous production of sheep and goats (Zenebe *et al.*, 2017). According to the pharmaceutical

companies the annual cost of antiparasitic compounds is proposed to be 10 billion dollars worldwide (Wolstenholme *et al.*, 2004). However, the yearly treatment cost of *H. contortus* has been estimated to be 26 million USD in Kenya, 46 million USD in South Africa and 103 million USD in India (Peter and Chandrawathani, 2005).

Gastrointestinal parasites are among the most economically important pathogens of small ruminants resulting into a serious economic losses and animal welfare problems for the industry of livestock worldwide (Kalule *et al.*, 2023). Goats are highly susceptible to gastrointestinal parasites (GIPs) (Sissay *et al.*, 2006) due to their lower innate immune response against specific helminths as a result of their evolution (Hoste *et al.*, 2008) and the nomadic nature of goat husbandry. In this regard, among the production constraints of goats that contribute to production losses in rural communities, GIPs constitute a major share (McRae *et al.*, 2014). Gastrointestinal parasites (GIPs) in goats may cause poor body conditions (Rahman *et al.*, 2017), reduction in feed intake and weight gain and weight loss (Win *et al.*, 2020; Paul *et al.*, 2020; Ghimire and Bhattarai, 2019), immunity (Bedada *et al.*, 2018; Paul *et al.*, 2020), reduced milk production and lactation duration (Win *et al.*, 2020; Bedada *et al.*, 2018), reduction in work capacity (Bedada *et al.*, 2018; Paul *et al.*, 2020), mortality (Ghimire and Bhattarai, 2019; Bedada *et al.*, 2018), abortion (Bedada *et al.*, 2018; Paul *et al.*, 2020), diarrhoea, dysentery and anemia (Das *et al.*, 2017). The important gastrointestinal helminths parasites belong to three different classes i.e. nematode (roundworm), trematode (flukes) and cestode (flatworm) (Dehuri *et al.*, 2021). The mortality and morbidity in animal populations due to infections caused by parasitic helminths are quickly enhanced globally (Nirmal *et al.*, 2007). It has been estimated that the gastrointestinal parasites are responsible for financial losses to the tune of 1191.71 millions of rupees every year (Swarnkar and Singh, 2012).

The helminths and coccidian are most common gastrointestinal (GI) parasites in goats (Ozung *et al.*, 2011). Gastrointestinal helminths and enteric protozoan parasites infections among goats are involved in significant economic losses including morbidity and mortality, mostly in young animals (Badran *et al.*, 2012; Majeed *et al.*, 2015). Coccidian parasites contribute to enteric disease particularly in young or goats under stress in poor farm conditions, which results into high mortality rate among goat kids (Ratanapob *et al.*, 2012).

Goats and sheep are infected with similar Gastrointestinal nematodes (GIN) species, mainly *Haemonchus contortus*, *Trichostrongylus axei*, *Teladorsagia circumcincta* (Abomasum), *T. colubriformis*, *Strongyloides papillosus*, *Nematodirus* spp. (Small intestine), *Oesophagostomum columbianum* and *O. venulosum* (Large intestine) (Hoste *et al.*, 2010; Bentounsi *et al.*, 2012; Moreno-Gonzalo *et al.*, 2013). The nematode of major concern is *H. contortus*, a most pathogenic parasite; due to its blood-sucking feeding habits results into anemia, submandibular edema and lethargy. In cases of enormous infections, high mortality rates are recorded (Molento *et al.*, 2011; Cantacessi *et al.*, 2012). *Haemonchus contortus* is most pathogenic gastrointestinal nematode parasite infecting in the goats population throughout the world (Shamim *et al.*, 2018; Dutta *et al.*, 2016; Selemon, 2018). Strongyle nematodes are observed as one of the highly pathogenic and economically notable parasites affecting the small ruminants (Jurasek *et al.*, 2010). Severe endoparasitemia is a slow procedure in goat which exerts its effects in grave manner in rural area (Maji *et al.*, 2018).

Gastrointestinal parasitism, particularly nematode infection, is a considerable health problem affecting the goat industry throughout the world, resulting in clinical diseases and loss in productivity (Zaros *et al.*, 2010; Rahman *et al.*, 2017). So, the infections from gastrointestinal nematodes like *Haemonchus contortus*, *Trichostrongylus* spp. and *Teladorsagia* spp. be a threat to the profitability and sustainability in production of the goat (Charlier *et al.*, 2021; Vineer *et al.*, 2020). Susceptibility of goats is more to gastrointestinal nematodes infections perhaps due to a deficiency in the mechanism of immune parasite expulsion. Moreover, goats metabolize anthelmintic drugs faster in comparison to sheep, decreasing the efficacy of anthelmintics when they are treated with the similar dose recommended for sheep (Hoste *et al.*, 2010). Due to climate changes, deforestation and expanded the facilities irrigation, ecological niche of the parasites as well as alteration in intermediate hosts resulting into alteration in prevalence or the epidemiology of the parasitic diseases (Dixit *et al.*, 2017). Though, parasites restrict goat productivity as they decrease fertility, cause irritation of skin and suck the blood, at last leading to death (Molefe *et al.*, 2012). Gastrointestinal nematode infections are predominant prevalent parasitic diseases influencing the productivity of small ruminant worldwide, particularly in tropics and sub-tropics (Torres-Acosta and Hoste, 2008). The management of intestinal parasites has become

more difficult in the small ruminants owing to the parasite's increasing resistance against various anthelmintics (Eke *et al.*, 2019).

Presently, the primary control plan for GIPs (Gastrointestinal parasites), particularly nematode infections, is the use of anthelmintic drugs (Sangster *et al.*, 2018), which are related with anthelmintic resistance (Fissiha and Kinde, 2021). The treatment of gastrointestinal parasites by farmers is depending on synthetic chemical products (Challaton *et al.*, 2022). The major anthelmintic drugs used for the treatment belong to the groups of benzimidazoles, imidazothiazoles, macrocyclic lactones (Hoste and Torres-Acosta, 2011), monepantel and derquantel (Kaminsky *et al.*, 2011). The control of GINs in various livestock systems is mainly via the use of anthelmintic drugs and this is likely to continue into expected in future, increasing the risk for anthelmintic resistance. Anthelmintic resistance in small ruminant gastrointestinal nematodes such as *H. contortus* has reached alarming levels worldwide as a result of the dependency on chemotherapy (Kaplan, 2004). The extensive use of anthelmintic has induced to the emergence of drug-resistant parasites strain (Charlier *et al.*, 2021). Resistance may become apparent to one anthelmintic class, or even to various or all classes of anthelmintics, referred to as MDR (Multidrug resistance). The spread of anthelmintic resistance seems to correspond to the popularity of anthelmintic classes utilized in veterinary practice (Rose *et al.*, 2015; Mickiewicz *et al.*, 2020). Consequently the increase in MDR among GINs and the unavailability of adequately effective alternative methods of control and prophylaxis of the parasitic infection are an increasing threat to health and production in the small ruminant across the globe (Holm *et al.*, 2014; Kaplan, 2004). In addition to anthelmintic resistance, insufficient availability and high expensive commercial anthelmintics are other important limitations of helminth control in developing nations (Mohan *et al.*, 2016).

The exploration of the antiparasitic activity of natural byproducts can contribute in the advancement of alternative therapy and the decrement of the dependence on the conventional chemotherapy (Santos *et al.*, 2017). Conventionally, two main approaches have been used in study of efficacy against helminths. The first one is via feeding of plants or their various parts to naturally or artificially infected animals (Iqbal *et al.*, 2004; Chandarawathani *et al.*, 2002). The second one is by testing extracts of medicinal plants and concoctions (compound) from medicinal plants through *in vivo* and *in vitro* systems (Githiori *et al.*, 2004; Gathuma *et al.*,

2004). Against anti-helminthic activities of plant extracts can be screened by several *in vitro* and *in vivo* procedures in host and non-hosts animals (Dehuri *et al.*, 2021). *In vitro* tests such as AMT (Adult Mortality Test), EHA/EHT (Egg Hatch Inhibition Assay/Test), LMA/LMT (Larval Mortality Assay/Test), LDA/LDT (Larval Development Assay/Test), LMIT (Larval Migration Inhibition Assay), LFIT (Larval Feeding Inhibition Test) and LEA (Larva Exsheathment Assay) are being used for assessment of anti-helminthic activities of plant extracts and products against gastrointestinal parasites (Tariq *et al.*, 2010). The most common test like adult mortality test and larval mortality test examines the effect of plant extract on the motility of the adult and larva of nematode respectively, while egg hatch inhibition test focuses on the inhibitory effect of the extracts on egg hatching. *In vitro* assays have an edge over *in vivo* methods owing to their relative low costing and quick results that permit large scale testing of plant materials. *In vitro* test are not capable to evaluate host factors since the substances are applied directly on the parasites (O'Grady and Kotze, 2004; Borges and Borges, 2016).

The *in vivo* evaluation of anthelmintic efficacy is carry out by the FECRT (Fecal Egg Count Reduction Test) or controlled test. The FECRT gives an estimate of the reduction in the excretion of eggs subsequent to treatment, whereas within the controlled test the effectiveness is evaluated by comparing the burdens of parasites in the treated group in comparison to control group. The controlled test is most authentic method, but the also the most costly analysis in terms of requirements labor and animal usage (Taylor *et al.*, 2002). *In vivo* studies were carried out mainly with the use of plants in the feed of animal and generally exhibited lower effectiveness in comparison to *in vitro* studies, probably due to interference of pharmacokinetic parameters of ruminants in the bioavailability of active compounds of plants (Santos *et al.*, 2019).

Numerous developing nations like India depend on plant based products for treating several diseases including helminth. India, being recognized as a botanical garden of the world is the biggest producers of herbal plants (Seth and Sharma, 2004). Medicinal plants are accessible source for parasiticides (Wangchuk *et al.*, 2016). The helminthes infections are still a major problem mainly due to warm temperature in linked with poor management practices and insufficient control measures (Akhtar *et al.*, 2000). The use of plants as medicine is

gradually get bigger day by day in the world because they have slight or no side effects (Jordan *et al.*, 2010). Nowadays, 80% of the world's population depends on the conventional medicines as a necessary source of their primary health care (Batiha *et al.*, 2020). The plants are a perfect source of naturally occurring compounds that can be utilized as alternative dewormers in livestock (Mahmoudvand *et al.*, 2016).

Herbal drugs are getting to be better known as cost-effective and sustainable alternatives against treatments with synthetic anthelmintic (Gurib-Fakim, 2006). Herbal medicines comprise herbs, herbal materials, herbal preparation and end an herbal product that contains parts of plants or other plant materials as active ingredients (Gupta, 1994). Additionally, an increase in synthetic anthelmintic drug resistance make necessary, they require to find a replacement for synthetic anthelmintic drug (Shalaby, 2013).

Utilizing plants having anthelmintic properties appears to have two advantages (Ali *et al.*, 2008). Plant products evolve resistance more gradually and a mixture of components that synergize, give rise to an anthelmintic effect in plants products (Pal *et al.*, 2011). Anthelmintic derived from plants are a promising area of research in effort to alleviate the challenges around utilize of synthetic anthelmintic drugs in controlling gastrointestinal parasites. Alternative ethno veterinary medicines, for example, plant extracts with anthelmintic activity are considered to be of enormous potential in get control of anthelmintic resistance (Lans *et al.*, 2007). The therapeutic armamentarium of plant origin in India comprises nearly 750 medicinal plants (Anjaria *et al.*, 2000) for both human and animal therapeutic management. Estimates of plant species known to have anthelmintic properties vary from below 100 (Tripathi, 1998; Akhtar *et al.*, 2000) to about 160 (Garg, 2007). Availability of scientific evidence on the anti-parasitic efficacy of most plant product is limited (Githiori *et al.*, 2006). Helminth parasites from all 3 major groups were utilized as experimental models to explore the anthelmintic activity of several plants, but gastrointestinal nematodes were subjected more often to *in vitro* as well as *in vivo* trials (Kumar *et al.*, 2012). Using plant extracts has resulted in decreased use of synthetic anthelmintics (Ferreira, 2009). Botanicals efficacy also improves when used in combination, indicating an additive effect may exist (Ferreira, 2009; Katiki *et al.*, 2017). Keeping in view the importance of

gastrointestinal parasites and increased anthelmintic drug resistance in goats, this study was conducted with the following objectives.

**Objectives:**

1. To study the prevalence of gastrointestinal parasites in goats from Meerut and its adjoining areas
2. To study *in vitro* anthelmintic efficacy of some selected medicinal plants
3. To study the cytotoxicity of some selected medicinal plants
4. To study *in vivo* anthelmintic efficacy of some selected non toxic medicinal plants

**2.1 Goat**

The goat belonging to Bovidae family is closely related to the sheep, both falling under the goat antelope subfamily Caprine (Dhara *et al.*, 2020a). Goat is among the earliest animals domesticated by human, globally are distributed, with higher concentrations in tropical and dry zone (Di Cerbo *et al.*, 2010). They possess a remarkable ability to convert low-quality feed into high-quality products, yielding higher economic returns compared to other animal species (Daramola *et al.*, 2021). Goats exhibit superior milk and meat production per unit of live body weight when compared to buffalo, camel and sheep (Vihan, 2010).

Goats are often regarded as a 'bank on hooves' for farmers because they provide valuable resource that can be exchanged for cash during difficult times (Joy *et al.*, 2020). Compared to cattle and sheep, goats are more economical when it comes to natural grazing and browsing (Sharma and Jindal, 2008). The rearing of goats ranks as second most important activity in livestock sector after cattle (Pawaiya *et al.*, 2023). Goat farming proves to be profitable for the marginal and landless farmers as it does not require expensive infrastructure or skilled persons (Ghaywat *et al.*, 2017). Goats have significant potential to boost economies of developing countries like India and can serve as major source of income, particularly for marginal farmers and landless laborers (Singh *et al.*, 2015). Presently, there are over 300 breeds of goats worldwide inhabiting every continent except Antarctica and thriving in a remarkably diverse range of environments, from humid tropical rainforests to arid deserts and cold, high-altitude regions (Dhara *et al.*, 2020a).

**2.2 Gastrointestinal parasitism in goats**

Parasitic diseases not only cause direct losses such as acute illness, death, organ damage and expenses on veterinary service but also lead to indirect losses. The indirect losses include decreased productive potentials, such as decreased growth rate, weight loss in young animals and delayed maturity in animals raised for slaughter (Blackburn *et al.*, 2011). Infected goats with internal parasites often exhibit symptoms such as rough dull-coat, weakness, diarrhea, loss of appetite, tail rubbing, signs of submandibular oedema (bottle jaw), hypo-proteinaemia loss of appetite and weight loss. Moreover, certain *Trichostrongyle*

nematodes can induce anemia by removing red blood cells and proteins, resulting in poor health and reduced productivity in animals (Risso *et al.*, 2015).

Gastrointestinal parasitism is linked to economic losses, decreased productivity, diminished animal performance (Badran *et al.*, 2012), as well as increased mortality and morbidity (Negasi *et al.*, 2012). Infections of gastrointestinal helminths and enteric protozoan parasites among goats have been associated with significant economic losses particularly due to morbidity and mortality among young animals (Waller, 1999; Badran *et al.*, 2012; Majeed *et al.*, 2015). Generally, severe gastrointestinal nematode pathogenesis is attributed to the migration of the infective larvae following ingestion rather than the adult worms in the gut (Dube *et al.*, 2002).

Various risk factors play a crucial role in the onset of gastrointestinal nematode (GIN) infections, influenced by both host and environmental factors. Environmental conditions, including agro-ecological factors and animal husbandry practices like system of housing, intervals of deworming and management of pasture (Ratanapob *et al.*, 2012). These largely determine the type, incidence and severity of several parasitic illnesses (Badran *et al.*, 2012). Moreover other risk factors such as the host species, sex, age, body condition and breed and genotype (Badaso and Addis, 2015), along with the parasite species and intensity of the worm population, significantly impact the development of gastrointestinal parasitic infections (Tariq *et al.*, 2010).

Over 150 species of internal and external parasites have been documented to infect goat and sheep across the world (Whitley *et al.*, 2014; Tariq *et al.*, 2010). Nematodes, second only to arthropods in terms of numbers and complexity of life cycles on earth, are particularly noteworthy. Both adult and larval stages of nematodes can inflict significant pathogenesis in domesticated animals (Urquhart *et al.*, 1996). Gastrointestinal nematodes (GINs) stand out as the most common and economically damaging parasites in ruminants (El-Alfy *et al.*, 2019), owing to their diversity and varying susceptibility to anthelmintic treatments (Han *et al.*, 2017; Rashid *et al.*, 2018).

Gastrointestinal nematodes (GINs), common parasite widely distributed worldwide (Saidi *et al.*, 2020), lead to reduced production and economic losses in husbandry (Barghandan *et al.*, 2020), posing a significant constraint on the survival and productivity of

animals (Yuan *et al.*, 2019). Among these parasites, *Haemonchus contortus* a major concern, is highly prevalent and pathogenic globally, causing substantial morbidity and mortality (Piedrafita *et al.*, 2012). The challenges were severe particularly in tropical regions due to favorable environmental conditions for GINs transmission (Zeryehun, 2012), inadequate nutrition of host animals (Mbuh *et al.*, 2008), and poor sanitation conditions in rural areas (Badran *et al.*, 2012). Consequently, controlling GINs becomes the paramount health issue in goats of all ages (Waller, 2006).

One of the most significant gastrointestinal helminth parasites in livestock is *Haemonchus contortus*, which primarily affects small ruminants in tropical, subtropical, and warm temperate regions (Qamar *et al.*, 2009; Sissay *et al.*, 2007). This parasite is highly virulent and poses a considerable threat to sheep, goats and other ruminants due to its blood-feeding behavior. Infestations can lead to various detrimental effects such as ascites, weight loss, anemia, and even death (Abakar *et al.*, 2004; Kelkele *et al.*, 2012). The economic impact of *H. contortus* on the global livestock industry is substantial, with estimated annual losses ranging from \$30 to \$300 million (Emery *et al.*, 2016). This parasite is particularly economically significant in its main endemic regions (Getachew *et al.*, 2007), underscoring the need for effective management and control strategies.

*H. contortus* is characterized is distinguished by its large size, twisted morphology, and distinctive spicule arrangement in males (Flay *et al.*, 2022). Female *Haemonchus* worms exhibit barber's pole appearance, characterized by white ovaries and uteri twisted around a red blood-filled intestine, while males display a uniform reddish-brown coloration (Das *et al.*, 2023). *Haemonchus* possesses a tooth or lancet in its underdeveloped oral cavity, aiding in perforating the gastric mucosa and extracting blood (Gareh *et al.*, 2021).

Gastrointestinal nematode infection poses a significant challenge for smallholder farmers and pastoralists due to its often-subclinical nature, making detection and prevention difficult. Subclinical nematode infections result in substantial economic losses through decreased production, suppressed immunity, and increased morbidity and mortality (Asrat *et al.*, 2018). Clinical diagnosis of GI nematodes is challenging because the signs are not pathognomonic. Ante mortem diagnosis of nematode infection in livestock has traditionally relied on detecting nematode eggs or larvae in fecal samples through microscopic

examination using flotation and/or larval culture methods. Quantifying the eggs per gram of feces provides the most accurate estimate of parasite burden (Roeder *et al.*, 2013a).

*Trichostrongylus* nematodes serve as causative agent of trichostrongyliasis in both humans and animals, including cattle, sheep, goats, deer, and rabbits. These nematodes are distributed globally (Khan *et al.*, 2010). Sheep and goats play a critical role in maintaining the parasite cycle as the primary reservoirs (Abede *et al.*, 2010). Infection with various *Trichostrongylus* species are typically asymptomatic, but severe cases, may lead to symptoms such as mild anemia and prolonged watery diarrhea, known as black scours, caused by the worms capillary heads embedding in the mucosa and feeding on blood (Craig, 2009).

Coccidiosis, caused by coccidian parasites of the genus *Eimeria*, is another significant parasitic infection affecting small ruminants. It is widespread across many regions worldwide, manifesting either clinically or subclinically. Particularly in young or stressed goats kept under inadequate farm management, coccidiosis significantly contributes to enteric diseases and leads to high mortality rates among goat kids (Ratanapob *et al.*, 2012). Furthermore, the presence of co-infections with other *Trichostrongyle* nematodes complicates the clinical diagnosis of coccidiosis (Zainalabidin *et al.*, 2015).

### **2.3 Prevalence of gastrointestinal parasites in goats**

Understanding the prevalence and specific composition of gastrointestinal fauna provides crucial baseline information for controlling parasite infections (Zvinorova *et al.*, 2016). However, parasitic infections particularly gastrointestinal nematode parasite (GINs) are widespread and persistent across India. Unlike bacterial and viral diseases, the subclinical nature of GI parasites, coupled with ineffective deworming implementation and the unavailability of vaccines, contribute to the enduring and extensive prevalence of parasitic diseases throughout the country (Kumar *et al.*, 2008). The gastrointestinal parasites epidemiology in livestock varies depending on the local climatic conditions, like humidity, rainfall, temperature, vegetation and management practices. These factors mainly determine the incidence and severity of different parasitic illness in an area (Takelye, 1991). Therefore, precise identification of different species, along with knowledge about the epidemiology of parasitic infections, is essential for developing sustainable parasite prevention and control strategies.

A study was conducted by Pathak and Pal (2008), on the prevalence of gastrointestinal parasites in goats in Durg district of Chhattisgarh reported an overall prevalence of infection was 85.22%. The identified various parasites and their respective prevalence rates: *Paramphistomum* spp. (80.68%), *Cotylophoron* spp. (45.45%), *Moniezia* spp. (17.04%), *Avitellina* spp. (3.40%), *Haemonchus* spp. (26.13%), *Trichostrongylus* spp. (5.68%), *Cooperia* spp. (3.40%), *Oesophagostomum* spp. (30.68%), *Bunostomum* spp. (5.68%), and *Trichuris* spp. (27.27%). Seasonal prevalence was highest during the monsoon season (94.60%), followed by summer (87.50%), and lowest during winter (63.15%).

An investigation was carried by Hassan *et al.* (2011), to assess the prevalence of ecto and endoparasites in semi-scavenging Black Bengal goats in Pahartali, Bangladesh. The study found that the overall prevalence of gastrointestinal helminths in goats was 63.41%. Among these positive samples, *Strongyloides* spp. (51.74%), were the most prevalent, while *Moniezia* spp. and *Capillaria* spp. were the least prevalent. Age emerged as a significant risk factor, with older goats showing higher rates of endoparasites infection compared to younger ones in this study.

The prevalence of GI parasite in Barbari and Jamunapari goats at Veterinary College DUVASU Mathura and Aurangabad farm was reported as follows: At the Veterinary College farm, 30 out of 40 goats (75%) tested positive for gastrointestinal parasites. Meanwhile, at the Aurangabad farm, out of 150 fecal samples collected from goats, 101 samples (67.33%) were found to be positive (Singh *et al.*, 2013).

Faecal samples (960) were collected from goats over an 8-month period from July 2011 to February 2012 in 3 districts of Madhya Pradesh, India. These samples were examined using sedimentation and flotation methods, followed by quantification of eggs per gram. 94.48% samples were positive against the one or more gastrointestinal parasite. Among these coccidian were most predominant (82.4%) coccidia were followed by *Strongyles* (69.27 %), *Amphistomes* (22.71%), *Strongyloides* spp. (9.17%), *Trichuris* spp. (3.85%), *Moniezia* spp. (3.02%), *Schistosomes* spp. (2.29%) and *Fasciola* spp. (1.77%). The seasonal incidence was highest during monsoon (98.06 %) and lowest during winter (91.67 %). Gastrointestinal parasitism was higher (96.25 %) in kids compared to (93.89 %) adult goats (Singh *et al.*, 2014).

The prevalence study aimed to determine the presence of gastrointestinal parasites and the severity of GINs infection in Black Bengal goat of Sundarban Delta, West Bengal. Overall prevalence of gastrointestinal parasites infection was found 73.34%. The highest prevalence (81.67%) and intensity of gastrointestinal nematode infection (606.5) were observed during monsoon season while the lowest prevalence (68.33%) and intensity (361.5) were recorded in summer season. *Haemonchus contortus* emerged as the as the predominant gastrointestinal parasite species with an overall prevalence of 63.25% (Brahma *et al.*, 2015).

A longitudinal study was undertaken in low-input and low-output farming systems to assess the prevalence of gastrointestinal parasitic infections among goats of varying ages, sex as well as associated risk factors. Indigenous goats (580) were randomly sampled across regions representing the five agro ecological zones of Zimbabwe, spanning both dry and wet seasons. The study revealed the highest prevalence was for *Eimeria* oocysts (43%) and *Strongyles* (31%), with lower levels observed for trematode and cestode (Zvinorova *et al.*, 2016).

A study was conducted by Das *et al.* (2017), on gastrointestinal parasitism among goats in 2 districts of Meghalaya. The overall prevalence of GI parasitic infections was 28.65% in goats. The highest infections were observed during rainy season (34.92%) followed by cool (26.87%), hot (26.62%), and cold (20.39%) seasons. The helminths infection was recorded 63.60% and protozoa infections 23.02%. Among the helminths, *Strongyle* spp. (32.63%) was the most prevalent, followed by *Trichuris* spp. (12.55%), *Moniezia* spp. (10.04%), and *Trichuris* spp. (8.36%). Mixed infections were found in 13.38% of the samples. Fecal culture revealed the presence of 72.16% (*Haemonchus contortus*) 14.41% (*Oesophagostomum* spp.), 8.91% (*Strongyloides* spp.) and 4.50% (*Trichostrongylus* spp.).

A study was conducted by Singh *et al.* (2017) to explore the prevalence of gastrointestinal parasitism in small ruminants in the western zone of Punjab examining various associated risk factors. The study found the on overall prevalence of endoparasitic infections was 83.08%, including 79.24% and 85.16% in goats and sheep respectively. Analysis of associated risk factors with revealed that females (85.97%) were significantly more susceptible compared to males (69.23%). Furthermore, adults were significantly more prone to parasitic infection compared to young individuals. Seasonal variation was observed

throughout the year, with the highest prevalence recorded during the monsoon season (90.10%), followed by winter (83.84%) and summer (78.35%).

A total 406 faecal samples were collected comprising 292 from goat and 114 from sheep to determine the prevalence of gastrointestinal parasites by Chikweto *et al.* (2018), across islands Grenada and Carriacou. The overall prevalence of gastrointestinal parasites was reported to be 95% (with a 95% confidence interval of 92% to 97%). The proportion of coccidia infection was 76% in goats and 75% in sheep. Regarding helminthes infections the proportions were as follows: *Moniezia* spp., 14% in goats and 4% in sheep; *Strongyloides* spp., 36% in goats and 21% in sheep; *Strongyle* type eggs 89% in goats and 66% in sheep. Mixed infections occurring in both sheep and goats were more common (92%) than single infections (8%).

Vohra *et al.* (2018), conducted a study aimed at detecting the incidence and severity of parasitic infection in small ruminants from the breeding farm of LUVASU, Haryana. In this study faecal samples from 100 sheep and 102 from goat were screening through qualitative, quantitative and coproculture examinations. The examination of faecal samples revealed a significantly higher infection rate in sheep (100%) compared to goats (96%). The eggs of *Strongyle*, *Strongyloides*, *Trichuris*, *Amphistome*, and *Moniezia* as well as Coccidian oocysts were observed as in sheep at rates of 100%, 13%, 46%, 29%, 15%, and 90%, respectively., In goats, the rates were 96.1%, 15.7%, 7.8%, 17.6%, and 87.2%, respectively. The majorities of animals were found to be infected with strongyles with an EPG count exceeding 1200 and also exhibited a light infection of coccidian. Coproculture analysis revealed the predominance of *Haemonchus contortus* along with other nematodes.

A prevalence evaluation of gastrointestinal parasitic infections among goats in Giza Governorate, Egypt was conducted. The fecal examination revealed an overall prevalence of gastrointestinal parasitic infections among the goats was 89.33%. The respective prevalence rates among kids, yearling, and adults were 89.16%, 98.44%, and 82.05%. The most common parasitic infections found were *Coccidia* spp. (76.89%), *Entamoeba* spp. (26.22%), *Moniezia* spp. (18.22%), *Strongyle* group (12.88%), *Trichuris ovis* (5.33%), *Strongyloides papillosus* (3.55%), *Balantidium coli* (2.66%), and *Fasciola* spp. (0.89%) in that order. Mixed infections were recorded in 61.77% of the examined goats. The study indicated that the prevalence of

infection of *Coccidia* spp., *Moniezia* spp. and Strongyle group was highest in the group of yearling (Hassan *et al.*, 2019).

A study investigating the prevalence and risk factors of gastrointestinal parasite (GIP) infections in goats in Sironko District, Eastern Uganda, was conducted. Two hundred twenty faecal and blood samples were analyzed. The overall prevalence of GIP was to be 74.5%. Among the various types of parasites, nematodes were the most prevalent, accounting for 61.8% of infections. Specifically, the infection rates were 37.7% for *Eimeria*, 36.4% for *Haemonchus contortus*, 43.6% for *Trichostrongylus*, 14.6% for *Strongyloides*, 12.7% for *Strongyle*, 0.9% for *Nematodirus*, 14.55% for *Moniezia*, and 11.82% for *Fasciola* (Namutosi *et al.*, 2019).

A study was conducted to investigate the prevalence of gastrointestinal parasites in small ruminants in the Central Part of Myanmar. A total of 380 faecal samples were collected comprising 280 from sheep and 100 from goats. The overall occurrence of gastrointestinal parasites in small ruminants was found to be 98.4%. Specifically, the prevalence in sheep (99.3%) was higher than that in goats (96%). The most prevalent parasites were *Eimeria* spp. (96%), followed by *Trichostrongyle* (77.1%), *Trichuris* spp. (35%), and *Moniezia expansa* (14%). The rate of mixed infection was 84.8%, while a single infection accounted for 15.2% (Win *et al.*, 2020).

A longitudinal study conducted by Mpofu *et al.* (2020a) aimed to assess the epidemiology common of gastrointestinal parasite infections affecting goats in South Africa examining how they are influenced by agro-ecological zone, sampling season, and age and sex. The study found an overall prevalence of GIP of 37.1%, with a mean prevalence of 30.0%, 26.4%, 31.1%, 36.6%, and 59.6% for *Eimeria* spp., *Trichuris*, *Strongyloides papillosus*, *Moniezia* spp., and *strongyles*, respectively. Specifically prevalence of *Eimeria* spp. infection was higher during winter (34.0%) compared to summer (26.0%).

A cross-sectional study was carried out to investigate the prevalence of gastrointestinal parasites in sheep and goats in AnLemo, Hadiya Zone Southern Ethiopia. The overall prevalence of gastrointestinal parasites was found to be 74.41%. Among this, 70.28% were found in goats, while 79.32% were found in sheep. The most prevalent infections identified were *Strongyles* (26.63%) followed by *Strongyloides* 3.65% which was the second highest

prevalent in the Woreda. Additionally, coccidian infection was found in 12.01% of the animals, *Moniezia* in 3.13% and *Fasciola* in 2.61%. Double infection was also observed with *Coccidia* and *Moniezia* at 3.65% being the most mixed infection followed by *Strongyle* and *Moniezia* 2.87% and co-infection of *Trichuris* is with coccidian 2.61% (Sebro *et al.*, 2022).

Kalwaghe *et al.* (2022) conducted a year-round epidemiological survey of gastrointestinal parasites in goats across various regions of Maharashtra during 2017-18. The study examined faecal samples from 667 goats and found overall parasitism prevalence 74.66%. Analysis of age-specific prevalence in goats from Western Maharashtra revealed a higher prevalence in adults, at 91.44%, compared to 22.22% in goats below one year old. There was no significant difference in parasitism prevalence between male and female goats across the state. Similarly, breed-specific prevalence did not show significant differences among the four breeds across all regions.

Female *Haemonchus contortus* worms have a higher prevalence compared to male worms, with a ratio of 6:1 in goats and 4:1 in sheep (Das *et al.*, 2023). Goats raised under intensive production system are at significant risk of infection of *Eimeria* spp. (Hassanen *et al.*, 2020; Cavalcante *et al.*, 2012). Coccidiosis is among the most prevalent parasitic affecting the goats worldwide (Kimbata *et al.*, 2009; Chartier and Paraud, 2012). This disease leads to economic losses due to high mortality and morbidity rates, stunted growth and treatment expenses (Kaya, 2004; Temizel *et al.*, 2011). Coccidiosis in caprine caused by protozoa of the genus *Eimeria* is a significant parasitic disease affecting goats (Abdelaziz *et al.*, 2021). *Eimeria* coccidian parasites cause enteric disease particularly in young or stressed goats under poor farm management resulting in high mortality rates in kids (Fayisa *et al.*, 2020). A clinical sign includes diarrhea, weight loss, anorexia and dehydration. Understanding the inherent aspects of the disease is crucial in defining the appropriate preventative measures (De Macedo *et al.*, 2020).

## **2.4 Molecular characterization of strongyles**

Gastrointestinal nematodes (GINs) are most common parasite widely distributed worldwide (Saidi *et al.*, 2020), cause significant reduction in the production and economic losses in husbandry (Barghandan *et al.*, 2020), posing a major constraint on the survival and productivity of animals (Yuan *et al.*, 2019). Strongyle infection of goat leads to economic

losses due to reduced productivity and increased mortality (Perry *et al.*, 2002). Reduced productivity stems from factors such as decreased food intake, stunted growth, diminished work capacity, and the cost associated with treatment and control of nematodes (Pedreira *et al.*, 2006; Odoi *et al.*, 2007). Nematodes rank second only to arthropods on earth in terms of their numbers and the complexity of their life cycles. Both adult and larval stages of nematodes may produce significant pathology in domesticated animals (Urquhart *et al.*, 1996). Typically, the diagnosis of GINs relies on microscopic technique to detect eggs or larvae in the faeces of animal (Zajac and Conby, 2006).

Traditional diagnostic procedure for nematodes necessitates laborious laboratory extraction, culturing and microscopic examinations of eggs or larvae from faecal samples. Advances in molecular technology offer the potential for more efficient and reliable methods (Learnmount *et al.*, 2009). However, differentiating nematodes based on larval stages has limitations. Many morphological characters overlap between larvae, making it challenging to identify them in mixed infections. Molecular methods provide a level of parasite identification beyond what is achievable with morphology alone (Veena *et al.*, 2020).

The utilization of molecular biology techniques has introduced new approaches for diagnosing nematodes. Methods for extracting DNA from various forms of parasites in animal faecal samples have enabled the use of molecular tools to diagnose several organisms (Schneider *et al.*, 1999). With the application of the polymerase chain reaction (PCR) these studies have been expanded. The PCR has been utilized to detect DNA from diverse organisms, thereby facilitating the biological diagnosis of parasite in tissues and secretions. Another significant application of the PCR is the differentiation of helminth species that are morphologically indistinct. Molecular techniques play a critical role in advancing of livestock sciences and can facilitate improved monitoring of the parasitic diseases and their drug resistance traits, which pose threats to livestock systems and food security worldwide (Whittaker *et al.*, 2017; Roeber *et al.*, 2013a). Various molecular approaches have been grown in recent years to monitor parasites of veterinary significance (Kotze *et al.*, 2020; Kumar *et al.*, 2021; Hassan and Ghazy, 2022).

*H. contortus*, *Oesophagostomum columbianum*, *Trichostrongylus colubriformis* and *Trichostrongylus axei* are most common and pathogenic species of strongyle. These species

cannot be readily distinguishable from each other or from less pathogenic species ones based solely on egg morphology. Given the presence of different GIN species can influence infections outcomes and the results of drug efficacy testing, it becomes essential to identify both the various species present and their relative plenty in co-infections. This identification is feasible through the morphological examinations of third-stage larvae (L3). However, this method is time taking and labor-intensive, need specific expertise, and crucially, can only be performed on fresh faecal material. Biobanking such material, particularly in tropical conditions, proves challenging (Airs *et al.*, 2023). Identifying *Haemonchus* species is particularly problematic due to the morphological overlap and genetic variation among populations (Arsenopoulos *et al.*, 2021).

Both morphological and molecular characteristic were play significant role in the identification and differentiation of the *H. contortus* parasites at the local levels. While morphological identification utilized as preliminary step, molecular identification utilizing the ITS sequences is crucial for species- specific identification isolates of *Haemonchus*. Sequence and phylogenetic analysis of ITSs give significant insights into the true taxonomic classification of various genotypes, enabling the establishment of precise strategies to detection of the parasite (Das *et al.*, 2023).

Conventional coprological methods involve identifying eggs or larvae based on morphology, which can be cumbersome and challenging due to similarities with other species or minor morphological variations. A multidisciplinary approach, combining both morphological and DNA-based techniques, offers a more reliable means of identification (Dorris *et al.*, 1999). Molecular methods have advantages such as rapidity and accuracy, making them particularly valuable for the specific identification of different nematode species (Callaghan and Beh, 1994; Roeber *et al.*, 2011).

Molecular techniques have become widely employed for identifying and diagnosing parasitic diseases (Dahlgren and Gjerde, 2010). They offer an alternative means to determine GINs from biobanked material with preserved DNA (Airs *et al.*, 2023). While molecular methods are considered gold standard for precisely identifying nematode species, morphometric based identification is more cost effective (Nath *et al.*, 2021). Sacrificing animals to obtain adult worms cannot used practice is not a practical practice; instead,

microscopic differentiation of infective stage larvae (L3) extracted from fecal cultures is utilized (Coles *et al.*, 2006). Accurately identifying nematode species, such as *Trichostrongylus* species in domestic animals, presents one of the most critical challenges in epidemiology and control studies for treating drug-resistant parasites (Chilton, 2004). Despite various morphological resemblance observed in the egg and larval stages of various *Trichostrongylus* species, depending solely on these characteristic to differentiate between them is neither practical nor efficient.

ITS-2 gene was targeted for confirming species identity. ITS-2 is widely utilized as a valuable tool for species identification due to its high specificity, conservative regions, and the availability of universal primers binding to the 5.8S and 28S ribosomal DNA genes of various nematodes (Heise *et al.*, 1999). A study conducted in the central part of Myanmar focused on the most common nematode, *Trichostrongyle* in sheep. Molecular characterization was pursued through the amplification of the ITS region of DNA. This approach was necessary because microscopic examinations alone could not reliably identify the specific species of *Trichostrongyle*, despite its significant impact on causing economic losses in sheep production (Win *et al.*, 2020).

In GINs, the nuclear gene internal transcribed spacers 1 and 2 (ITS-1 and 2) serve as crucial markers for various purposes such as constructing phylogenetic trees, estimating genetic population structures, assessing population-level evolutionary processes, and determining taxonomic identity (Powers *et al.*, 1997). Particularly, ITS-2 region stands out as most utilized marker for discriminating among nematode species and finds widespread application in species identification within the genus *Haemonchus* (Gasser and Newton, 2000). This region exhibits high variability and is subject to concerted evolution (Luton *et al.*, 1992).

The ITS region harbors dependable genetic markers that effectively differentiate closely related species. It's also utilized in identifying strongylid nematodes down to the species level (Newton *et al.*, 1998). Sequences of the ITS2 (Internal Transcribed Spacer) of rDNA are extensively employed as genetic markers for distinguishing various nematodes and for identification purposes (Lin *et al.*, 2012). This marker is commonly utilized to elucidate the classification and molecular relationships among parasites (Marigo *et al.*, 2011).

*Trichostrongylus* species infecting small ruminants in Dakahlia governorate, Egypt were identified using a molecular- based technique. Faecal samples were collected from 340 sheep and 115 goats from rural areas across three cities in Dakahlia governorate and two cities bordering it. Strongyle type eggs were detected in 33.2% of sheep samples and 14.7% of goat samples. PCR amplification of internal transcribed spacer of ribosomal DNA was for three *Trichostrongylus* spp., *T. axei*, *T. colubriformis* and *T. vitrinus*. Eggs harvested from 25 sheep samples and 16 goat samples were analyzed. Two species were detected at expected gel band size; *T. axei* (186bp) and *T. colubriformis* (232bp). *Trichostrongylus axei* was most prevalent species, found in 64% of sheep samples and 87.5% of samples tested in goat. *Trichostrongylus colubriformis* was detected in two samples from two different sheep herds but in combination with *T. axei*, no *T. colubriformis* was found in any sample from tested goats (Elseadawy *et al.*, 2021).

The study was focused on the morphological and molecular characterization of *Oesophagostomum columbianum* in goat in the Lucknow district of Uttar Pradesh. *Oesophagostomum* species were identified genetically using the second internal transcribed spacers of ribosomal (ITS2) regions of ribosomal DNA. The fact that the amplified PCR product range was approximately 117 bp indicates the size of the DNA fragment that was amplified using polymerase chain reaction (PCR) targeting the ITS2 region. This information is crucial for understanding the genetic makeup and diversity of *Oesophagostomum* species in the goat population studied (Jaiswal *et al.*, 2020a).

A study was conducted for to molecularly characterize strongylide eggs in the Nyala Area South Darfur State Sudan. Faecal samples were confirmed using PCR techniques, revealing a prevalence of *Haemonchus contortus* (47%), *Trichostrongylus* spp. (4%) and *Oesophagostomum columbianum* (0%). It was found that small ruminants are commonly affected by the multiple *strongylide* nematodes with a recorded 3% incidence of mixed infection between *Haemonchus contortus* and *Trichostrongylus* spp. (Hassan *et al.*, 2017).

A study was conducted to determine the prevalence of gastrointestinal *Strongyles* of goats in mid Himalayas regions of Uttarakhand, India. The researchers reported a prevalence of gastrointestinal nematode infection was to be 95.78% and EPG was 1982±452. Using PCR-RFLP results differentiated: *Haemonchus contortus*, *Teladorsagia circumcincta* and

*Trichostrongylus colubriformis*. According to the PCR-RFLP, results the predominant strongyle infections in high altitude of Uttarakhand were *H. contortus* and *T. circumcincta* (Sankar *et al.*, 2020).

In Thailand a study found high prevalence of *Strongyle* nematode infection in goats reaching 86.3%. Researchers successfully employed DNA sequencing and in-house semi-nested PCR utilizing primers specific to a region within the internal transcribed spacer 2 (ITS2), to identify the genera of strongyle present randomly selected egg-positive goat samples. Notably, *Haemonchus* spp. was found to be predominant genus accounting for 100% of the infections (Income *et al.*, 2021).

In Africa populations *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, and *Oesophagostomum columbianum* were the dominant gastrointestinal nematodes. Both multiplex PCR and qPCR showed high predictability in determining GIN species proportions compared to nemabiome amplicon sequencing. However, it was noted that high-resolution melt curve analysis was less reliable than PCR in predicting the presence of specific species (Airs *et al.*, 2023).

The overall prevalence rate of *Strongyle* from a slaughter house of Mhow, Madhya Pradesh was recorded 65%. The faecal samples that tested positive were pooled separately and then subjected to coproculture. Larvae obtained were identified up to genus level. Among the samples positive for strongyle type, seven genera of parasites were identified. *Haemonchus* spp. (32.33%) emerged as the predominant gastrointestinal nematode followed by *Oesophagostomum* spp. (22.51%), *Trichostrongylus* spp. (18.67%), *Cooperia* spp. (15.03%), *Nematodirus* spp. (12.65%), *Ostertagia* spp. (3.34%) and *Bunostomum* spp. (3.12%). *Haemonchus* was the most commonly encountered type of strongyle affecting 32.33% goats, while *Bunostomum* was the least frequently found affecting only 3.12% of goats (Rajpoot *et al.*, 2017).

## **2.5 Extraction of medicinal plants**

Extraction of medicinal plants involves the process of isolating active plant materials or secondary metabolites like flavonoids, alkaloids, saponins, terpenes, glycosides, and steroids from inert or inactive material using a suitable solvent and standardized extraction methods. Medicinal plants are extracted and prepared for direct consumption as herbal or

traditional medicine or prepared purposes of various experimentations (Abubakar and Haque, 2020).

The fundamental principle behind this process is to finely grind the plant material whether dry or wet thereby increasing its surface area for extraction and consequently enhancing the rate of extraction (Das *et al.*, 2010). The objective of extraction is to separate the soluble plant metabolites while leaving behind insoluble cellular residue (Azwanida, 2015). During the extraction process, solvents permeate the solid plant material and dissolve compounds with identical polarity (Pandey and Tripathi, 2014). To initiate the extraction process, dried or wet plant parts are finely grounded in a blender to produce fine particles. These particles are then combined with a specific quantity of solvent and vigorously shaken for 5-10 minutes or allowed for 24 hours. Subsequently, the resulting extract is filtered to remove any solid remnants (Das *et al.*, 2010).

The solvent employed for the extracting of medicinal plants is commonly referred as menstruum. Various solvents are utilized to isolate the medicinally active molecule from different plant materials (Handa, 2008). Typically, solvents are selected based on the polarity of the desired solute effectively dissolve it (Dai and Mumper, 2010). In the literature, a broad spectrum of solvents and solvent mixtures including water, methanol, ethanol, hexane, dichloromethane, ethyl acetate, chloroform, petroleum ether, and tetrahydrofuran have been employed to obtain crude extracts. Methanol stands out as the most frequently used solvent due to its high efficacy in extracting high polar phytochemicals like phenolic and alkaloids, resulting in substantial extraction yields (Truong *et al.*, 2019). The choice of solvent hinges on the factors such as type of plant, the plant part to be extracted, the nature of the bioactive compounds, and the availability of the solvent.

Earlier studies such as Green, (2004) suggested that a solvent to sample ratio of 10:1(v/w) is ideal. Solvents commonly employed in the extraction of medicinal plants ranges from polar (water and alcohols), to intermediate polar (acetone and dichloromethane), and nonpolar (n-hexane, ether and chloroform). Extraction procedures typically encompass maceration, digestion, decoction, infusion, percolation, soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions (Abubakar and Haque, 2020). Methanol emerges as the preferred solvent for plant extraction, likely due to its polar

nature, which facilitate the release of various bioactive compounds from plants. Scientific evidence supports the use of highly polar solvents for the accurate extraction of different bioactive compounds (Altemimi *et al.*, 2017). The efficacy of herbal formulations in yielding active compounds from plants significantly relies on the choice of solvents.

Water serves as a quintessential polar solvent, employed in extracting a broad spectrum of polar compounds due to its remarkable dissolving capabilities. This universal solvent boasts affordability, non-toxicity, non-flammability, and high polarity. However, its solvent properties can inadvertently foster bacterial and mold proliferation, potentially induce hydrolysis, and necessitate substantial heat input for extract concentration (Das *et al.*, 2010; Tiwari *et al.*, 2011).

In the cold extraction process, plant parts undergo drying in a carefully regulated environment at low temperatures, followed by milling into a powder, which is then precisely weighed. This powder is subsequently introduced into a beaker containing solvents and left at room temperature for thirty minutes. Over the course of seven days, the mixture is intermittently shaken every twenty-four hours. Following this period, the extract is subjected to filtration using Whatman filter paper under vacuum conditions. The resulting filtrate is then dried at room temperature on a watch glass dish. The weight of the powder is meticulously recorded both before and after the drying process (Harborne, 1998).

In maceration method, a whole or coarsely powdered plant material is immersed in a solvent within a container and continuously agitated until the biomass dissolve (Velavan, 2015). In the decoction extraction method, plant parts are boiled in water then cooled, strained, and rinsed with cold water to achieve the desire volume. For the infusion extraction method, plant parts undergo maceration with either cold or boiling water. In digestion extraction method, plant parts are macerated while under gently heated. In the percolation extraction method, the raw material is placed in an appropriate amount in a closed container for approximately 4 hours. Additional solvent is then added to cover the raw material and mixture is left to macerate in a closed container for 24 hours. Subsequently, the percolator is opened, and the extract is collected drop by drop. Additional solvent is added until the collected liquid measures about three-quarters of the final product's required volume. The remaining solid material is pressed and the resulting liquid is combined with collected

percolate. More solvent is added to reach the desired volume, and the mixture is clarified through filtration or decantation (Handa, *et al.*, 2008).

## **2.6 Medicinal plants**

Medicinal plants also known as medicinal herbs are plants whose various parts contains substances that can be utilized for therapeutic purpose or serve as precursors for synthesis of beneficial drugs (Sofowora *et al.*, 2013). They represent the most ancient form of medications, having been utilized for thousands of year in traditional medicine across numerous countries worldwide. The knowledge of their beneficial effect has been passed down through generations within human communities (Khan, 2014). In rural areas of India, sixty five percent of the populations rely on Ayurveda and medicinal plants to fulfill their primary health care needs (WHO, 2003). India boasts a rich flora of medicinal plants with more than 7500 species documented (Kumar *et al.*, 2018). Additionally, over 50% of the synthetic drugs currently available are derived from plant products (Butler, 2005).

Medicinal plants play a crucial role in indigenous medical systems worldwide (Srivastava and Singh, 2016) and often referred as chemical goldmines due to their natural compounds, which are compatible with human and animal physiology (Dhanik *et al.*, 2017). These plant-derived remedies, known as phytomedicines, have been utilized for their health benefits since ancient times (Gilani, 2005). In recent years, there has been a growing global interest in plant-based medicine owing to its natural origin, accessibility within local communities, affordability, ease of administration, and potentially fewer adverse effects. Herbal remedies may serve as valuable alternative treatments, particularly in cases of medication side effects and drug resistance (Ingle *et al.*, 2017; Azwanida, 2015; Sasidharan *et al.*, 2011). Natural product or compound separated from the plants offer significant advantage over synthetic drugs such as cost effectiveness, easy availability and minimal side effects (Wang *et al.*, 2007).

## **2.7 Anthelmintic activity of medicinal plants**

Medicinal plants are recognized their potential as significant sources of bioactive compounds, which lead to the development of novel drugs (Azwanida, 2015; Ali *et al.*, 2020). Scientist have analyzed and evaluated the effects of various types of solvents, for extractings these bioactive compounds from different parts of plant (Altemini *et al.*, 2017). Medicinal

plants have been utilized for centuries to combat parasitism and various human and veterinary illness for centuries and they are still employed for these purposes in many regions worldwide (Kumsa and Hagos, 2020). Phytotherapeutic drugs, particularly plant anthelmintics, are deemed safe, non-toxic, biodegradable and do not leave residues in animal products. Hence plants with anthelmintic properties have been included in the pharmacopoeias (Pathak and Chhabra, 2014). Various extracts have exhibit potential for development as anthelmintic agents (Ndlela *et al.*, 2021; Soren *et al.*, 2020).

Various *in vitro* techniques are commonly employed to assess the nematicidal activity of drugs or plant extracts prior to *in vivo* testing (Sangster and Gill, 1999). The criteria for determining anthelmintic efficacy *in vitro* have ranged from inhibition of motility or inducing paralysis to affecting glucose uptake or enzyme activity, and even to complete disruption of the parasites. However, it's worth nothing that there often exist as disparity between the efficacies observed *in vitro* studies due to differences in conditions and concentrations utilized in the assay (Pathak and Chhabra, 2014). The adult Indian earthworm (*Pheretima posthuma*) has commonly been utilized as a test organism in many anthelmintic screenings. This choice is based on its anatomical and physiological similarity to the intestinal roundworm parasites found in human. *Pheretima posthuma* worms are readily available and serve as a suitable model for screening anthelmintic drugs (Das *et al.*, 2011).

There are several advantages to conducting *in vitro* assay prior to *in vivo* studies. These include being less time consuming, more cost effective requiring smaller number of animals, and enabling the evaluation of the efficacy of various anthelmintic compounds throughout parasite's lifecycle (Demeler *et al.*, 2013). Reliable results obtained from *in vitro* analysis allow for further selection of extract or pure compound for *in vivo* evaluation (Zips *et al.*, 2005). *In vivo* studies are primarily conducted to assess the mechanism of action of the desired extract or compound, host animal immune response, levels of toxicity and overall effectiveness. Despite many advantages of *in vivo* studies, they also have some drawbacks such as being more time consuming, expensive and having lower precision and reproducibility (Lacey *et al.*, 1990).

Comparative analysis also indicated that extracts and essential oils were exhibit grater efficacy *in vitro* compared to *in vivo* against various stages of the parasites. Similar disparities between *in vitro* and *in vivo* outcome with plant treatments have been documented in prior

studies (Peneluc *et al.*, 2009; Nogueira *et al.*, 2012). These differences may be attributed to the varying bioavailability of plant chemical constituents across different segments of ruminant gastrointestinal tract (Athanasiadou *et al.*, 2007; Eguale *et al.*, 2007). Additionally, adult nematodes might display increased resistance to the active components or the presence of rumen micro biota could diminish the efficacy of metabolites (Nogueira *et al.*, 2012) along with other factors such as ruminal pH.

Most studies on the anthelmintic activity of plants in small ruminants have been conducted in sheep. However, findings from these studies cannot be directly applied to goats due to differences in their immunological, physiological and behavioral characteristics (Torres-Acosta *et al.*, 2004). Anthelmintic treatments are typically less effective in goats which may contribute higher nematode infections. Goats are more susceptible than sheep to gastrointestinal nematode infections exhibiting higher worm eggs production and increased numbers of adult parasites in their gastrointestinal tract. These factors suggested that goats may play a significant role in dissemination of worm eggs from resistant nematodes within the population small ruminants (Torina *et al.*, 2004).

## **2.8 Anthelmintics**

Anthelmintics or antihelminthics comprise a group of drugs designed to expel helminth worm from the body by effectively killing them without causing harm to host (Mahajan *et al.*, 2021). These drugs alternatively termed vermifuges or vermicides operate through the mechanisms that either kill or stun parasites, facilitating their removal from the host (Singla and Kaur, 2021). With their crucial role in veterinary medicine, anthelmintics represent a significant portion of the drugs utilized globally, estimated at 53% (Diaz Lira *et al.*, 2008). In veterinary practices anthelmintics find extensive use both as preventive measures and in the treatment of both chronic and acute infections (Sandhu, 2020). However, the excessive utilization of drugs within a singular chemical group can lead to the development of resistance among parasites to the active compounds resulting in treatment failure (Zanzani *et al.*, 2014; Michalski, 2001). Furthermore, administering inadequate doses, particularly in case of sheep may also contribute to the emergence of drug resistance in the nematode population owing to the rapid breakdown and elimination of drugs (McKenna and Watson, 1987).

Unfortunately, farmers often underestimate the impact of parasitic diseases, leading to deworming practices without prior faecal examinations for the parasites. Additionally, veterinarian sometimes assumes the goats will respond similarly to sheep or cows and administer incorrect drug dosing. Goats have a more rapid metabolism of anthelmintics than sheep. However, goats metabolize anthelmintics more rapidly than sheep, meaning that using the same dosages for both species may accelerate the development of parasite resistance (Pilarczyk *et al.*, 2021). Plants derived anthelmintic exhibit minimal to no toxicity, possess wide spectrum of activity, and are more environmentally friendly (Hammond *et al.*, 1997). Secondary metabolites extracted from plants through various isolation and purification techniques serve as viable alternative to synthetic drugs (Singla and Kaur, 2021).

## **2.9 Mode of action of medicinal plants extracts**

Morphological or motility changes may serve as indicators to discern the mode of action of plant products (Monzote *et al.*, 2012). The tegument/cuticle of helminth parasites has been identified as one of the primary target sites for antiparasitic drugs (Mottier *et al.*, 2006). The mechanism by which plant extracts affect human or animal cells is generally elucidated through the action of secondary metabolites. These metabolites often interact with specific molecular targets within cells, such as proteins, biomembranes, or nucleic acids (Wink, 2015) resulting in disrupted membrane permeability, neurotoxic effects, or antioxidant activity. Compounds found in essential oils, such as terpenoids (thymol and geraniol) or flavonoids, may also disrupt membrane permeability by disturbing cellular equilibrium, pH, and the balance of inorganic ions (Carnesecchi *et al.*, 2002; Lambert *et al.*, 2001).

Alkaloid compounds found in nearly all plant families are recognized as neurotoxic agents acting as agonists or antagonists at neuroreceptors and ion channels (Wink, 2000; Wink and Schimmer, 2018). They can inhibit the acetylcholine receptors (AChR) of multi cellular parasites inducing muscular paralysis. Consequently, parasitic worms attached to intestinal walls lose their grip and can be easily expelled from the gut (Wink and Schimmer, 2018). It has been suggested that the nematocidal activity of phenolic compounds such as thymol may stem from their inherent antioxidant properties (Ali *et al.*, 2013). Phenolic compounds are categorized as primary antioxidants, capable of forming a phenoxy radical upon donating a hydrogen atom thus exhibiting antioxidant properties through radical scavenging mechanisms

(Kiokias *et al.*, 2008). Recent studies have shown that the efficacy of thymol against *H. contortus* is comparable to that of the anthelmintic drugs class macro cyclic lactones (Lynagh *et al.*, 2014). These drugs induce cell hyper polarization by enhancing chloride ion reflux, thereby inhibiting parasite development larval stages and adult motility (Kotze *et al.*, 2002; Wolstenholme, 2011).

## **2.10 Secondary metabolites of plants having anthelmintic activity**

Medicinal plants serve as rich source of both primary and secondary metabolites. While primary metabolites, like carbohydrates, proteins and lipids primary provides nutrients, secondary metabolites constitute the main bioactive compounds (Silva Soares *et al.*, 2018). Secondary metabolites can be categorized into three primary groups: terpenes (including mono- and sesquiterpenes, saponins, and glycosides), phenolic compounds (comprising tannins and flavonoids such as anthocyanins, flavones, flavonols, and isoflavonoids) and nitrogen-containing compounds (encompassing alkaloids and nonprotein amino acids). These secondary metabolites are often attributed to the anthelmintic effects of medicinal plants (Idris *et al.*, 2019; Symeonidou, *et al.*, 2018; Mengistu, *et al.*, 2017).

Different mechanism of actions is responsible for the anthelmintic effect of secondary metabolites. Allelochemicals, produced from primary metabolites in plants serve as precursors for various secondary metabolites (Saltveit, 2017). Anthelmintic activity of medicinal plants has been attributed to secondary metabolites. Plants produce a diverse array of secondary metabolites including flavonoids, alkaloids, polyphenols, saponins, chalcones, terpenoids, coumarins, and tannins (Mukherjee *et al.*, 2016). The major secondary metabolites of plant accompanying with anthelmintic effect are 68% of condensed tannins, 12.5% of saponins and 8.9% of flavonoids (Sanatos *et al.*, 2019). These secondary metabolites can act through various mechanisms including inhibition of egg hatching enzymes, competition with membrane receptors and binding to proteins in the membrane (Chan-Pèrez *et al.*, 2016).

### **2.10.1 Tannins**

Tannins are water-soluble, polyphenolic group of compounds that aid in the elimination of nematodes. They achieve this by disrupting the absorptions of nutrients by the worms from the host cells observed by Symeonidou *et al.* (2018). Additionally larvae ingest condensed tannins these compounds bind to the intestinal mucosa of the parasitic worms leading to

autolysis described by Del Carmen Acevedo-Ramirez *et al.* (2019). Tannins have been extensively researched as a natural solution for nematode control in small ruminants (Hoste and Torres-Acosta, 2011).

The anthelmintic effect of tannins is hold responsible for its capability to bind to the proteins found in the cuticle, oral cavity, esophagus, cloaca and vulva of the nematodes, thereby, altering their physical and chemical properties. One more probability is associated to an indirect action of these compounds, which can increase the host immune response by binding dietary with protecting them from ruminal degradation and thus increasing protein availability in the small intestine (Hoste *et al.*, 2006). The application of polyvinylpolypyrrolidone (PVPP), an inhibitor of tannins, confirmed that this metabolite is accountable for the anthelmintic activity of the tannin rich plants (Alonso-Díaz *et al.*, 2008).

Feeding with tannin rich plants (containing 30 to 40 gm of condensed tannins per kg dry matter) promotes antiparasitic effects in the small ruminants. Although, over consumption of tannin can lead antinutritional effect in animals (Hoste *et al.*, 2006). The inclusion of tannins in ruminant diets at levels exceeding 4-5% of dry matter has been associated with reduced food intake, decreased dry matter digestibility, and impaired rumen metabolism (Barry *et al.*, 1999). Certain types of condensed tannins can also significantly impact the mucosa of the digestive tract in sheep and rats, leading to decreased nutrient absorption (Dawson *et al.*, 1999).

### **2.10.2 Terpenes**

Terpenes are compounds formed various isoprene units ( $C_5H_8$ ) (Chanda and Ramachandra, 2019). They exhibit anthelmintic activities inducing intestinal damage in parasites (Mukherjee *et al.*, 2016).

### **2.10.3 Glycosides**

Glycosides exhibit potent activity against various helminths (Overend, 2012; Kaingu *et al.*, 2013). Cardenolide disrupts the transportation of sodium and potassium ions in helminths leading to their demise (Hussein and El-Anssary, 2018).

### **2.10.4 Saponins**

Saponins consist of triterpene or sometimes steroidal aglycones attached to sugar chains (Güçlü-Üstündağ and Mazza, 2007). They exhibit anthelmintic activity by inhibiting acetylcholinesterase, which can result in worm paralysis and eventual death (Ali *et al.*, 2011).

They are known to have inhibitory effects against animal parasitic nematodes such as *Haemonchus contortus* (Cavalcante *et al.*, 2016). The anthelmintic effect of saponins is attributed to their ability to form complexes with cellular membrane components, leading to formation of pore and an increase in membrane permeability (Santos *et al.*, 2018).

#### **2.10.5 Flavonoids**

Flavonoids play various roles such as UV protection, flower coloring, allelopathy, and inhibition of auxin transport (Ohri and Pannu, 2010). In flavonoid-rich plants, activity is observed through the blocking of phosphorylation reactions, which ultimately inhibits energy production in parasitic worms, leading to their demise (Symeonidou *et al.*, 2018).

#### **2.10.6 Alkaloids**

Alkaloids have demonstrated anthelmintic activity by targeting acetylcholine receptors and suppressing glucose uptake. This leads to the death of helminths due to starvation, as they are deprived of essential nutrients (Badarina *et al.*, 2017).

#### **2.10.7 Non-protein amino acids**

Non-protein amino acids are the nitrogen -containing compounds with derivatives of ammonia hydrogen atoms. They damage parasitic worms by affecting the central nervous system of, leading to paralysis and eventual death (Huang *et al.*, 2011).

### **2.11 Chemotherapy**

Chemotherapy remains the cornerstone for controlling all types of helminthiasis, including gastrointestinal nematodes. However, the market offers a limited array of drugs for this purpose. Livestock are commonly administered four main classes of anthelmintics to combat nematode infections: benzimidazoles (such as mebendazole, albendazole, fenbendazole, and flubendazole), imidazothiazoles (like levamisole), macrocyclic lactones (such as ivermectin and moxidectin) and the amino-acetonitrile derivative known as monepantel (Taman and Azab, 2014; Geary *et al.*, 2015).

Closantel a salicylanilide anthelmintic disrupts oxidative phosphorylation in parasite mitochondria, thereby interfering with electron transport associated processes and halting ATP production. Depletion of energy kills the susceptible parasites. Closantel has a broad - spectrum of anthelmintic activity. This energy depletion ultimately leads to the demise of susceptible parasites.

With a broad spectrum of anthelmintic activity, it effectively targets adult and juvenile (6-10 weeks old) *Fasciola* flukes, blood-sucking nematodes (such as adult hookworms and *Haemonchus*) and certain tapeworms. Additionally, Closantel demonstrates efficacy against specific arthropods like mange, mites, ticks and parasitic fly larvae. After oral administration, closantel is readily absorbed reaching peak plasma concentrations within 24 hours. It is generally well- tolerated at recommended dosages, boasting safety margins up to six times the recommended dose (Sandhu, 2020).

## **2.12 Needs for phyto anthelmintics**

Phyto anthelmintics derived from plants are frequently used to manage helminthic infections in livestock. The management of these infections primarily relies on the preventive or curative application of chemotherapeutics. However, the use of antiparasitic drugs is often hindered by factors such as limited availability, treatments side effects and parasites resistance. Prolonged use and high-dose treatment regimens can also pose toxicity risks of host (Ranasinghe *et al.*, 2023). Drug resistance arises due to genetic changes in a parasite population, spurred by the selective pressure exerted by antiparasitic drugs, thereby hindering the treatment and control of parasitic infections (Bloland, 2001). The frequent and often excessive use of the same drug compounds to manage parasites in livestock has resulted in elevated resistance levels, posing a serious threat to the sustainability of livestock industries (Roeber *et al.*, 2013b).

Anthelmintic resistance is a growing concern in sheep, goats, and horses within industrial livestock systems globally (Van den Brom, *et al.*, 2013; Wirtherle *et al.*, 2004). For example *Haemonchus contortus*, has demonstrated a remarkable ability to develop resistance against majority of anthelmintic drug classes globally (Kotze, and Prichard, 2016), with resistance emerging in most cases within ten years of the introduction of each drug group. Additionally, the occurrence of multiple anthelmintic resistances in sheep and cattle is a significant worry (Sargison *et al.*, 2001). Consequently; there is an urgent need for new drug compounds that are safe, effective and affordable to effectively combat the current global burdens of parasitic diseases in both humans and livestock. The exploration of naturally occurring antiparasitic substances, such as those found in plants, which are plentiful and cost-effective, holds promise as a viable alternative in this regard.

## **2.13 *In vitro* and *in vivo* tests of for screening of plants for anthelmintic activity**

Numerous screening assays for anthelmintics, both *in vitro* and *in vivo*, assess the viability and motility of parasites (Murthy *et al.*, 2011). Several researchers have validated the anthelmintic properties of various plants through *in vitro* and *in vivo* studies (Iqbal *et al.*, 2007; Sunandhadevi *et al.*, 2017). However, the majority of research evidence in the literature focuses on *in vitro* rather than *in vivo* evaluations of plants against gastrointestinal parasites. This preference is primarily due to lower costs, higher throughput, quicker results turnover, and ethical considerations aimed at reducing the use of animals (Athanasiadou *et al.*, 2007).

### **2.13.1 *In vitro* tests**

Several *in vitro* tests were employed to screen plants for anthelmintic activity against various stages of parasites, including the egg hatch assay (EHA), larval development assay (LDA), larval feeding inhibition assay (LFIA), larval migration inhibition assay (LMIA), larval exsheathment inhibition assay (LEIA), and adult motility inhibition assay (AMIA). Few assays were established for *in vitro* drug screening or drug resistance testing in parasitic stages (Borges and Borges, 2016). The adult motility inhibition assay is the sole *in vitro* test designed to evaluate the anthelmintic effects on the parasitic life stage of the nematodes (adult worms), which were target of synthetic anthelmintic drugs. Although, this test has certain limitations such as euthanize an animal infected with gastrointestinal nematodes and requirement to maintain nematodes in a CO<sub>2</sub> incubator (due to short viability of parasite) (Hounzangbe-Adote *et al.*, 2005; Andre *et al.*, 2016).

*In vitro* assays offer several advantages over *in vivo* methods primarily due to their lower cost and quicker enabling large scale testing of plant materials. These tests are characterized by their affordability, rapid data collection, high sensitivity, repeatability and utilization of free-living stages such as eggs, first and third stage larvae, and adult nematodes species. However, they do have limitations, notably their inability to assess host factors since substances are directly applied to the parasite (O'Grady and Kotze, 2004; Borges and Borges, 2016). The adaptations of standardized *in vitro* methods are crucial for evaluating the efficacy of plant products, particularly in determining EC<sub>50</sub> and EC<sub>90</sub>. These measures facilitate the comparison of the activities of various plants (Borges and Borges, 2016).

*In vitro* studies are often used as screening tests, preceding *in vivo* evaluations. However, they, alone cannot conclusively confirm the anthelmintic efficacy of plants. This is because the conditions *in vitro* are differ significantly from those *in vivo*, particularly considering the complex environment of the gastrointestinal tract in ruminants. Therefore, it is essential to conduct pharmacokinetic studies to determine the bioavailability of the natural products in small ruminants. *In vitro* treatment involves direct contact with parasites, and the concentrations of potentially active substances may not always reflect their bioavailability *in vivo* (Githiori *et al.*, 2006). While, successful results from *in vitro* tests are promising indicators for *in vivo* assays, it's important to note that compounds showing efficacy *in vitro* may not necessarily exhibit the same level of effectiveness *in vivo* (Ranasinghe *et al.*, 2023).

### **2.13.2 *In vivo* tests**

Compared to *in vitro* studies, *in vivo* studies offer a more realistic assessment; however, they are also more time-consuming, expensive, and difficult to replicate. *In vivo* studies are tailored to assess the safety, toxicity, and efficacy of a drug candidate within the relevant host or model organism. However, it's worth noting that the majority of *in vivo* studies identified in this review have primarily centered on evaluating the efficacy of the candidate (Ranasinghe *et al.*, 2023). The evaluation of anthelmintic efficacy *in vivo* is typically conducted through either the fecal egg count reduction test (FECRT) or the controlled test method (Githiori *et al.*, 2006). However, it's worth noting that the FECRT may not always provide accurate estimates of efficacy due to the inconsistent correlation between egg count and actual worm numbers. While the FECRT offers an indication of the reduction in egg excretion post-treatment, the controlled test method is deemed more reliable as it involves comparing parasite burdens between treated and control groups. Nonetheless, it's important to recognize that the controlled test is also the most costly in terms of labor and animal utilization (Taylor *et al.*, 2002).

Majority of studies on potential *in vivo* anti-parasitic plants have shown a lower efficacy percentage compared to synthetic drugs. An anthelmintic product is considered effective when it achieves a reduction percentage of over 90% in EPG (egg per gram of faeces) and adult parasites (Vercruyssen *et al.*, 2001). For evaluation tests of *in vivo* herbal preparation, a lower level of reduction should be established (at least 70%), as products with moderate anthelmintic activity can still contribute to integrated parasite control programs in ruminant production

systems (Githiori *et al.*, 2006). Integrated management refers to the combination of chemical and nonchemical methods of parasite control aimed at maintaining acceptable levels of production without entirely eliminating the parasites (Hoste and Torres-Acosta, 2011).

The potential for biotransformation of these compounds within the animal's gastrointestinal tract, altered by rumen microorganisms, could result in a reduction of their biological activity (Athanasidou and Kyriazakis, 2004). *In vivo* testing of herbal drugs offers substantial evidence of their anthelmintic properties, ideally culminating in the examination of slaughtered animals to assess the presence of in gastrointestinal tract. Standardization of *in vivo* trials should be conducted according to both host and target parasite characteristics (Dehuri *et al.*, 2021).

#### **2.14 Limitations of the use of plant-based medicines**

Plant based medicines show promising potential in preventing and treating of gastrointestinal parasitic diseases. However, their application may be limited in utilizations due to issues with bioavailability. The major phytochemicals consisting of glycosides, tannins and flavonoids have not good solubility in water and lipid, thereby limiting their capabilities to cross biological membranes and leading to poor absorption (Manach *et al.*, 2004).

Additionally, the pharmacokinetics of these compounds may be further affected by the highly acidic gastric pH (Gao and Hu, 2010). Moreover, plants undergo various procedures including extraction, distillation, purification, concentration or fermentation to get bioactive compounds. During these processes, active components are susceptible to oxidation and hydrolysis, enhance regarding their stability (Rangari, 2009). Moreover, plant products are frequently prone to deterioration, especially during storage, leading loss in active components and the generations of metabolites without activity (Thakur *et al.*, 2011). Despite the promising potential and extensive uses of plants many of them remain not prove in term of safety or toxicity. This limited knowledge of their potential adverse effects poses difficulties in identifying safe and effective therapies (WHO, 2002).

#### **2.15 *In vitro* anthelmintic activity of aqueous extract of selected medicinal plants**

##### **2.15.1 *Coriandrum sativum***

Coriander is among the oldest herbs utilized for over 3,000 years as evidenced by the Ebers papyrus of 1550 BC. It has been valued both for culinary and medicinal purposes

(Laribi *et al.*, 2015; Adams *et al.*, 2012; Meena *et al.*, 2014). *Coriandrum sativum* commonly known as dhaniya belongs to Apiaceae family (Thakur *et al.*, 2021). It is primarily cultivated for its yellowish-brown globular fruits called as seeds (Sobhani *et al.*, 2022). *Coriandrum sativum* an herbaceous plant originally hailing from the Mediterranean region, has found widespread cultivation across Asia, Central Europe, and North Africa for various purposes (Laribi *et al.*, 2015). The genus is comprised of only two known species: *C. sativum* L. and *C. toridylum* (Fenzl) Bornm commonly known as wild coriander (Sahib *et al.*, 2013).

*Coriandrum sativum* has demonstrated pharmacological effects such as antioxidants, antimutagenic, antidiabetic, anthelmintic, anxiolytic, anticonvulsant and hepatoprotective properties (Laribi *et al.*, 2015). These effects are likely mediated by potent antioxidant activity of this plants and its primary constituents, linalool (Sobhani *et al.*, 2022). The enticing aromas and flavors of coriander have long captivated human interest due to the presence of a pleasantly aromatic essential oil rich in linalool, which is found in the stems, leaves, and fruits of the plant (Beyzi *et al.*, 2017). *C. sativum*, commonly known as coriander, harbors diverse classes of bioactive compounds, encompassing polyphenols, lipids, essential oils, isoprenoids, flavonoids, alkaloids, monoterpenoids, tannins, and glycosides (Prachayasittikul *et al.*, 2018). Extraction techniques notably affected by several factors, including extraction duration, solvent-to plant ratio and granulometry of the sample being extracted (Shi *et al.*, 2022). The extract comprises various phenolic compounds such as phenolic acids, flavonoids, and other substances (Zeković *et al.*, 2016). Both aqueous and hydro-alcoholic extracts of *Coriandrum sativum* demonstrate anthelmintic activity (Debella *et al.*, 2007).

Aqueous extracts of *Coriandrum sativum* exhibited strong inhibition of egg hatching. Complete inhibition of egg hatching occurred at concentration below 0.5 mg/ml with an estimated effective dose (ED<sub>50</sub>) of 0.12 mg/ml. additionally; the aqueous extract induced only 45% mortality in adult parasites of *Haemonchus contortus* at a concentration of 8 mg/ml. *In vitro* studies have shown that aqueous extracts of *C. sativum* possess significant anthelmintic activity against nematode parasites, particularly *Haemonchus* spp. (Egualé *et al.*, 2007).

Various concentrations of extracts (1 mg/ ml, 2.5 mg/ ml, 5 mg/ml and 10 mg/ml) were tested on *Haemonchus* spp. to observe the time of paralysis and time of death of the parasite. Albendazole (1 mg/ ml) was utilized as standard with PBS serving as control group.

The shortest time of paralysis was observed at the highest concentration (10 mg/ml) of aqueous extracts at  $388.3 \pm 3.8$  (Jaiswal *et al.*, 2020 b). Essential oils derived from *Coriandrum sativum* L. were found to be highly effective against *H. contortus in vitro*. This efficacy may stem from a combination of different chemical constituents whose interactions lead to compounds inhibits or disrupt vital functions from the early stages of development onwards. They may interfere with the parasites metabolic activities and disrupts its motility mechanisms, possibly by the affecting the nervous system (Oka *et al.*, 2000). Moreover, essential oils can alter the permeability of cell membranes and induce depolarization by interacting with and disrupting the chemical structures of lipids, polysaccharides and phospholipids (Bakkali *et al.*, 2008).

### **2.15.2 *Allium sativum***

Garlic is scientifically named as *Allium sativum* L. (Liliaceae) (Vanaclocha and Cañigüeral, 2019). *Allium sativum* is one of the oldest cultivated medicinal plants (Imo and Zàaku, 2019), belongs to the family Amaryllidaceae (Londhe *et al.*, 2011). The bulb is primarily utilized to treat the illness and the perennial herbaceous plants is large, with upright flowering systems that can extends up to one meter (Meriza *et al.*, 2012). Garlic was initially discovered in Central Asia and later spread throughout the China, the Near East and the Mediterranean before reaching Southern and central parts of Europe, Mexico and northern Africa particularly Egypt (Londhe *et al.*, 2011). Garlic contains a higher concentration of sulfur compounds such as allicin, diallyl disulfide, S-allylcysteine and diallyl trisulfide, which are accountable for its therapeutic properties (Singh and Singh, 2019; Tesfaye and Mengesha, 2015). A wide range of therapeutic effects of garlic with its low toxicity are significant interest to researchers scrutinized its medicinal properties (Bayan *et al.*, 2014).

Garlic is known for its ability to inhibit and destroy bacteria, fungus and parasites while also aiding in lowering blood pressure, cholesterol and sugar levels, protecting the liver as well as preventing blood clotting. Additionally, it exhibits anticancer effects (Singh and Singh, 2019). Allicin found predominantly in aqueous garlic extract and raw garlic products is considered the primary bioactive compound (Elosta *et al.*, 2017). The most commonly utilized method for garlic extraction is maceration (Santander, 2021). Allicin, along with its breakdown products such as diallyl disulfide and diallyl trisulfide, are recognized as the main

contributors to the garlic's characteristic odour along with other allicin derived compounds like vinylthiols and ajoene (Newall *et al.*, 1996). The antiparasitic activity of *A. sativum* can be attributed to the sulfur containing compounds such as ajoene and allicin. These compounds can potentially form disulfide bonds with free thiol groups, thereby inhibiting enzymes or other proteins crucial for parasites survival (Krstin *et al.*, 2018).

Exposure of cercariae of flukes against aqueous extracts from *Allium sativum* bulbs resulted in their death (Nama and Bhatnagar, 1990). *In vitro* studies demonstrated significant anthelmintic activity of aqueous extracts of *Allium sativum* was observed at 16 hours with lowest concentration (10%) and at 10 hours with the highest concentration (50%) against *Ascaridia galli* (Singh, 2014). Anthelmintic activity of the aqueous extract of *Allium sativum* exhibited a dose dependent response against Indian earthworms. The potency of test sample was exhibited inversely proportional to take time for paralysis and death of worms. The activity was evaluated to determining the time of paralysis and death of worms (Prajakta *et al.*, 2015). Aqueous extract of bulb of *Allium sativum* demonstrated better efficacy regarding the egg hatch assay and larval development test to naturally occurring ovine gastrointestinal nematodiosis. However, in larval paralysis test, reverse trend was seen as methanolic extract was more potent than the aqueous counterpart (Kanojiya *et al.*, 2015).

The Aqueous extract of *Allium sativum* bulbs *in vitro* has shown moderate effects on both the eggs and the larvae of *Ancylostoma caninum* and *Toxocara canis* (Orengo *et al.*, 2016). Additionally, garlic's aqueous extract exhibit good activity against nematodes such as *Trichuris muris* and *Angiostrongylus cantonensis* (Kirha *et al.*, 2016). Furthermore, the aqueous extracts of *Allium sativum* L. have demonstrated a significant anthelmintic effect on *Capilaria* spp. in both fish and sheep (Khalil *et al.*, 2016). The *ethanolic* extract of *A. sativum* inhibits the motility of *H. contortus* by exerting a destructive and inhibitory effect on the enzyme acetyl cholinesterase. The enzyme plays a crucial role in hydrolyzing acetylcholine a neurotransmitter involved in cholinergic synaptic transmission (Taylor, 1990; Lee, 1996). Inhibition of AChE leads to the accumulation of acetylcholine, disrupting neuromuscular transmission and causing paralysis of musculature. Consequently, muscular discoordination/paralysis halts food swallowing and movement through digestive system. Thus ultimately induces a state of starvation and energy deprivations in the parasites

rendering them unable survive inside the host (Kaur and Sood, 1982; Opperman and Chang, 1992).

### **2.15.3 *Carica papaya***

The common name of *Carica papaya* is Papaya (Yogiraj *et al.*, 2014). Scientifically, it is referred to as *Carica papaya* Linn. (Milind and Gurditta, 2011; Da Silva *et al.*, 2007). Belonging to family Caricaceae, *Carica papaya* is native to Mexico and northern South America, but has naturalized in various parts of the world particularly in tropical and subtropical regions (Sharma *et al.*, 2020). Papaya is recognized as the medicinal plants providing significant of therapeutic remedies based on the traditional or indigenous healing system and widely utilized by the population in several countries (Sharifi-Rad *et al.*, 2018). Various parts of papaya plant including the fruit, bark, roots, seeds, peel, pulp and leaves have been recognized for their therapeutic uses worldwide (Saran and Choudhary, 2013). Papaya is known for its several health benefits attributed to the presence of key phytochemicals such as phenolic, flavonoids and alkaloids (Sharma *et al.*, 2020). The seeds, which are numerous, small and black round, covered with gelatinous aril, account for about 16% of the fresh fruit weight. Each seed comprises sarcotesta and endosperm and has been found to possess several medicinal and nutritional properties (Wadekar *et al.*, 2020).

Papaya seeds exhibit pharmacological activities such as anthelmintic, contraceptive, antifertility, anti-analgesic, inflammatory, and antimicrobial properties (Agarwal *et al.*, 2016). They are edible and have a sharp, spicy taste, sometimes used as substitute for black pepper (Arvind *et al.*, 2013). Alkaloids significant compounds known for their physiological effects on animals are present in papaya seeds (Jayakumar and Kanthimathi, 2011). Benzyl isothiocyanate is main constituents for a papaya seed has anthelmintic properties (Rumiyati, 2006). Moreover, the efficacy of papaya latex and cysteine proteinases against the gastrointestinal nematodes *Heligmosomoides polygyrus* has been suggested offering a novel class of anthelmintics (Shackel Fordd *et al.*, 2000). The phytoprinciple benzyl isothiocyanate has been isolated from the extract due to its role as an active agent responsible for anthelmintic activity. According to Sharma (1987), the primary target sites of anthelmintic compounds including benzyl isothiocyanate are the metabolic pathways in general with particular emphasis on carbohydrate pathways as well as neuromuscular coordination. *In*

*vitro* studies conducted by Kumar *et al.* (1991), have revealed that benzyl isothiocyanate exerted its action by inhibiting energy metabolism and by influencing motor activity of the parasites. Additionally, Kermanshai *et al.* (2001) conducted a study where benzyl isothiocyanate isolated from *C. papaya* Linn. seed extract was tested for anthelmintic activity using a viability assay with *Caenorhabditis elegans*. This study claimed and was claimed benzyl isothiocyanate as the primary anthelmintic agent.

The seeds of *C. papaya* have been used to manage the helminthes infections in traditional medicine (Srivastava and Singh, 2016). The anthelmintic properties of the aqueous extract of *Carica papaya* seeds (Carbicaeaceae) against *Ascaris lumbricoides* and *Ascaridia galli* have been well established (Yadav and Tangpu, 2006). *In vitro* studies of anthelmintic activity of *Carica papaya* seed ethanolic and hydro ethanolic extracts on *Pheretima posthuma* showed grater effectiveness in comparisons to extracts from stem bark and leaves both in reducing in times regarding paralysis and death (Goku *et al.*, 2020). *C. papaya* seeds are inexpensive; naturally, occurring harmless, readily available and can serve as a monotherapy for preventing the intestinal parasitosis (Okeniyi *et al.*, 2007). The anthelmintic properties of the aqueous extract of *Carica papaya* seeds of the Carbicaeaceae family against *Ascaris lumbricoides* and *Ascaridia galli* have also well established (Dhar *et al.*, 1965; Yadav and Tangpu, 2006).

The aqueous extract of papaya seeds demonstrated over 90% efficacy against *Oesophagostomum*, *Trichuris* and *Trichostrongylus* (Fajimi *et al.*, 2001). Additionally, *in vitro* tests showed that a concentration of 50 mg/ml of the aqueous extract exhibited 70-80% efficacy against gastrointestinal nematodes in cattle (Amin *et al.*, 2009). Crude aqueous extracts of *C. papaya* have shown anthelmintic activity *in vitro* against gastrointestinal nematodes in sheep. The egg hatch assay revealed a reduction of more than 95.8% in egg hatch at concentration of 500 mg/ml for *C. papaya* seeds. Results from the larval development inhibition assay indicated that the aqueous extract of *C. papaya* seeds yielded more than 98% inhibition at a concentration of 500 mg/ml. Based on the LD<sub>50</sub> values dried extract of *C. papaya* seeds were the most potent for inhibiting both egg hatching (49.94 mg/ml) and larval development (49.32 mg/ml) (Odhong *et al.*, 2014).

The aqueous extract of *C. papaya* was observed 93.33% effective against deshelled larvae of *A. galli* at the concentration of 60 mg/ml. Adult motility assay, *C. papaya* aqueous

extract exhibited 100% effectiveness in killing adult *A. galli* worms at concentration of 20 mg/ml in poultry. This anthelmintic effect was observed to be dependent on both concentration and time (Sen *et al.*, 2020). Furthermore, the mean paralysis time for aqueous extract of *Carica papaya* as well as its combination with albendazole was found to be shorter than that of albendazole alone ( $p < 0.05$ ) indicating a greater efficacy in paralyzing the worm compared to albendazole alone (Muundaa *et al.*, 2020). *In vitro* experiments with aqueous seed extract of *C. papaya* demonstrated efficacy ranging from 51 to 100% in controlling *Ascaridia galli* eggs in chickens. Results after 21 days of incubation showed that *C. papaya* seed extract effectively inhibited the development of *A. galli* eggs, particularly during the 7th day of incubation (Salvedia, 2023). When tested against *Haemonchus contortus* in goats, the corrected mortality of aqueous extract of *Carica papaya* seed at concentration of 15 mg/ml was found 29%, 27% and 25% at 1 hour, 2 hour and 3 hour of trial respectively, compared to the control (Kumar *et al.*, 2023).

#### **2.15.4 *Zingiber officinale***

*Zingiber officinale* commonly known as ginger (Rehman and Fatima, 2018), belongs to the Zingiberaceae family and the genus Zingiber. It has been widely utilized both as a spice and an herbal medicine for centuries (Han *et al.*, 2013). Its medicinal properties have been recognized for over 2000 years in India and neighboring countries establishing it as one of the most versatile medicinal plants (Gupta and Sharma, 2014). The underground edible part of ginger is called rhizome. It is encased in a thick, brownish outer layer, revealing a yellowish interior (Hart, 2005). Scientifically known as *Zingiber officinale* Roscoe (Dhanik *et al.*, 2017), ginger is an herbaceous perennial that grows annual pseudo stems reaching approximately a meter in height adorned narrow leaf blade (Azmat *et al.*, 2019). Originating in Southeast Asia, ginger is cultivated extensively in various regions including India, Australia, Nigeria, China and Jamaica.

The plant is called as Sringavera in Sanskrit (Vasala, 2004) and Sunthi in Ayurveda has been depicted in the ancient texts such as Charaka, Sushruta, Vagbhatta and Chakra-dutta (Agrahari *et al.*, 2015). In traditional medicine the gnarly, thick underground stem of ginger is commonly utilized (Rehman and Fatima, 2018). Ginger is rich in active constituents, including phenolic and terpene compounds (Prasad and Tyagi, 2015). Ginger rhizomes major constituents

are 50-70% (carbohydrates), 3-8% (lipids), terpenes and phenolic compounds (Grzanna *et al.*, 2005). Phytochemical constituents in *Zingiber officinale* that act as an anthelmintic includes flavonoids, tannins, saponins, phenols and terpenoids (Abdullahi *et al.*, 2017). In traditional medicine, the rhizome has been used for the treating various diseases, including inflammatory conditions and has demonstrated several pharmacological activities like antiulcer, antiemetic, anti-inflammatory, antiplatelet, antioxidant, glucose and lipid lowering, cardiovascular, antimicrobial, gastro protective, neuroprotection effects and respiratory protection and anti-cancer activities (Jafarzadeh and Nemati, 2018). The relative composition in ginger extraction is determined by ginger species, rhizome maturity, climate conditions during growth time of harvest and extraction methods (Grzanna *et al.*, 2005).

The anthelmintic activity of aqueous extract of rhizome of *Zingiber officinale* was investigated against the adult earthworm (*Pheretima posthuma*). The study revealed paralysis occurring within 32 to 34 minutes, 28 to 31 minutes and 25 to 29 minute with subsequent death occurring 86 minute, 78 minute and 65 minute respectively, at concentration of 25, 50 and 100mg/ml. A comparable effect to piperazine citrate at the concentration of 10 mg/ml, was observed inducing paralysis within 23 to 25 minute and subsequent death within 62 minute (Dubey *et al.*, 2010). Moreover, the crude aqueous extract of ginger against exhibited activity antischistosomal activity against *Schistosoma mansoni*, resulting in partial loss of tubercle spines, extensive erosion in the tegumental region, and alterations in normal surface topography (Osama *et al.*, 2011).

*Zingiber officinale* aqueous extract exhibited concentration dependent efficacy on the *Ascaridia galli* roundworm in the domestic fowl. Significant mortality rate was recorded at 16 hours at the lowest (10%) concentration and at 12 hours in the highest (50%) concentration (Singh, 2014). During the *in vitro* tests, ginger was observed a dose-dependent antiparasitic effect exhibited against *Dactylogyrus* spp. (Van *et al.*, 2021). *Zingiber officinale* aqueous extract exhibited paralysis time of 1.22 hours, 1.08 hours and 1.0 hours at the concentrations of 20 mg/ml, 40 mg/ml and 60 mg/ml respectively. The death time was recorded 1.5 hours at all concentrations against the earthworms. The capability of the extracts resulted into paralysis and death was depending on increase the concentration (Marwa *et al.*, 2024).

### 2.15.5 *Azadirachta indica*

*Azadirachta indica*, belongs to family Meliaceae, is commonly called as neem and has been recognized from ancient times for its medicinal properties (Rahmani *et al.*, 2018). The term *Azadirachta indica* is derived from Persian where ‘Azad’ means free, ‘dirakht’ means tree and ‘I’ signifies Hind, indicating its Indian origin (Latif *et al.*, 2020). Neem is originated from Indian subcontinent and is widely cultivated, especially in drier (arid) tropical and subtropical parts of Asia, Africa, Australia, Americas, and the South Pacific islands (Devi and Sharma, 2023). The leaves, stem, seeds, bark, roots, gum, fruits and flowers have been utilized in traditional medicines for d treating various type of human diseases from ancient times (Islas *et al.*, 2020). The medicinal properties of neem are primarily attributed to its leaves. Both whole neem leaves and active components extracted from them have been widely used for their multiple health benefits (Asghar *et al.*, 2022). The tree has been extensively used in Naturopathy, Unani and Homoeopathic medicine and has become cornerstone of modern medicine (Srinivasa Reddy and Neelima, 2022). Neem consist of a variety of chemicals across its various plant components, such as quercetin, azadirachtin, a number of limonoids and nimbosterol (Kumar *et al.*, 2018). Nimbin, nimbolide, ascorbic acid, n-hexacosanol and nimbiol are just a few of the substances founds in the leaves (Rinaldi *et al.*, 2017). Additionally, nimbinin, nimbin, nimbidin, salannin, zafaral, and azadirachtin are also found neem. The diverse chemical constituents of neem exhibits various properties such as antibacterial, antitumor, antifungal, hepatoprotective, antifertility, antidiabetic, nephroprotective and neuroprotective (Devi and Sharma, 2023).

Neem has been reported to exhibit activity against insects and parasites affecting both plants and animals. Its insecticidal activity arises from its ability to inhibit the enzymes ecdysone 20-monoxygenase, which are crucial for the larval development of insects like *Drosophila melanogaster*, *Aedes aegypti* and *Manduca sexta* (Mitchell *et al.*, 1997). The anthelmintic properties of neem are likely attributed to the presence of an active alkaloid, azadirachtin, which disrupts the central nervous system of the parasites by inhibition excitatory cholinergic transmission and partially blocking the calcium channel leading to the expulsion of parasites from the host body (Qiao *et al.*, 2013; Veerakumari and Priya, 2006). *Azadirachta indica* and *A. juss*, leaves contains condensed tannins (CT) (Saktia *et al.*, 2018), which make

possible diffusion of flavonoids by binding to the cuticle proteins (Kerboeuf *et al.*, 2008). Flavonoids and condensed tannins inhibit the secretion of key enzymes like esterase, tyrosin kinase and non specific cholinesterase which can result in fatal intracellular instability, neuromuscular disorganization, energy depletion, paralysis and ultimately death of the parasites (Hoste *et al.*, 2006; Kerboeuf *et al.*, 2008).

Aqueous extracts of *Azadirachta indica* leaf, stem, root and bark demonstrated significant inhibition of nematode egg hatch (Okwudiri *et al.*, 2006). This reduction in egg hatch was concentration dependent with the highest inhibition was observed at the concentration of (100 mg/ml) of the extracts reaching 51 % and 50 % for the leaf and stem bark extracts respectively. However these effects were inferior to those produced by albendazole which achieved 100% inhibitions at 40 mg/ml, larval mortality followed a similar pattern in both extract types, increasing with higher concentrations of the leaf and root bark extracts. The decrease in larval survival caused by the extracts was comparable to that achieved by Albendazole (Nwosu *et al.*, 2006). Furthermore the aqueous leaves extract of *Azadirachta indica* exhibited significant anthelmintic activity against various species including earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*) species (Rabiu and Subhasish, 2011).

The aqueous leaf infusion of *A. indica* at doses of 6% exhibited *in vitro* anthelmintic activities against *H. contortus* through reducing egg hatch and adult worm motility, indicating its potential as a bio-anthelmintic against *H. contortus* (Saktia *et al.*, 2018). The *in vitro* anthelmintic properties of *Azadirachta indica* aqueous leaf extract was conducted on *Paramphistomum cervi* and *Fasciola hepatica*. Result showed a mortality rate over 50% for both parasites within a 5 hours duration starting from a concentration of 4 mg/ml of the extract and above. The anthelmintic activity of *Azadirachta indica* was observed to be dependent on both time and concentration (Ibekwe, 2019). The crude aqueous extract of leaves of *A. indica* demonstrated *in vitro* anthelmintic activity against gastrointestinal nematodes in sheep, goats and cattle with, an effective concentration 0.06 mg/ml though it is not effective for cattle (Correa Herrera *et al.*, 2023).

### 2.15.6 *Swertia chirata*

*Swertia chirata* is also known as *Chirayta*, is highly regarded Unani medicinal herb with a rich history of traditional use across the various medicinal properties (Zakir *et al.*, 2021). Sometimes it is also called Nepali Neem owing in the forests of Nepal. *Swertia chirata* was native to the temperate Himalaya this is annual or biennial herb is esteemed for its bitter taste and versatile application in the traditional medicines (Aleem and Kabir, 2018). It is often referred to as *Chirayta Shherin* and *Chirata talkh* due to its pronounced bitterness, attributed to a compounds such as amarogentin, swerchirin, swertiamarin and other bioactive, with amarogentin being the most bitter compound identified as yet (Greenish, 1999). The genus *Swertia*, belonging to the Gentianaceae family was initially described by the Roxburgh in 1814 under the name of *Gentiana chyrayta* (Kumar and Van Staden, 2016; Scartezzini and Speroni, 2000). In India alone records approximately 40 species of *Swertia chirata* (Kumar and Van Staden, 2016). This herbaceous plant or shrub thrives in the sub-temperate regions of Himalayas, typically found at altitudes ranging from 1200 to 1500 meters and can grows up to 1.5 meters in height from Bhutan to Kashmir (Kumar and Van Staden, 2016).

The crude extract of the various parts of the plants contains major bioactive such as mangiferin, amarogentin and swertimarin along sides secondary metabolites like glycosides, secoirrioid, xanthenes, phenolics, flavonoids, alkaloids, tannins, triterpenes, carbohydrates and sterols. These phytochemicals contributes to its pharmacological effects (Phoboo *et al.*, 2010; Mahjabeen *et al.*, 2017). *Swertia chirata* exhibits significant medicinal properties including anti-inflammatory, hepatoprotective, hypoglycemic, antibacterial and wound healing effect (Laxmi *et al.*, 2011).It also demonstrates antioxidant, antispasmodic, antipyretic anti-diabetic, and antitussive activities (Tabassum *et al.*, 2012; Saha *et al.*, 2004). Traditionally, *Swertia chirata* is used as a bitter tonic for gastrointestinal disorders such as dyspepsia/anorexia, acting as a digestive aid, laxative and preventive measure against malaria induced fever. It is also effective against intestinal worms, body burns, bronchial asthma and bowel regulating (Khanal *et al.*, 2014). All parts of the chirata plant hold therapeutic value (Aleem and Kabir, 2018).Chemical constituents of *Swertia chirata* such as amarogentin and sweroside contribute to its anthelmintic activity along with other biological effects (Sinha *et al.*, 2023).

*In vitro* studies on anthelmintic properties of *Swertia chirata* have demonstrated that at 25 mg/ml the crude aqueous (CAE) derived from *S. chirata* whole plant exhibited an anthelmintic effect against live *Haemonchus contortus* (Iqbal *et al.*, 2006) albeit statistically insignificant ( $P \geq 0.05$ ). Additionally, steam distillates of *Swertia chirata*, both individually and in conjunction with cow urine, exhibited significant anthelmintic activity against the Indian earthworm (*Pheretima posthuma*) *in vitro* (Kekuda *et al.*, 2009).

### **2.15.7 *Prunus persica***

The *Prunus persica*, commonly known as the peach, is a perennial, deciduous tree characterized by its rounded crown and upward- reaching branches adorned with 3 to 6 inch long, dark green and deciduous leaves (Kumar and Chaudhary, 2017). The term *Prunus* may have its origins in Greek words *Prounos* or *Proumnos* (Biswajit Das *et al.*, 2011). This tree exhibits a wide variety of fruit with flesh colors and shapes that are distinctive (Kant *et al.*, 2018). In Zhejiang province of Eastern China *Prunus persica* was first domesticated and cultivated there (Zheng *et al.*, 2014). The particular name *persica* alludes to its extensive cultivation in Persica (modern day- Iran) from where it was later transplanted to Europe and subsequently in 16<sup>th</sup> century to the Americas.

Peaches exhibit essential biological activities and are recognized for their prevent properties against various diseases due to their abundance in secondary metabolites, including phenolic compounds, carotenoids and tocopherols (Haleema *et al.*, 2020). Beyond their nutritional and nutraceutical value, peach byproducts such as peel and residual pulp demonstrate hypoglycemic and hypotriglyceridemic effects (Rodríguez-gonzález *et al.*, 2018). Additionally, peach leaves display pharmaceutical potential and are traditionally employed as antihelminthics, sedative and laxative (Kazan *et al.*, 2014). The leaves contain various compounds such as caffeic acid, chlorogenic acid, p- coumaric acid, quercetin, kaempferol, quercetin-3- glycoside, quercetin-3-rhamnoside, urosolic acid, tannin, and zeaxanthin (Haleema *et al.*, 2020). The leaves possess anthelmintic, diuretic, insecticidal, demulcent, sedative, expectorant and vermifugal and are utilized in treating conditions such as leucoderma and piles (Aziz and Rahman, 2013). Leaf paste is applied externally combat worms infestations and fungal infections. Internally, treatment for gastritis, whooping cough and chronic bronchitis are administered using leaves (Kirtikar and Basu, 2001).

The leaves of *P. persica* were tested for their effectiveness against helminths, specifically, *Pheretima posthuma* (Indian earthworm), similar in anatomy and physiology to intestinal roundworms found in humans, and *Ascaridia galli*, a roundworm posing significant challenges in poultry industry (Kumar *et al.*, 2015). The anthelmintic activity of crude powder, aqueous, diethyl ether and methanol extracts of *Prunus persica* leaves was examined at concentration of 0.25%, 0.5%, 1 % and 2 % against adult *Haemonchus contortus*. All extracts exhibited 100% efficacy after 24 hours. Overall, crude powder and aqueous extracts demonstrated superior anthelmintic activity compared to diethyl ether and methanol extracts (Kumar *et al.*, 2014).

A study was conducted to compare the anthelmintic potential of methanolic and aqueous extract of *Prunus persica* leaves on adult Indian earthworm. The researchers tested various concentrations (15, 30, 45, 60, 75 and 100 mg/ml) of both extracts methanolic and aqueous to evaluate their anthelmintic activity by measuring time it took for paralysis and death of the worms. The result showed significant anthelmintic effects on the live adult *Pheretima posthuma* across different concentrations. The aqueous extract particularly demonstrated very significant results compared to the methanolic extracts (Ch. Usharani *et al.*, 2014).

## **2.16 Cytotoxicity of selected medicinal plants**

Cytotoxicity stands out as a paramount parameter in the assessment of biological studies conducted *in vitro*. In these controlled environments, substances like pharmaceuticals and pesticides exhibit diverse mechanisms of cytotoxicity, including but not limited to cell membrane disruption, inhibition of protein synthesis, and irreversible binding to receptors. To ascertain the extent of cell demise resulting from such damages, there exists a pressing demand for cost-effective, dependable and consistently reproducible short-term assays for cytotoxicity and cell viability. These assays rely on the evaluation of assorted cellular functions to gauge the health and viability of cells under experimental conditions.

A variety of cytotoxicity assays are utilized in the fields of toxicology and pharmacology, each with its own classification such as dye exclusion, colorimetric, fluorometric and luminometric assays. Selecting the appropriate method among these assays is crucial for obtaining precise and dependable results (Aslantürk, Ö. S., 2018). Viability levels and proliferation rates of cells serve as reliable indicators of cell health, which can be

influenced by both physical and chemical agents. These agents may induce toxicity in cells through various mechanisms, including cell membrane damage, inhibition of protein synthesis, irreversible binding to receptors, interference with polydeoxynucleotide elongation, and disruption of enzymatic reactions (Ishiyama *et al.*, 1996).

*In vitro* cell viability and cytotoxicity assays using cultured cells have become increasingly popular for cytotoxicity testing of chemicals and drug screening in recent years. These assays offer several advantages, including rapidity, cost-effectiveness, and elimination of the need for animal testing. Additionally, they enable the testing of a large number of samples efficiently. Cell viability and cytotoxicity assays rely on various cellular functions such as cell membrane permeability, enzyme activity, cell adherence, ATP production, coenzyme production, and nucleotide uptake activity (Ishiyama *et al.*, 1996). While *in vitro* assays offer benefits like speed, reduced cost and potential for automation, tests utilizing human cells may be more relevant than certain *in vivo* animal tests.

Certainly, it's crucial to recognize that despite their benefits, *in vitro* cytotoxicity and cell viability assays still come with limitations. They haven't reached a level of technological sophistication where they can entirely supplant animal testing (Chrzanowska *et al.*, 1990). Ongoing progress in technology and methodology is essential to address these limitations and enhance the accuracy and applicability of *in vitro* assays in toxicological and pharmaceutical research (Chrzanowska *et al.*, 1990). Understanding the quantity of viable cells remaining and the number of decreased cells following the experiment is crucial. In the realms of toxicology and pharmacology, a diverse array of cytotoxicity and cell viability assays are employed for this purpose. The selection of appropriate assay method is paramount for accurately assessing the nature of interaction (Sliwka *et al.*, 2016).

The SRB (Sulforhodamine B) assay stands as a rapid and highly sensitive colorimetric method for computing the drug-induced cytotoxicity in both adherent and suspension cell cultures. Originating from the pioneering work of Skehan and colleagues, this assay emerged within the ambit of the National Cancer Institute's comprehensive anticancer drug discovery program, inaugurated in 1985. SRB, a vibrant pink aminoxanthene dye featuring two sulfonic groups exhibits an affinity for basic amino acid residues within proteins of TCA-fixed (trichloroacetic acid) cells thereby furnishing discerning measures of cellular protein content.

Moreover, the SRB assay serves as a versatile tool for assessing colony formation and extinction. Renowned for its simplicity, swiftness and sensitivity, the SRB assay demonstrates excellent linearity with cell count, allowing for the utilization of saturating dye concentrations. Furthermore, its robustness renders it less susceptible to environmental fluctuations, while its independence from intermediary metabolism simplifies experimental interpretation. Notably, the assay culminates in a fixed endpoint, obviating the need for time-sensitive measurements of initial reaction velocity (Skehan *et al.*, 1990). The high reproducibility of this method underscores its reliability; however, it is imperative to maintain a uniform cell suspension, avoiding the formation of cellular aggregates to ensure optimal assay performance.

Medicinal plants are distinguished by their capacity to synthesize a broad array of bioactive chemical substances or their precursors, which can carry out specific physiological functions (Sofowora *et al.* 2013). Cellular cytotoxicity refers to disturbances in basic cellular function that leads to cell damage observable at a microscopic level (Cureño *et al.*, 2017). As per National Cancer Institute (NCI, USA) plant screening programme, crude plant extracts are generally deemed cytotoxic to normal cells *in vitro*, of the LC<sub>50</sub> value after 48-72 hours incubation is < 0.03 mg/ml (Fadeyi *et al.* 2013). The decline in cell viability resulting from an increase in concentration of plant extract indicates that overdosing, on most plant extracts can render them cytotoxic (Abd'quadri-Abojukoro *et al.*, 2022).

More than 40% of the medicinal plants examined were identified to be potentially toxic reported by Tamokou and Kuete (2014). The toxicity of plant compounds can be influenced by various factors such as the concentration used, duration of use, and route of exposure, among others (McGaw *et al.*, 2014). It's essential to recognized that *in vitro* cytotoxicity results serve as initial screening tool to identifying potentially safe or toxic plant material extracts or compounds and do not definitively determine general toxicity. Therefore, caution should be exercised when given even the least toxic plant extracts in animal models with close monitoring required of animal performance (Abd'quadri-Abojukoro *et al.*, 2022). To ensure the safety of traditional herbal extracts or remedies, it's crucial to evaluate their toxicity due to the presence of potentially harmful secondary metabolites. This assessment helps in understanding not only the adverse effects but also determining the lethal

dose (Bahuguna *et al.*, 2017). Evaluating the cytotoxicity of these extracts or remedies can be conducted using various cell types (Misra *et al.*, 2013; Ajaiyeoba *et al.*, 2006).

The hexane and aqueous seed extracts of *Coriandrum sativum* showed values of more than 20 µg/ml indicating that the samples were non cytotoxic to vero cell line. According to the MTT assay, both hexane and aqueous extracts show inhibition of vero cells with CC<sub>50</sub> 600 µg/ml and 700µg/ml. The minimum inhibition of HepG2 (hepatocarcinoma) cells was observed at a concentration of 350 µg/ml for the three extracts (Fayyad *et al.*, 2017).

Cytotoxic activity was not observed even with a concentration of 1mg/ml of aqueous garlic extract SCC -15 reported by Szychowski *et al.* (2016). In their study, cell lines were treated separately with 50 mg and 100 mg aqueous extract from *Allium cepa* and *Allium sativum* and the cell viability of treated cell was compared to that of the non-treated control cell lines. The data indicated that onion exhibited a superior tumor cell inhibition property compared to garlic. Furthermore, it was noted that the South Indian garlic at concentrations of 100 mg and 50 mg, followed by Nasik onion at concentrations of 100 mg and 50 mg, demonstrated notably effective tumor cell inhibition properties compared to other varieties of garlic and onion (Shrivastava and Ganesh, 2010).

The aqueous extract of *Carica papaya* seeds was preliminary examined for cytotoxicity testing. Across all incubation periods, neither of the two concentrations used (1 and 2 mg/ml) showed any signs of toxicity. Notably, at 4 hours of incubation, the 1 mg/ml extract actually boosted cell viability by approximately 43% compared to the control. These findings suggest that aqueous extracts from *C. papaya* seeds is not toxic and, overall demonstrate greater efficacy in mitigating H<sub>2</sub>O<sub>2</sub>oxidative damage compared to vitamin C (Panzarini *et al.*, 2014).

The methanolic (80%) ginger extract showed a significant cytotoxic effect and demonstrated potent inhibitory activity against the hepatocellular carcinoma cell line (HePG2) and breast carcinoma cell lines (MCF7). The IC<sub>50</sub> of this extract against both (HePG2) and (MCF7) cell lines decreased reactive oxygen species levels (El-Sayeh *et al.*, 2018).

The aqueous extracts of *Azadirachta indica* leaves were found to be non-cytotoxic, exhibiting over 80% cell viability at the highest tested concentration of 1200 µg/ml. This evaluation was conducted on WI38 cells, which are human noncancerous fibroblast cell lines

(Tchetan *et al.*, 2022). The cytotoxicity of the *Azadirachta indica* leaf extracts was further assessed by determining LC<sub>50</sub> values. The results indicated that the acetone extract demonstrated higher cytotoxicity compared to the chloroform extract with the LC<sub>50</sub> values of 6.00 µg/ml and 11.92 µg/ml respectively. The extracts *S. chirayita*, did not induce evident toxic effects in mice as evidenced by the absence of significant differences in BW and body temperature between the treated and control groups (Alam *et al.*, 2011; Das *et al.*, 2012).

Conventional and supercritical carbon dioxide extracts of *P. persica* leaves exhibited reduced cytotoxicity on healthy cells compared to parthenolide (used as positive control), suggesting the therapeutic potential of *P. persica* extracts. This cytotoxic activity was attributed to higher total phenolic and flavonoids contents found in ethyl acetate and butanol fractions. Conversely, the weakly active hexane and aqueous fractions had lower total phenol content, further supporting these findings (Koyu *et al.*, 2019).

## **2.17 *In vivo* anthelmintic activity of aqueous extract of selected medicinal plants**

### **2.17.1 *Coriandrum sativum***

In an *in vivo* study, 24 sheep were artificially infected with *Haemonchus contortus* and randomly divided into four groups, each consisting of six animals. The first two groups received treatment with crude aqueous extract of *Coriandrum sativum* at dose levels of 0.45 and 0.9 g/kg respectively. The third group was treated with albendazole at 3.8 mg/kg body weight, while the fourth group remains untreated. The efficacy of treatment was evaluated using faecal egg count reduction and total worm count reduction. On the second day after treatment, significant FECR was observed in the groups treated with higher dose of *Coriandrum sativum* ( $p < 0.05$ ). Significant total worm count reduction ( $p < 0.05$ ) was only detected in the group treated with the higher dose of *Coriandrum sativum* compared to the untreated group. It's worth nothing that reduction in male worms was greater than that of female worms (Egualé *et al.*, 2007).

The administration of aqueous extract of seed of Coriander (at a dosage of 0.9g/ kg body weight) has been found to enhance the reduction in fecal egg count among sheep infested with Haemonchosis, thereby reducing the intensity of the infection. However, the observed efficacy of the coriander extract falls short of reaching the therapeutically required level. This limitation could potentially be addressed by either repeating the dosage or

combining the extract with anthelmintic drugs. Doing so may lead to reduction in the frequency of anthelmintic treatment (Ahmed *et al.*, 2019).

### **2.17.2 *Swertia chirata***

An *in vivo* study was conducted to investigate the anthelmintic activity of *Swertia chirata*. The study administered different forms of the plant-crude powder, crude aqueous extract, and crude methanolic extract-at a dose of 3 g/kg to sheep naturally infected with a variety of gastrointestinal nematodes. The results indicated a significant reduction in egg count per gram of feces across all treatments. However, crude aqueous extract was found to be the least effective, resulting in only a 34% reduction in egg count per gram of feces by day 14 post-treatment (Iqbal *et al.*, 2006).

Study was conducted to evaluate the *in vivo* anthelmintic efficacy of an aqueous extract of *Swertia chirata* seeds against common pathogenic gastrointestinal nematodes consisting of *Haemonchus*, *Bunostomum* and *Oesophagostomum* spp. in naturally infected goats. The extract was administered orally at a dose of 550 mg/kg body weight for 10 consecutive days to adults goats naturally infected from gastrointestinal nematodes *S. chirata* anthelmintic activity was compared from albendazole administered orally at a dose of 5 mg/kg body weight. The anthelmintic efficacy of the aqueous extract was evaluated by counting the mean eggs per grams on day 0, 3, 6, 9 and 12 posts-treatments. Faecal sample examination revealed a significant and progressive decrease in the number of eggs in faeces from second day post - treatment, counting until day 12. On day 12 of the experiment anthelmintic efficacy depend on fecal scores in goats treated with *S. chirata* and albendazole was found 62.5% and 100% respectively. These finding indicate that *Swertia chirata* exhibited a significant *in vivo* anthelmintic efficacy in goats against gastrointestinal nematodes (Arora *et al.*, 2009).

Goats were administered with crude powdered and cold aqueous extracts of *S. chirata* a dosage of 500 mg/kg, body wt, orally for seven consecutive days. The egg per gram count was measured on 0 day (pretreatment), 7 days and 15 day (post treatment). The results indicated significant anthelmintic efficacy of *Swertia chirata* against various gastrointestinal nematodes including *Trichostrongyle* spp., *Bunostomum* spp., *Oesophagostomum* spp. and *Haemonchus* spp. to the extent of 70 % to 90 %. Moreover the study further revealed that cold

aqueous extract of indigenous herb exhibited superior anthelmintic efficacy in compared to the respective crude powdered extract. Among various treatments tested for anthelmintic efficacy of *Swertia chirata* showed demonstrated the highest effectiveness against *Trichostrongyle* spp., which achieving an efficacy of 86.88 % (Jain and Sahni, 2009).

### **2.18 Haemato-biochemical study**

*C. sativum* was determined to be safe, with no adverse effects detected on hematological profiles, histology, relative organ weights, or plasma markers of damage to vital organs (Patel *et al.*, 2012). However, there was a notable decrease in body weight attributed to reduce food intake, indicating potential disruptions in carbohydrate, protein, and fat metabolism (Ecobichon and Klaassen, 2001).

The study was conducted by Jain and Sahni, (2010), treating goats infected with parasitic infestations using crude powdered and aqueous extracts of specific herbs. These extracts were administered orally at dosages of 500 mg/kg, body weight for seven consecutive days. Remarkably, the treatment lead to a notable increase in the levels of blood glucose, total protein and albumin in treated goats. This suggests that the herbal extracts might have therapeutic potential in managing parasitic infestations in goats possibly by enhancing these physiological parameters.

A single orally dose 5 g/animal of aqueous extract of *Allium sativum* bulb was administered in naturally infected ovine gastrointestinal nematodiosis. The toxicity study conducted revealed no adverse effects on any of the haematological and biochemical parameters examined indicating the safety of plant for use in sheep. Although significant changes were noted in SGPT, RBCs, Hb, RDWc, PLT, PDWC, lymphocyte % and granulocyte % values, other parameters exhibit non-significant alterations throughout the trial (Kanojiya *et al.*, 2015).

The study on haemato-biochemical gastrointestinal parasitism in goats unveiled noteworthy findings. Among infected animals, there was a considerable decrease observed in the mean levels of Hb, PCV, TEC, and TSP. Conversely, there was a significant increase in TLC, neutrophil, lymphocyte, eosinophil, and monocyte count in infected goats (Ahmed *et al.*, 2015).

Haematological and biochemical analysis were conducted on 20 goats affected by helminth parasites in Bikaner region of Rajasthan. The findings suggested alterations in various blood parameters, indicating the impact of the parasitic infection. Among hematological parameters, there was a substantial decrease observed in total erythrocyte count, packed cell volume, hemoglobin and lymphocyte count, while there was a marked increase in total leukocyte count, eosinophils and monocytes. Although there was a non-significant increase in neutrophils, basophils exhibited a non-significant decrease. Notably, there was a significant rise in total eosinophil counts, further emphasizing the eosinophilia commonly associated with helminth infestation. Biochemical analyses reflected pronounced decreases in total serum proteins, albumin levels, and the albumin to globulin ratio, indicating impaired protein metabolism likely attributed to the parasitic burden. Conversely there was a considerable increase in a globulin levels suggestive of an inflammatory response or immune reactions against the parasites (Ashfaque *et al.*, 2016).

A combined herbal anthelmintic dose was formulated a ratio of 1 part neem, 2 part bitter and 2 clove to achieve superior result in compared to a single dose. *In vivo* evaluation, of the efficacy of these extracts was studied at a dose rate of 1ml/kg body weight with a concentration of 100 mg/ml on day 0 and day 7 using naturally gastrointestinal parasites infected sheep. Analysis of hematological and biochemical parameters revealed a reduction in the percentages of eosinophils and basophils, indicative of a decrease in endoparasites and improvement in anemic conditions. Furthermore, significant variations were observed in AST, ALT, and creatinine values in the treated groups on days 7, 14, 21 and 28, indicating no toxic effects. Importantly, no harmful effects on the animals' bodies were observed throughout the study period (Biswas *et al.*, 2017).

Significant reductions in the value of mean haemoglobin, packed cell volume, total erythrocyte counts and lymphocytes, total serum proteins, albumin levels were recorded in gastrointestinal nematodiasis goats. Conversely, total leukocyte counts, eosinophils, monocytes, globulin, and alanine aminotransferase values showed significant elevation in goats infected with gastrointestinal nematodiasis. Furthermore, it was suggested that decreases in hemoglobin, total erythrocyte counts, total serum proteins, and albumin were

crucial indicators of gastrointestinal nematodiasis infestations in goats (Shashank *et al.*, 2019).

In case of gastrointestinal parasitic infection such as oesophagostomosis or mixed parasitic infestations, notable decreases have been noted in total erythrocyte count, hemoglobin levels, and packed cell volume (Rajpoot *et al.*, 2022).

Both aqueous and alcoholic extracts of seeds of *Carica papaya* were orally administered daily at a dose of 100 mg/kg body weight for 30 days to goats naturally infected with gastrointestinal parasites. Throughout the study, on the 0th, 10th, 20th and 30th days, evaluations of fecal egg count, hematological parameters, and biochemical levels were conducted. Treated goats exhibited a significant increase in hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), and lymphocytes, while a significant reduction was observed in total leukocyte count (TLC), neutrophil, and eosinophil levels on the 20th and 30th days of the trial (Singh *et al.*, 2023).

In gastrointestinal parasitic infections in goats, hematological evaluations reveal a decrease in red blood cell count, hemoglobin levels and packed cell volume, indicating the presence of anemia. Biochemical analyses show a reduction in total protein and albumin levels, suggesting a potential link to malnutrition or malabsorption due to parasitic infections (Sarkar *et al.*, 2024).

Infestations of helminths in goats have been shown to lead to a notable decrease in total protein and albumin levels, accompanied by a slight reduction in globulin levels (Faran *et al.*, 2024).

## **2.19 Antioxidants**

Antioxidant was playing a crucial role in our bodies by scavenging and preventing the formation of reactive oxygen species (ROS). When the production of ROS exceeds the body's antioxidant capacity, it leads to oxidative stress. This imbalance between free radicals and antioxidants is where oxidative stress occurs. Free radicals are produced during metabolic processes, and antioxidants neutralize them (Jain and Shakkarpude, 2024). Antioxidants are generally defined as substances that can delay, prevent or mitigate damage caused by oxidative stress (Halliwell, 2007). The cellular antioxidant defense mechanism operates through three distinct each fulfilling specific functions consisting quenching oxidants, repairing or removing

oxidative damage and encapsulating non repairable damage (Lykkesfeldt and Svendsen, 2007). Antioxidants are molecules capable of donating electrons to oxidants, thereby dampening their reactivity and rendering them harmless to macromolecules. The entire antioxidant system operates under homeostatic control. However, when metabolic processes become overloaded, the buildup of free radicals can disrupt redox homeostasis, leading to oxidative stress (Celi, 2011), which in turn can contribute to various diseases and diminish animal performance. Antioxidants intervene in radical chain reactions, thereby averting the damage caused by oxidative stress (Da Pozzo *et al.*, 2018).

Antioxidants can be categorized into enzymatic and non-enzymatic types. However, from a nutritional standpoint, a more informative classification distinguishes between endogenous and exogenous antioxidants. Additionally, antioxidants can be further classified based on their solubility into two categories: water-soluble and fat-soluble (Lazzarino *et al.*, 2019). Endogenous antioxidants are categorized into 3 major groups (Miller *et al.*, 1993).

The first group encompasses enzymatic antioxidants, among which superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) stand out as key components of intracellular antioxidant defense. Specifically, glutathione peroxidase plays a pivotal role in cellular oxidation-reduction reactions, safeguarding the cell membrane against oxidative harm induced by free radicals (Liu *et al.*, 2017). SOD plays a pivotal role in catalyzing the dismutation of superoxide into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) representing the initial line of defense against pro-oxidants (Halliwell and Chirico, 1993).

The second group comprises non-enzymatic protein antioxidants primarily found in plasma. They are predominantly represented by sulfhydryl (SH) groups found in albumin and are considered a significant component of the extracellular antioxidant defense system against oxidative stress. The reducing properties of SH residues are known to be oxidized under conditions of oxidative stress and other physiological circumstances. The total thiol groups in plasma include SH groups from albumin, L-cysteine, and homocysteine. Under physiological conditions, SH groups are the most chemically reactive sites and exhibit strong reducing properties (Tan *et al.*, 2018; Deters and Hensen, 2020).

The third category consists of non-enzymatic, low molecular-weight antioxidants, primarily located in plasma but also present in other extracellular and intracellular fluids.

Serum's principal antioxidant prowess stems from non-enzymatic antioxidants like glutathione,  $\alpha$ -tocopherol,  $\beta$ -carotene, and uric acid. Glutathione (GSH) holds particular significance in shielding cells from oxidative stress and harmful substances. It serves as a substrate or co-substrate in enzymatic reactions and directly interacts with free radicals and lipid peroxides, bolstering cellular defense mechanisms (Briviba and Sies, 1994).

The FRAP (Ferric Reducing Ability of Plasma) assay is a method used to assess the antioxidant levels within an animal's body. It provides insight into the overall plasma antioxidant capacity, incorporating various compounds such as uric acid, ascorbic acid, vitamin E, bilirubin, and albumin. The primary mechanism involves the reduction of ferric ions to ferrous ions. The concentration of FRAP in the aqueous phase of plasma serves as a measure of antioxidant capacity, reflecting the organism's ability to counteract oxidative stress (Wood *et al.*, 2003; Yeum *et al.*, 2004).

#### **2.19.1 Antioxidant activity of Coriander (*Coriandrum sativum* L.) extract**

Naturally occurring antioxidants are consistently favored over synthetic counterparts, with safety being a paramount concern. Therefore, significant efforts are underway to explore and discover natural sources of antioxidants (Reische *et al.*, 2002). Coriander boasts potent natural antioxidant properties, effectively thwarting unwanted oxidation processes. Notably, coriander leaves exhibit more robust antioxidant activity compared to the seeds (Wangenstein *et al.*, 2004). The effects are notably stronger in extracts derived from coriander leaves compared to those from its seeds. Additionally, it appears that compounds with moderate polarity exhibit the highest potency, despite their relatively minor contribution to the plant's overall antioxidant content (Dorman *et al.*, 2008).

The aqueous extracts of coriander seeds exhibited antioxidant activity *in vitro* and *in vivo* (Satyanarayana *et al.*, 2004). Naveen and Farhath (2010) further observed that coriander seed extract reduced the drug induced oxidative stress and shield the body from potential toxicity. This antioxidant activity of coriander seed was attributed to the abundant presence of tocopherols, carotenoids and phospholipids which functions through various mechanisms (Ramadan and Morsel, 2004). The coriander seeds demonstrated scavenging activity against superoxide and hydroxyl radicals in a concentration-dependent manner. The highest levels of

free radical-scavenging action and free radical reducing power were observed in coriander seed extracts at a concentration of 50 µg gallic acid equivalent (Chahal *et al.*, 2017).

Numerous studies have unequivocally shown that coriander, harbors robust antioxidant properties. This efficacy predominantly stems from the presence and interplay of polyphenols, vitamins and sterols within the herb (Ananthan *et al.*, 2004; Patel *et al.*, 2013; Kousar *et al.*, 2011). Insufficient levels of natural antioxidant components in the body, such as the superoxide dismutase (SOD) enzyme and glutathione peroxidase (GSH-px), can lead to an oxidative chain reaction (Ananthan *et al.*, 2004), which may result in tissue damage (Ramadan and Moersel, 2006). However, it has been observed that coriander possesses a strong antioxidant potential, possibly attributed to the synergistic effect of various antioxidant compounds, including tocopherols and sterols (Ramadan *et al.*, 2008).

The principal activities associated with polyphenols include their antioxidant and antiradical properties. The substantial concentration of polyphenols found in *C. sativum* extracts renders it effective as a reducing agent, inhibitor of lipid peroxidation, scavenger of free radicals, and quencher of singlet oxygen (Thiviya *et al.*, 2021). Beyond solvent selection and extraction techniques, various other factors play a role in determining the antioxidant potency of an extract. Among these, the drying method applied to the plant material holds significance. Varied drying conditions, including duration and temperature, can impact the stability of secondary metabolites, consequently influencing the biological efficacy of the extracts (Scandar *et al.*, 2023).

The aqueous extract of coriander seeds, rich in flavonoids, demonstrated a remarkable ability to mitigate oxidative damage in animals afflicted with lead-induced toxicity in the liver and kidneys. Oral administration of these extracts resulted in heightened levels of crucial antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) while concurrently reducing lipid peroxidation (Samojlik *et al.*, 2010). The methanol extract of coriander fruits has demonstrated a noteworthy capacity for scavenging, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. This suggests that coriander fruits hold promise as a natural source of antioxidant compounds suitable for application in the food industry (Msaada *et al.*, 2017).

### 2.19.2 Antioxidant activity of *Swertia chirata*

Laboratory investigations grounded in evidence demonstrate that aqueous, alcoholic, and methanolic extracts of *S. chirayita* exhibit numerous promising pharmacological properties (Kumar and Van Staden, 2016). Mangiferin, an additional bioactive compound found in *chirayita* species, is renowned for its antioxidant properties and diverse therapeutic potential (Du *et al.*, 2018). Researchers have conducted investigations into the antioxidant properties of extracts from various species of *Swertia*. For instance, a comparative study was undertaken to evaluate the antioxidant activity of aqueous and ethanolic extracts from *S. chirayita*, *S. nervosa*, and *Andrographis paniculata*. The results indicated that *S. chirayita* exhibited the highest antioxidant activity, followed by *S. nervosa* and *Andrographis paniculata* (Phoboo *et al.*, 2010). Additionally, they noted that the antioxidant activity of both aqueous and ethanolic extracts was comparable in *S. chirayita*, whereas it was higher in ethanolic extracts than in aqueous extracts for the remaining two species.

Phoboo *et al.* (2012) conducted a comparison of antioxidant activity in aqueous and ethanolic extracts from various parts of *S. chirayita*. They reported relatively higher activity in a mixture of leaves and inflorescence, followed by stems, and the lowest in roots. In another study, Roy *et al.* (2015) investigated the antioxidant activity of *S. chirayita* and *S. cordata* using aqueous and methanolic solvents, finding superior antioxidant activity in methanolic extracts. Additionally, they highlighted the superiority of *S. chirayita* over *S. cordata* in terms of antioxidant activity.

The ethanolic extract of *chirata* demonstrates both *in vitro* and *in vivo* antioxidant effects, suggesting *Swertia chirata* potential efficacy in treating liver diseases (Chen *et al.*, 2011). An activity-guided isolation and purification process was employed to identify components of *Swertia chirata* responsible for scavenging the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. Dry whole plants of *Swertia chirata* were extracted using various solvents and evaluated for their DPPH radical scavenging activity. The acetone: water (8:2) exhibited the highest total phenolic content and DPPH radical scavenging activity. This extract was then subjected to column chromatography, leading to the isolation of decussating, swertianin, bellidifolin, isobellidifolin, amarogentin, swertianolin and mangiferin as active components (Singh *et al.*, 2012).

The methanolic extract of *S. chirata* demonstrates antioxidant activity owing to the presence of flavonoids and secoiridoids. These compounds act as substrates and scavengers for free radicals, neutralizing their toxic effects (Ekka *et al.*, 2008). Additionally, the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* exhibit remarkable antioxidant properties. Consequently, these extracts can serve as valuable sources of antioxidants for therapeutic purposes in the management of various illnesses (Kumar *et al.*, 2016).

### 3.1 Ethical Approval

This study design was approved by Institutional Animal Ethics Committee (IAEC), Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110 (U.P.), India by a vide letter no. of IAEC/SVPUAT/2022/125 dated on 03/12/2022.

### 3.2. Prevalence study of gastrointestinal parasites in goats

#### 3.2.1 Study area

The faecal samples were collected from 4 districts in Western Uttar Pradesh of India, namely Meerut, Baghpat, Saharanpur and Muzaffarnagar, during the period from March 2023 to February 2024 (Table 3.2.1 & Figure 3.2.1).

**Table 3.2.1: Total number of samples collected**

S.No.	Animal	Districts	No. of collected samples
1.	Goat	Meerut	312
2.	Goat	Baghpat	300
3.	Goat	Saharanpur	248
4.	Goat	Muzaffarnagar	257
Total number of faecal samples			1117



**Figure 3.2.1: Map showing sample collection districts**

### **3.2.2 Collection of faecal sample**

A total of 1117 faecal samples were randomly collected in goat from different parts of the targeted 4 districts such as Meerut, Baghpat, Saharanpur and Muzaffarnagar, which are situated in the Western region of Uttar Pradesh, India. Fresh faecal samples were collected directly from the rectum of goat using lubricated disposable gloves. Separate gloves were used to prevent potential cross-contamination between faecal samples. The gross examination of faecal samples was done for colour, consistency, odour and for presence of larvae or adult worms. At the time of sample collection breed, age, sex and month were also recorded for each animal. From each goat 10 to 20 gram of faeces was collected. Immediately after collection faecal samples were put in clean labeled plastic bags and kept in icebox and carried to the laboratory of Department of Veterinary Parasitology, SVPUAT Meerut to examine the parasitic eggs/ova/larvae/ cysts and trophozoites. Samples were processed and analyzed on the same day. However, in the cases where faecal samples could not be analyzed on the same day, they were kept at 2 to 4°C in a refrigerator until further analysis the next day. The faecal samples were examined using 10× and 40× objectives lenses of compound microscope (Nikon Eclipse E200, Tokyo, Japan) by direct faecal wet smears, flotation, sedimentation and modified Ziehl-Neelsen (MZN) staining technique to detect the eggs/ova/larvae/ cysts and trophozoites. The diagnostic stages of the parasites were identified from their unique morphological characters (Soulsby, 1982). The age of goat was estimated by dental examination. Age of goats were classified as young (<1 year), 1-2 years and more than 2 years. The year is divided into 3 seasons such as winter, summer and rainy.

### **3.2.3 Faecal examinations**

#### **3.2.3.1 Direct wet smear method**

A small quantity of faeces is placed on a slide, mix it gently with a few drops of tap water and spread it evenly to get a translucent film on the slide. Then kept a coverslip over it and examined under the microscope.

#### **3.2.3.2 Faecal flotation method**

Floation method was performed for determining the eggs of nematode, cestode as well as coccidian oocysts. For this method, 5 gram of faecal sample was thoroughly mixed in 50 ml of saturated solution of common salt in mortar and pestle after that, strained through a

sieve to remove the coarse faecal material. Then the mixture was placed in 15 ml tube in order to form convex surface at the top of the tube and gently placed a coverslip over on it and allowed to stand for 30 minutes and then gently take over the coverslip and kept on slide in order to touch the lower surface of coverslip to slide (Soulsby, 1982).

#### **3.2.3.3 Sedimentation method**

Sedimentation method was performed for determining the presence and absence of eggs of trematode. For this method, 5 gram of faecal material was mixed in 200 ml of tap water in mortal and pestle and after homogenization and filtrations the mixture poured into a conical bottom of urinary flask. Then allow stand for 30 min. after that supernatant fluid was discarded in single swift action and again refilled the urinary flask with water likewise performed 2 to 3 times until the colour of sediment become colorless. This step was repeated three to five times until the supernatant fluid was clear and lastly few drops of sediment taken on slide and a coverslip was put on it and examination was performed under a microscope (Soulsby, 1982).

#### **3.2.3.4 Modified Ziehl-Neelsen (MZN)**

Direct faecal smears were prepared on clean grease-free glass slides for detection of *Cryptosporidium* spp. oocysts and stained by the modified Ziehl-Neelsen (MZN) staining technique (Henriksen and Pohlenz, 1981) for confirmation. In short, smears were dried in air, then fixed with absolute methanol for the duration of 5 minute after that smears were transiently passed over a flame and kept on staining rack. Strong carbol fuchsin poured on smears and allowed to stay for 40 min. The slide were then washed under running tap water for 2-5 min, decolorized with 10 % H<sub>2</sub>SO<sub>4</sub> for fraction of second and then washed again under running tap water. Counter staining were performed with 5% malachite green for 2-5 minute and then washed under running tap water for 5 minute. After drying, the smears were screened under 40× and 100× objectives of microscope for the presence of *Cryptosporidium* oocysts.

#### **3.2.3.5 Direct faecal wet smears method**

*Giardia* spp. was identified by direct faecal wet smears method (Alharbi *et al.*, 2020). In briefly, approximately 2 mg for individual faecal samples were mixed with few drop of

normal saline solution and then put one drop of lugol's iodine after that cover slip was kept gently on slide and then examined under light microscopy.

### **3.3 Molecular characterization of strongyle nematode infection in goats**

#### **3.3.1 Collection of faecal sample**

Total 1117 faecal samples were collected in goat from various regions of district Meerut, Baghpat, Saharanpur and Muzaffarnagar. Randomly fresh faecal samples were collected from the rectum of the individual goats irrespective of age, sex breed and seasons. The macroscopic examination of faecal samples was performed for colour, consistency and odour and for presence of immature and adult worms. Approximately fifty gram of faeces was collected from every goat. After collection of faecal samples were kept in plastic bags and put in icebox and brought to the laboratory to examine the parasitic eggs and larvae. Analyses of samples were performed on the same day. The faecal samples were screened under 10× and 40× objectives of compound microscope (Nikon Eclipse E200, Tokyo, Japan) by direct faecal wet smears and flotation methods. The strongyle types of eggs were identified from their distinctive morphological features (Soulsby, 1982).

#### **3.3.2 Faecal examination**

##### **3.3.2.1 Direct wet smear method**

A small amount of faecal material was put on greases free slide, mix it properly with a few drops of water and spread it uniformly to obtain a translucent film on the slide. After that, a coverslip kept over it and examined under the microscope.

##### **3.3.2.2 Faecal flotation method**

Floatation method was performed according to Soulsby (1982), for identification of nematode eggs. In this method, 5 gram of faecal sample was properly mixed in 50 ml of saturated solution of table salt in mortar and pestle. After that material was strained via a sieve to separate debris from faecal material. Then the filtrate was poured in 15ml plastic tube in order to form convex meniscus at the top of the tube and then properly placed a coverslip over on it and stand for 30 minutes and subsequently lifted the coverslip and put on slide.

##### **3.3.2.3 Coproculture:**

The positive faecal samples for strongyle type of eggs were separately pooled and cultured according to Roberts and Sullivan (1950). Approximately 40 grams of faecal materials

were broken up finely, employing a mortar pestle and together with glass rod. If the faecal material remained too harder then, a little amount of water was sprinkled to adjust desirably consistency and if the faeces were very soft in consistency in this situation charcoal was added to obtain the needed consistency. Faeces of required consistency was put in a small sized petri dish and spread uniformly and this dish was put down in another bigger size petri dish having a little amount of water in it. The bigger sized petri dish was then covered with another petri dish to reduce the evaporative losses and incubated at 27°C in B.O.D. incubator (Biogen Scientific) for a period of 7 days. The water in bigger petri dish contained larvae which migrated from the faecal mass from the small petri dish subsequently hatching out from the eggs. Several larvae reached the infective third stage by that time facilitating specific diagnosis. The infective larvae migrating to water in outer petri dish were pipetted out and centrifuged at 1500 rpm for 3 minutes. Sediment contained larvae used for DNA extraction.

### **3.4 Molecular characterization**

#### **3.4.1 DNA template preparation**

Five larvae from the sediment were selected for extraction of DNA. The larvae were exsheathed by incubating in 3.5% sodium hypochlorite (aqueous solution, 4% active chlorine) for 20 minutes. The exsheathed larvae were washed in distilled water for 3 to 5 times and then used for DNA extraction.

#### **3.4.2 DNA extraction**

The genomic of DNA was extracted from the infective larvae by using a kit (QIAamp<sup>®</sup> Blood Mini Kit- QIAGEN) performed according to manufacturer's instruction with slight modification. The DNA was stored at -20°C until used as a template for genus and species identification of strongyle worm.

##### **3.4.2.1 Procedure**

1. First, pipette 20µl QIAGEN protease into a 1.5 ml microcentrifuge tube. Then, add 200µl of the sample. If the sample volume is less than 200µl, compensate by adding an approximate volume of PBS.
2. Add 200µl of buffer AL. Ensure thorough mix by vortexing.
3. Incubate at 56 °C for 10 minute. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove the droplets adhering to the inner surface of the lids.

4. Add 200µl of ethanol (96-100%). Thoroughly mix by vortexing. Briefly centrifuge the tube to ensure any drops adhering to the lid are removed.
5. Pipet the mixture on to the QIAamp mini spin column placed in a 2ml collection tube. Centrifuge at  $6000 \times g$  (8000rpm) for 1 minute. Discard the flow-through along with collection tube.
6. Place the QIAamp mini spin column in a fresh 2ml collection tube and add 500µl buffer AW1. Centrifuge at  $6000 \times g$  (8000rpm) for 1 minute. Discard the flow-through along with the used collection tube.
7. Place the QIAamp mini spin column in a new 2ml collection tube and add 500µl buffer AW2. Centrifuge setup at maximum speed ( $20,000 \times g$  14000rpm) for 3 minute. Discard both the flow- through and collection tube.
8. Place the QIAamp mini spin column in a new 2ml collection tube. Centrifuge at maximum speed for 1 minute. This step effectively eliminates the chance of possible buffer AW2 carryover.
9. Place the QIAamp mini spin column into a new 1.5 ml microcentrifuge tube. Add 200µl of buffer AE or distilled water, then incubate at room temperature (15-25°C) for 1 minute. Centrifuge at  $6000 \times g$  (8000 rpm) for 1 minute for elute the DNA.

### **3.4.3 PCR (Polymerase chain reaction) amplification**

The PCR amplifications was performed in thermo cycler (Bio-Rad), reaction mixture of 25 µl composed of 12.5 µl Master Mix (Promega), 1 µl of 10 pmol of each primer (Xploregen Discoveries Pvt. Ltd. Bengaluru, Karnataka, India) 2 µl of DNA template and 8.5 µl of nuclease free water.

### **3.4.4 Gel electrophoresis**

For PCR product visualization, 5 µl of PCR product and 10 µl 100 pb plus ladder (BR Biochem) was loaded into 1.5% agarose gels using TAE buffer (Bio-Rad) and ethidium bromide and then run at 90 V for 60 min in horizontal gel electrophoresis (HiMedia), resolution pattern was examined and image was captured in Gel Doc (Bio-Rad, Molecular Imager<sup>®</sup> Gel Doc<sup>™</sup> XR+ Imaging System).

### **3.5 *Haemonchus contortus***

Primer amplified the second internal transcribed spacer region-2 (ITS-2) of the ribosomal DNA were utilized. PCR was conducted by using species specific forward and universal reverse primer to strongylide species Bott *et al.* (2009).

HAE forward primer: 5' CAAATGGCATTGTCTTTTAG3' and HAE reverse primer: 5' TTAGTTTCTTTTCCTCCGCT 3'

#### **3.5.1 The conditions of reaction**

Initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30s (denaturation), 55°C for 30s (annealing) and 72°C for 30s (extension), followed by a final extension at 72°C for 7 minutes (Bott *et al.*, 2009; Tan *et al.*, 2014).

### **3.6 Genus *Trichostrongylus***

Genus *Trichostrongylus* were amplified targeted ITS-2 gene according to Waghorn *et al.* (2013). Generic forward: 5' CACGAATTGCAGACGCTTAG 3' and Generic reverse 5' GCTAAATGATATGCTTAAGTTCAGC 3' used for amplification of ITS-2 gene of genus *Trichostrongylus*.

#### **3.6.1 Touchdown PCR conditions**

Eight minutes at 95°C, 12 cycles at 94°C for 15 seconds, 60°C (decreasing by 0.5°C per cycle) for 15 seconds, 30 seconds at 72°C, 25 cycles at 94°C for 15 seconds, 15 seconds at 54°C, 30 seconds at 72°C, subsequently final extension at for 7 minutes at 72°C.

### **3.7 Genus *Oesophagostomum***

Genus *Oesophagostomum* were amplified targeted internal transcribed spacer-2 (ITS2) with upstream flanking region about 117 bp of ribosomal DNA (rDNA) and the cycling conditions developed by Kumar *et al.* (2018). Oeso forward primer: 5' TCG ACT AGC TTC AGC GAT G 3' and Oeso reverse primer: 5' CCA AAG CAT TCT TAG TCG CT 3'.

The cyclic conditions were, initial denaturation at 94°C for 2 minutes was followed by 36 cycles each for 30s at 94°C, for 30s at 53°C and for 30s at 72°C and final extension was done followed at 72°C for 5 min.

### **3.8 Collection of plant materials**

The selection of plants for this study was based on the literature survey concerning their traditional uses of the plants across the various regions of the world. In present study

seven medicinal plants were selected namely seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica* (Table 3.8.2 & Figure 3.8.2). Adequate quantities of these plant materials were meticulously collected and transported to the laboratory for the further process. Plants materials were collected from January 2023 to June 2023. These medicinal plants are widespread distribution and are commonly recognized in households for their diverse array of uses. The procurement of plant materials was conducted after a thorough assessment of their quality.

**Table 3.8.2: Selected medicinal plants**

S. No.	Name of the plants	Common name	Parts used
1.	<i>Coriandrum sativum</i>	Coriander	Seeds
2.	<i>Allium sativum</i>	Garlic	Bulbs
3.	<i>Carica papaya</i>	Papaya	Seeds
4.	<i>Zingiber officinale</i>	Ginger	Rhizomes
5.	<i>Azadirachta indica</i>	Neem	Leaves
6.	<i>Swertia chirata</i>	Chirayta	Whole plants
7.	<i>Prunus persica</i>	Peach	Leaves

### 3.8.1 Collection of seeds of *Coriandrum sativum*

Seeds of *Coriandrum sativum* were procured from the local market of Meerut. These seeds were cleaned properly and thoroughly washed under running tap water. Subsequently, seeds were air-dried at room temperature until completely devoid of moisture.

### 3.8.2 Collection of bulbs of *Allium sativum*

The bulbs of *Allium sativum* were purchased from the local market of Meerut. The bulbs were manually peeled, ensuring meticulous removal of the outer layers. After peeling, the bulbs were cleaned properly to eliminate any adulterants. Following this, bulbs were finely sliced into smaller pieces to facilitate the drying process. The prepared bulbs of *Allium sativum* pieces were then subjected to air drying under shade conditions in well-ventilated place for duration of 15 days.



**Figure 3.8.2: Plants or their parts used for the preparation of extracts**

### **3.8.3 Collection of seeds of *Carica papaya***

Ripe papaya (*Carica papaya*) fruits were procured from the local market of Meerut for the purpose of collecting the fresh seeds. The papaya fruits were carefully sliced into pieces. The seeds were meticulously removed by hands from ripe fruits. Subsequently, the collected seeds underwent a thorough cleansing process using clean water to eliminate any impurities or foreign substances. Once cleaned the seeds were subjected to shade drying for duration of 2 weeks.

### **3.8.4 Collection of rhizomes of *Zingiber officinale***

Rhizomes of *Zingiber officinale* were procured from the local market in Meerut. The rhizomes were washed thoroughly under clean running tap water to remove any impurities. Following this, the rhizomes were meticulously peeled and sliced into smaller pieces to facilitate the drying process. Subsequently, the prepared rhizomes pieces were air dried under shade area with ample ventilation place for duration of 15 days.

### **3.8.5 Collection of leaves of *Azadirachta indica***

Fresh mature and immature healthy leaves of *Azadirachta indica* were carefully collected from adult trees located within the SVPUAT campus in Meerut. Subsequently, the leaves were carefully detached from their leafstalk and subjected to a through washing process using clean tap water to remove the any traces of dirt. Following this cleansing procedure, the leaves were air- dried under shade for duration of one week.

### **3.8.6 Collection of whole plants of *Swertia chirata***

The whole plants of *Swertia chirata* was purchased from authorized herbal medicine dealer. The whole plant was cleaned properly to remove any other foreign materials. Subsequently; the plants were dried using a hot air oven at the temperature of 50°C to effectively remove the moisture.

### **3.8.7 Collection of leaves of *Prunus persica***

The leaves of *Prunus persica* were collected from the SVPUAT campus in Meerut. These leaves were cleaned, where coarse impurities were manually removed followed by thorough washing with clean tap water. Subsequently, cleaned leaves of *Prunus persica* were dried under shade for duration of one week.

### **3.9 Preparation of powder from plant materials**

The powdered of plant materials were carefully prepared from dried seeds of *Coriandrum sativum*, dried bulbs of *Allium sativum*, dried seeds of *Carica papaya*, dried rhizomes of *Zingiber officinale* dried leaves of *Azadirachta indica*, dried whole plant of *Swertia chirata* and dried leaves of *Prunus persica*. Initially the above mentioned dried plant materials were grinded and finely was grinded by using electric grinder machine. The resulting powdered material was further refinement through sieving, which eliminate any coarse particles. The sieved powder was then accurately weighted and stored in clean airtight labeled to their contents in a plastic containers. Theses plastic containers were securely kept in dry place until to the start of extraction process.

### **3.10 Aqueous extract preparation from plant materials**

#### **3.10.1 Aqueous extract preparation from seeds of *Coriandrum sativum***

The aqueous extracts of seeds of *Coriandrum sativum* were prepared by soaking a 100 gram of the dry powder in 1000 ml distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. Subsequently, the suspension was first filtered through muslin cloth and then through the Whatman filters paper No.1. The resulting filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with a water bath temperature 42.6°C. Following concentration, the extract was dried at 40°C in hot air oven to obtained dry extract. This solid residue obtained after extraction was carefully weighed and stored in airtight and water proof container. Finally containers of extracts were stored in a refrigerator at 4°C until further use.

#### **3.10.2 Aqueous extract preparation from bulb of *Allium sativum***

Initially, 100 grams of dry powder of bulbs of *Allium sativum* was mixed with 1000 milliliters of distilled water. This mixture was then heated in water bath at 100°C for 30 minutes. After cooling, the mixture was filtered through muslin cloth and subsequently through Whatman filter paper No.1. The resulting filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with a water bath temperature 51.8°C. After that the extract was dried at 40°C in hot air oven to obtained dry extract. This solid residue obtained after extraction was weighed and stored in airtight, water proof container and preserved in a refrigerator at 4°C until required for further use.

### **3.10.3 Aqueous extract preparation from seeds of *Carica papaya***

To prepare the aqueous seed extract, 100 grams of powdered seeds of *Carica papaya* was mixed in 1000 milliliters of distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. The resulting mixture was then filtered first through a piece of muslin cloth and then through Whatman filters paper No.1. The filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with water bath temperature 40°C. After that the extract was dried at 40°C in hot air oven to obtain dry extract. The solid residue obtained after extraction was carefully weighed and transferred into an airtight and water proof container. Then, extract containing container was stored in a refrigerator at 4°C until needed.

### **3.10.4 Aqueous extract preparation from rhizomes of *Zingiber officinale***

The aqueous extract of rhizome of *Zingiber officinale* was prepared by soaking 100 grams of the dry powder in 1000 milliliters distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. Afterward, the suspension was filtered first through muslin cloth and then through Whatman filters paper No.1. The resulting filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with a water bath temperature 50°C. Subsequently, the concentrated solution was dried at 40°C in hot air oven to obtain dry extract. This solid residue obtained from this process was weighed post extraction and securely stored in airtight, water-proof container. Finally, extract containing container was preserved in a refrigerator at 4°C until needed.

### **3.10.5 Aqueous extract preparation from leaves of *Azadirachta indica***

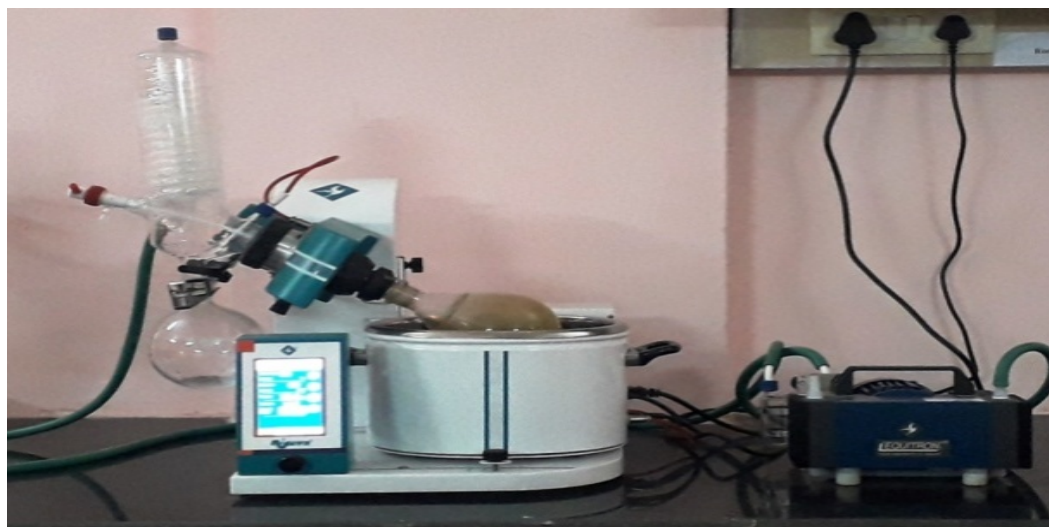
The aqueous extract of leaves of *Azadirachta indica* was prepared by soaking 100 grams of the dry powder in 1000 milliliters of distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. Subsequently, the aqueous suspension was filtered first through muslin cloth and then through Whatman filters paper No.1. The resulting filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with a water bath temperature 55°C. The concentrated paste obtained was further dried at 45°C using a hot air oven. Then, extract containing container was preserved in a refrigerator at 4°C until required for further use.

### 3.10.6 Aqueous extract preparation from whole plants of *Swertia chirata*

The aqueous extract of whole plant of *Swertia chirata* was prepared by initially; soaking 100 grams of the dry powder in 1000 ml distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. Subsequently, the suspension was filtered first through muslin cloth and then through Whatman filters paper No.1. The filtrate was then concentrated using a rotary evaporator (Equitron, Medica Private Limited Figure 3.10.1.3) with a water bath temperature 55°C. Following the concentration, the resulting solution was dried at 40°C by using hot air oven to obtain a solid residue. This solid residue was carefully weighed and transferred into an airtight, water proof container. Finally, the container was stored in a refrigerator at 4°C until further use.

### 3.10.7 Aqueous extract preparation from leaves of *Prunus persica*

The process involved preparing an aqueous extract from the leaves of *Prunus persica* by soaking 100 grams of the dry powder in 1000 ml distilled water. This mixture was then heated in a water bath at 100°C for duration of 30 minutes. The resulting suspension was filtered first through muslin cloth and then through Whatman filters paper No.1. The obtained filtrate was concentrated using a rotary evaporator (Equitron, Medica Private Limited, Figure 3.10.1.3) with a water bath temperature of 39.7°C. Then the extract was dried at 40 °C in hot air oven to obtained dry extract. This solid residue obtained after extraction was weighed carefully and stored in airtight, water proof container in a refrigerator at 4°C until needed.



**Figure 3.10.1.3: Rotary evaporator (Equitron, Medica Private Limited)**

### 3.11 Determination of extraction yield (% yield)

The percentage yield from all the dried extracts was calculated using formula given below:

$$\text{Yield (\%)} = (W_1 \times 100) / W_2$$

Where  $W_1$  represents the weight of the extract obtained after evaporation of solvent and  $W_2$  is the weight of the plant powder.

### 3.12 *In vitro* anthelmintic efficacy of selected medicinal plants

This evaluation was conducted utilizing the following test:

1. Egg hatch assay (EHA)
2. Larval paralysis test
3. Adult worm mortality assay (AWMA)

#### 3.12.1 Egg Hatch Assay (EHA)

This test was carried out the aqueous extracts of seven medicinal plants have been tested *in vitro* to explore their potential for inhibiting egg hatching. The Barbari breed of goats of Instructional Livestock Farm Complex-II, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110, was selected for the study. Prior to three to four months of trials periods the selected goat for the study did not received any anthelmintics treatment.

Faecal samples were directly collected from the rectum of the goat using sterilized gloves and kept in well labeled collecting bags. Subsequently, these faecal samples were examined under the microscope using 10× and 40× objectives lenses of compound microscope (Nikon Eclipse E200, Tokyo, Japan). Faecal samples were revealing the presence of strongyle eggs. To process the positive faecal samples, they were mixed with warm distilled water (37°C) to get the initial suspensions of the egg. This suspension was then filtered through a sieve battery with mesh sizes of 250, 125 and 25 µm following the guidelines outlined by Thienpont *et al.* (1986). Strongyle eggs being retained on the 25 µm sieve were washed and collected under pressure using distilled water. After three washes with distilled water, the eggs were adjusted to a concentration of 100-200 eggs/ml employed the McMaster technique (Soulsby, 1982). The suspension was centrifuged for duration of 5

minutes at 1500 rpm and then supernatant was discarded. Approximately, 100 eggs in 200  $\mu$ l of distilled water were then pipetted into each well of tissue culture plate.

### 3.12.2 Procedure of egg hatch assay (EHA)

The EHA was performed following the method previously outlined by Coles *et al.* (2006), albeit with some modifications. To conduct the experiments, distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous plant extract (1000  $\mu$ l) were added in the each well of tissue culture plate 24-well (Tissue culture plate 24-well, Axygen Scientific, Figure 3.12.2.4). The aqueous plant extracts were tested at concentrations of 12.5, 25, 50, and 100 mg/ml. Each concentration was tested in three replicates. Closantel, utilized as positive control was dissolved in dimethyl sulfoxide (DMSO) and then diluted in distilled water to achieve a concentration of 0.50 mg/ml. The estimated number of eggs contained within 200  $\mu$ l was 100. Following the preparation, the plates were then incubated at 27°C for 48 hours. Test was conducted across in nine groups.

**Group 1:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of seeds of *Coriandrum sativum* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 2:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of bulb of *Allium sativum* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 3:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of seeds of *Carica papaya* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 4:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of rhizome of *Zingiber officinale* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 5:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and of aqueous extract (1000  $\mu$ l) of leave of *Azadirachta indica* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 6:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of whole plant of *Swertia chirata* at 12.5, 25, 50 and 100 mg/ml.

**Group 7:** Distilled water (800  $\mu$ l), egg suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of leaves of *Prunus persica* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 8:** Closantel at 0.5 mg/ml (Positive control)

**Group 9:** Egg suspension (200  $\mu$ l) and PBS (Negative control)

Lugol's iodine one drop solution was added to each well to cessation of further hatching. Unhatched eggs and L1 larvae in each well were then counted using an optical microscope at 40× and 100× magnifications. Finally, percent inhibition of egg hatching was calculated:

$$\text{Percent of inhibition}(\%) = 100 \left( 1 - \frac{P_{\text{test}}}{P_{\text{nontreated}}} \right)$$

Where P=number of eggs hatched



**Figure 3.12.2.4: Tissue culture plate 24-well**

### **3.12.3 Larval paralysis test**

This test is utilized to evaluate the capacity of plant extracts with the anthelmintic activity to immobilize the larvae.

To get L3 larvae, coproculture was conducted. Fresh faecal samples were collected from the rectum of the Barbari goats at Instructional Livestock Farm Complex- II, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110. The faecal samples positive for strongyle type of eggs were pooled separately and cultured following the method outlined by Roberts and Sullivan (1950). The L3 larvae (Figure 3.12.3.5) migrating to water in outer petri dish were pipetted out and centrifuged at 1500 rpm for the durations of 3 minutes.



**Figure 3.12.3.5: L3 larvae**

#### **3.12.3.1 Procedure of larval paralysis assay (LPA)**

The larval paralysis assay was conducted within 3 h of collecting infective larvae (L3) following the method outlined by Varady and Corba (1999), along with some modifications. For the experiments distilled water (800  $\mu$ l), larvae suspension (200  $\mu$ l) and aqueous plant extract (1000  $\mu$ l) were added in the tissue culture plate 24-well (Tissue culture plate 24-well, Axygen Scientific Figure 3.12.2.4). Plant extracts were tested at the concentrations of 12.5, 25, 50, and 100 mg/ml. Each experiment was conducted in triplicates. Each 100  $\mu$ l suspension was contained 50 L3 larvae. Closantel dissolved in dimethyl sulfoxide (DMSO) and diluted in distilled water to a concentration of 0.50 mg/ml, was used as a positive control while PBS served as negative control. The experiment was conducted in nine groups.

**Group 1:** Distilled water (800  $\mu$ l), larvae suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of seeds of *Coriandrum sativum* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 2:** Distilled water (800  $\mu$ l), larvae suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of bulb of *Allium sativum* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 3:** Distilled water (800  $\mu$ l), larvae suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of seeds of *Carica papaya* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 4:** Distilled water (800  $\mu$ l), larvae suspension (200  $\mu$ l) and aqueous extract (1000  $\mu$ l) of rhizome of *Zingiber officinale* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 5:** Distilled water (800 µl), larvae suspension (200 µl) and aqueous extract (1000 µl) of leave of *Azadirachta indica* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 6:** Distilled water (800 µl), larvae suspension (200 µl) and aqueous extract (1000 µl) of whole plant of *Swertia chirata* at 12.5, 25, 50 and 100 mg/ml.

**Group 7:** Distilled water (800 µl), larvae suspension (200 µl) and aqueous extract (1000 µl) of leaves of *Prunus persica* at the concentration of 12.5, 25, 50 and 100 mg/ml.

**Group 8:** Closantel at 0.5 mg/ml (Positive control)

**Group 9:** Egg suspension (200 µl) and PBS (Negative control)

The plates were incubated at 27 °C for duration of 24 hours. Following this incubation periods, the plates were examined and the larvae were categorized as either normal (exhibiting the movement) or paralyzed (showing no observable motion for 5 seconds) with particular attention given to the presence or absence of smooth sinusoidal movement, respectively. The experiment was conducted in three replicates and the results were reported as the percentage (%) of larval motility inhibition.

### **3.12.3 Adult worm mortality assay (AWMA)**

In this study, examine aqueous extracts of seven medicinal plants were carried out. These medicinal plants or its parts were seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica*. These selections were made based on information gathered from available literature surveys. The Experimental investigation aimed to assess the *in vitro* anthelmintic activities of the aforementioned plants aqueous extracts using the adult worm mortality (AWM) assay.

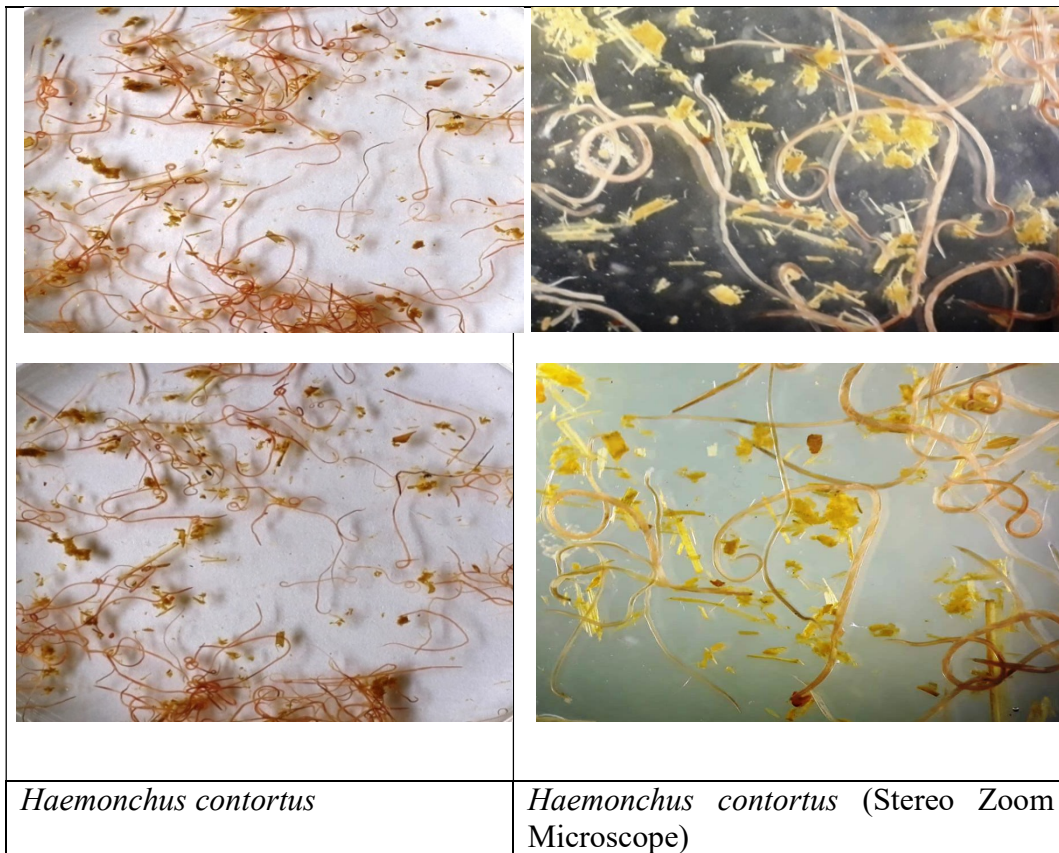
#### **3.12.3.1 In Vitro experiments**

The *in vitro* anthelmintic activity was carried out following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) as outlined by Coles *et al.* (1992), with slight modifications to the procedures for parasites collection, preparation and adult worm mortality assay.

#### **3.12.3.2 Collection of parasite and preparation:**

Adult mature parasites of *Haemonchus contortus* were harvested from abomasum of naturally infected Barbari breeds of goats (Figure 3.12.3.6) following their death and

subsequent postmortem examination at Instructional Livestock Farm Complex- II, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110.



**Figure 3.12.3.6: Collected *H. contortus* from the abomasum of the Barbari goat**

The collection of *Haemonchus contortus* were to standard procedure as outlined by Rahman and Collins, (1990). The both ends of the collected abomasums were ligated after collections and they were then brought to the laboratory. The abomasum was rinsed thoroughly with running water then, opened along its greater curvature and its contents emptied into a 5 liter plastic bucket containing 2 and half liters of water. The parasites were separated by passing the contents through a sieve with a diameter of 100 micrometers and were subsequently collected using a wire loop. Adult mature *Haemonchus contortus* was identified and isolated from other parasites depend on their morphological characteristics utilizing the keys and description described by Taylor *et al.* (2007). The parasites were then, gathered, washed, and kept in phosphate buffered saline (PBS) until required for *in vitro* testing.

### 3.12.3.3 Procedure of adult worm mortality assay (AWMA)

The experiment followed the protocol outlined in prior studies (Sharma *et al.*, 1971; Coles *et al.*, 1992). The test was carried out in 50 mm diameter glass petri dish. A total of 1080 adult *Haemonchus contortus* parasites were utilized. Four concentrations were examined for each plant extract. In each petri dish, 10 actively moving parasites were introduced with varying concentration (50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml) of the aqueous extracts from seven plants materials, dissolved in distilled water or phosphate buffered saline. A negative control group was consisting of ten actively moving parasites in phosphate buffer saline in a total volume of 4 ml. In a positive control group closantel initially dissolved in DMSO (dimethyl sulfoxide) and then diluted in distilled water at the concentration of 1.25 mg/ml along with ten parasites. Each treatment concentrations were conducted in three replicates at room temperature (25-30°C). The assay was comprised in nine groups.

**Group 1:** Aqueous extract of seeds of *Coriandrum sativum* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 2:** Aqueous extract of bulb of *Allium sativum* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 3:** Aqueous extract of seeds of *Carica papaya* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 4:** Aqueous extract of rhizome of *Zingiber officinale* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 5:** Aqueous extract of leaves of *Azadirachta indica* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 6:** Aqueous extract of whole plant of *Swertia chirata* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 7:** Aqueous extract of leaves of *Prunus persica* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml

**Group 8:** Closantel at the concentration of 1.25 mg/ml (Positive control)

**Group 9:** PBS (Negative control)

The inhibitions of worm motility served as an indicator of worm mortality or paralysis. Worm motility was observed, and motile worms were counted at a regular time

intervals of 0, 2, 4, 6 and 8 hours of post treatment. Worms showing no motility were isolated and placed in lukewarm PBS for duration of 10 minutes. If there was a revival in motility during this time the worms were considered alive; otherwise, they were considered dead. Worm death was confirmed by the absence of motility for observed over a period of 5 to 6 seconds. After 8 hours post treatment, the plant extracts and closantel were washed away, and the parasites were suspended in PBS for duration of 30 minutes to allow for possible recovery of the motility of the parasite. Finally, the number of alive (motile) and dead (immotile) worms were counted under microscope. This was recorded for every concentration. Dead worms were simply identified by their straight, flat appearance with absence of movements observed at the head and tail regions of the body. The percent mortality of worms was calculated for each extract concentration using the formula: the mortality index was determined as the total number of dead worms divided by the total number of worms per petri dish.

### **3.13 *In vitro* cytotoxicity study of medicinal plants**

The procedure was conducted at CDRI, located in Lucknow, India. A cytotoxicity study was performed on the aqueous extract of selected medicinal plant employing the SRB (Sulforhodamine B) assay protocols.

#### **3.13.1 SRB assay protocol**

##### **3.13.1.1 Addition of cells**

Normal epithelial cell lines were cultured in RPMI (Roswell Park Memorial Institute)-1640 medium and maintained in DMEM (Dulbecco's Modified Eagle Medium). The cells are enumerated and dispensed in a 96-well tissue culture plate with each well receiving 100  $\mu$ l of the cell suspension containing 10,000 to 20,000 cells, depending on the cell line nature. The plate is then incubated at 37°C in 5% CO<sub>2</sub> concentration for 24 hours before adding the test samples or standard drugs.

##### **3.13.1.2 Addition of test samples**

To achieve the desired final extract concentration in each sample, 100 $\mu$ l of the working solution was added to the cell monolayer. Duplicate wells were utilized and in the control wells, DMSO is added as the vehicle. The final concentration of DMSO (Dimethyl

sulfoxide) in all assay wells was 0.1%. Subsequently, the plate was incubated at 37°C with a 5% CO<sub>2</sub> concentration for 48 hours.

### **3.13.1.3 Addition of SRB and colorimetric reading**

After 48 hours of incubation, the cells attached to the substratum of the plate are fixed by adding 50 µl/well of cold 50% Tri-chloroacetic acid (TCA) to each well on top of the medium followed by incubation at 4°C for 1 hours. Subsequently, the plate was gently washed five times with slow-running tap water using plastic tubing to remove TCA, culture medium and dead cells. It's important to avoid injecting the water stream too rapidly or directly on to the bottom of the wells, as this could lead to detachment of the cell monolayer. After washing, the plates were allowed to air-dry (plates can be stored for extended periods at room temperature after the fixing and drying step). To dry the plates, 50 µl/well of SRB solution was added to each well and left at room temperature for duration of 30 minutes.

At the end of the staining duration, unbound SRB was removed by quickly rinsing plates 4 to 5 times with 1% (v/v) acetic acid. The plates are then allowed to air-dry at room temperature stained and dried plates may be stored indefinitely at room temperature. Next, 150 µl of 10 mMTris base solution is added to each well and plate is shaken for duration of 15 minute on a gyratory shaker to solubilize the protein-bound dye. Alternatively, if a shaker is unavailable, SRB can be solubilized after 30 minutes in tris base solution. Finally, absorbance is measured at 510 nm in using a micro plate spectrophotometer.

### **3.13.1.4 Data analysis**

Percentage (%) of cell growth inhibition in presence of the test sample is calculated as follows:

$$\% \text{ of cells killed} = 100 - \left[ \frac{(\text{Mean OD test})}{(\text{Mean OD cont})} \times 100 \right]$$

## **3.14 *In vivo* studies of anthelmintic activity of selected medicinal plants**

### **3.14.1 Experimental place**

An *in vivo* experiment was conducted at Instructional Livestock Farm Complex- II located within Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110 (Figure 3.14.1.7).



**Figure 3.14.1.7: Experimental shed at ILFC-11**

### **3.14.2 Selection of experimental goats**

The Barbari goats acclimatized to the pen conditions before the study commenced. Each group was kept separate from others, ensuring no physical contact between goats from different treatment groups. Each pen was equipped with its own feeder and watering system. Prior to the trial, the selected goats had not received anthelmintic medication for 2 to 3 months. Their weights were recorded on day 0 before treatment, and anthelmintics were administered based on individual body weight. A thorough clinical examination was conducted on all selected goats before the commenced experiment (Figure 3.14.2.8). In the current study only female Barbari goats were included.

### **3.14.3 Collection of faecal samples**

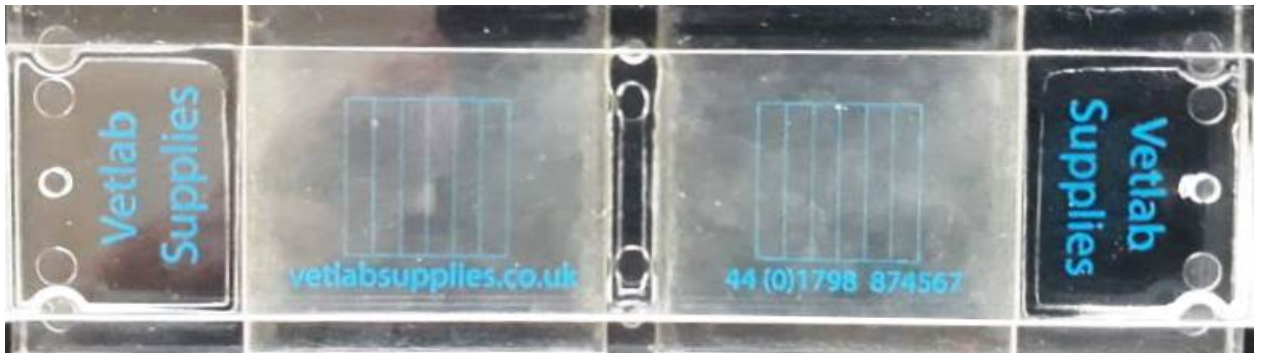
Approximately 20 to 30 grams faecal samples were individually collected from each goat directly from the rectum in the morning with wearing disposable gloves to avoid contamination. Following collection, the faecal samples were carefully placed into properly labeled zip-lock polythene bag. The faecal samples were collected on day 0 prior to treatment initiation and subsequently at 7, 14, 21, and 28 days of post-treatment. These fecal samples were transported to the laboratory in an icebox and promptly stored in refrigerators set at 4°C until further analysis.



**Figure 3.14.2.8: Clinical examination of goat**

#### **3.14.4. Examination of faecal samples**

A qualitative analysis of faecal samples was conducted using both floatation and sedimentation methods following the procedure according to Soulsby (1982) to detect strongyle infection. Fecal egg count (FEC) was determined utilizing the Vetlab Worm Egg Counting Method (Vetlab Supplies Ltd Unit 13 Broomers Hill Park Broomers Lane Pulborough West Sussex RH20 2 RY Figure 3.14.4.9).



**Figure 3.14.4.9: McMaster 2 cell counting slide**

#### **3.14.5 Vetlab worm egg counting method**

##### **3.14.5.1 Equipment required:**

- Microscope equipped with magnifications of 40 × and 100 × and mechanical stage
- 50 ml plastic graduated measuring cylinder
- Ready-made flotation solution with specific gravity of 1.200
- McMaster 2 cell counting slide

- Plastic transfer pipettes
- Plastic bowl
- Tea strainer and tea spoon

### 3.14.5.2 Procedure

1. Pour flotation solution into the measuring cylinder up until it reaches the 26 ml mark.
2. Add faeces until the level reaches the 30 ml mark.
3. Pour the contents into the tea strainer whilst holding over the bowl.
4. Dip the tea strainer in and out of the bowl whilst also mixing faeces retained in strainer with the tea spoon.
5. Discard faecal matter retained in the tea strainer.
6. Thoroughly mix faecal solution in the bowl using the tea spoon and promptly aspirate into a transfer pipette.
7. Carefully transfer the sample to fill the both chambers of the McMaster slide.
8. Allow the slides to stand on bench for minimum 2 minutes and not maximum 5 minutes.
9. Transfer McMaster slide to the microscope stage.
10. Utilizing the 10× objective, adjust the focus on any corner of the first grid.
11. Count the number of worm eggs within the entire area of both grids and then multiply by 25 to obtain final result in egg per gram.

### 3.14.5.3 The number of eggs per gram can be calculated as follows

- Count the number of eggs within each chamber's grid, ignoring any eggs located outside the squares.
- Next, calculate the total count and multiply it by 25. This gives the eggs per gram of faeces (e.p.g.).

### 3.15 Faecal egg count reduction test (FECRT)

The modified McMaster technique was employed to conduct faecal egg counts as outlined by Coles *et al.* (1992) and Zajac and Conboy (2012). Anthelmintic efficacy was determined through the faecal egg count reduction (FECR) test (Wood *et al.*, 1995). This calculation was performed using the following formula:

$$FECR(\%) = \frac{\text{Pre treatment EPG} - \text{Post treatment EPG}}{\text{Pre treatment EPG}} \times 100$$

Following the guidelines outlined by WAAVP, an anthelmintic is classified as highly effective if it achieves FECR (Fecal Egg Count Reduction) percentage more than 98%. It was effective at 90-98% FECR and moderately effective within the 80-89% FECR. Anthelmintics falling below 80% FECR are not recommended for use.

### **3.16 Efficacy of the drugs**

The efficacy of administered anthelmintics was evaluated through multiple criteria, including the percentage reduction in fecal egg count, restoration of hemogram and various biochemical parameters and enhancement in overall condition of post-treatment.

### **3.17 Experimental protocol**

The experimental protocol was presented in Table 3.17.3. Initially, 90 goats were screening for strongyle infections through fecal examination, revealing that 48 of them positive for strongyle infections. From this pool, 48 female Barbari goats aged between 18 to 30 months were selected. These goats were chosen randomly and categorized into 8 groups, each consisting of six goats. Selection criteria included goats with egg per gram (EPG) counts exceeding 1000.

Dosages regimens for plant extracts of *Coriandrum sativum* and *Swertia chirata* were divided into three categories: low (50 mg/kg), medium (250 mg/kg) and high (500 mg/kg). Group 1 acted as a control and received no treatment while, Group 2 served as positive control, and receiving a single oral dose of Closantel (ZenVet™, Intas Pharmaceutical LTD.) at @ 10 mg/kg body weight.

Groups 3, 4 and 5 received individual a single oral doses of an aqueous extract derived from of *Coriandrum sativum* seeds, at concentrations of 50 mg/kg, 250 mg/kg and 500 mg/kg, body weight respectively. Similarly, Groups 6, 7 and 8 were administered single oral doses of an aqueous extract obtained from whole plant of *Swertia chirata* at concentrations of 50 mg/kg, 250 mg/kg and 500 mg/kg body weight respectively. Anthelmintic dosing for goats was based on their individual body weight. To administer the plant extracts orally, 10 to 15 grams of honey was added to ensure easy and complete administration (Figure 3.17.10).



**Figure 3.17.10: Administering of plant extracts**

**Table 3.17.3: Experimental protocol**

<b>Group</b>	<b>Goats</b>	<b>Dosages regimens</b>
Group 1	6	Control group: Received no treatment
Group 2	6	Positive control: Received a single dose of Closantel @10 mg/kg body weight
Groups 3	6	Low dose: Aqueous extract of seed of <i>Coriandrum sativum</i> administered orally @ 50 mg/ kg body weight (single dose)
Group 4	6	Medium dose: Aqueous extract of seed of <i>Coriandrum sativum</i> administered orally @ 250 mg/kg body weight (single dose)
Groups 5	6	High dose: Aqueous extract of seed of <i>Coriandrum sativum</i> administered orally @ 500 mg/kg body weight (single dose)
Group 6	6	Low dose: Aqueous extract of whole plants of <i>Swertia chirata</i> administered orally @ 50mg/ kg body weight (single dose)
Group 7	6	Medium dose: Aqueous extract of whole plants of <i>Swertia chirata</i> administered orally @ 250 mg/kg body weight (single dose)
Groups 8	6	High dose: Aqueous extract of whole plants of <i>Swertia chirata</i> administered orally @ 500 mg/kg body weight (single dose)

### 3.18 Collection of blood samples for hematology and biochemical study

Blood samples were aseptically collected from the jugular vein using a 5 ml disposable syringe for the analysis of various haematological and biochemical parameters.

#### 3.18.1 Haematological estimations

Blood samples (2 ml) were collected in EDTA containing vials from jugular vein of each goats on day zero (pre-treatment) and on day's 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup> and 28<sup>th</sup> post-treatment for haematological analysis. The haematological parameters including haemoglobin (Hb), total erythrocyte counts (TEC), total leukocyte count (TLC) and packed cell volume (PCV), were determined using an Auto Hematology Analyzer (mindray BC-30Vet, Figure 3.18.1.11). Blood smears for differential leukocyte count (DLC) were prepared from fresh blood. The DLC was conducted manually.



Figure 3.18.1.11: Auto Hematology Analyzer (mindray BC-30 vet)

#### 3.18.1. Procedure for Giemsa staining

1. Carefully a thin blood smear on a clean, grease free microscopic glass slides were prepared using the freshly collected blood.
2. Allow the smear to air dried.
3. Absolute methanol was poured on blood smear, ensuring complete coverage, and let it fix for approximately 5 minutes.

4. A working solution of Giemsa stain was poured on the blood smear, allowing it to for about 40 minutes.
5. Blood smears were rinse thoroughly under running tap water to remove excess stain.
6. Once blood smear was washed then, allow the smear to air dry again.
7. Apply a drop of immersion oil to the dried smear and examine in under microscope utilizing the 100× objective for observations.

### 3.18.2 Biochemical estimation

Blood samples (3ml) were collected in plain tubes for the estimation of serum biochemical parameters. Subsequently, serum was obtained by centrifugation at 3000 rpm for 10 minutes. The separated serum samples were then stored at -20°C until analysis. Biochemical parameters including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) albumin, BUN and creatinine were determined using a fully Automatic Biochemical Auto Analyzer (mindray BS-240, Figure 3.18.2.12).The value of globulin was obtained by subtracting the albumin value from the total protein value. This calculation was performed using the following formula: Globulin (g/dl) = Total Protein (g/dl) - Albumin (g/dl). Albumin and globulin ratio was calculating by dividing the albumin value by the globulin value.



**Figure 3.18.2.12: Biochemical Auto Analyzer (mindray BS-240)**

### **3.19 Antioxidant Activity**

1. SOD
2. Catalase
3. Total antioxidant activity

#### **3.19.1 Preparation of plasma and haemolysate**

The blood samples were collected into a graduated centrifuge (6ml) tube, filling it up to the marked level, and then centrifuge it. After centrifugation, the plasma was separated and stored. The remaining erythrocytes underwent washing and centrifuged three times with normal saline (0.9% NaCl) solution. Subsequently, distilled water was slowly added to the erythrocyte pellet with continuous stirring until reaching the marked level, thus creating the haemolysate. The remainder of the haemolysate was promptly stored at -20°C until enzyme activity could be assessed.

#### **3.19.2 Superoxide dismutase (SOD)**

##### **Reagents required**

- a) Pyrogallol (2mM)

25.2 mg was dissolved in 100ml of 10mM HCl (Hydrochloric acids)

- b) Tris buffer (50mM)

Tris buffer 605 mg of dissolved in 100 ml of distilled water. Subsequently, 39 mg of Diethylene triamine pentaacetic acid was added into 100 ml of the buffer solution. The pH of resultant solution was ten adjusted to 8.2 utilizing hydrochloric acids.

The enzyme activity was assayed by the method outlined by Marklund and Marklund (1974). The reaction mixture comprised varying concentrations of appropriately diluted lysate, ranging from 0.2 to 2.0 ml, which were adjusted to a total volume of 3 ml with tris - HCl buffer (50mM, pH 8.2) containing 1mM diethylene triamine pentaacetic acid. Additionally, 0.2 ml of 2mM pyrogallol was included in the mixture. A standard solution was prepared without the presence of sample. The rate of pyrogallol auto oxidation was measured by monitoring the increase in absorbance at 420 nm against a reference cuvette containing 3.0 ml of tris buffer using a UV-VIS Double Beam Spectrophotometer 2201 (SYSTRONICS, Figure 3.19.2.13). The absorbance increased was  $0.02 \text{ min}^{-1}$  in the absence of superoxide dismutase enzyme. Superoxide dismutase was employed to inhibit the auto-oxidations of pyrogallol,

thereby facilitating the determination of enzyme activity. An enzyme unit was defined as the amount of enzyme required to inhibit the reaction by 50 percent.



**Figure 3.19.2.13: UV-VIS Double Beam Spectrophotometer 2201**

### 3.19.3 Catalase

The enzyme activity was assessed spectrophotometrically utilizing the methodology described by Aebi (1984).

#### Reagents

1. Phosphate buffer (50 mM)

#### Dissolve

- (a) 6.81 gm  $\text{KH}_2\text{PO}_4/\text{L}$
- (b) 8.90 gm  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}/\text{L}$

Solution (a) was placed in a beaker, followed by the gradual addition of solution (b) to solution (a) in a 1: 1.5 (v/v). The pH was subsequently adjusted to pH 7.0.

2.  $\text{H}_2\text{O}_2$  (30 mM)

Dilute 0.34 ml of 30 %  $\text{H}_2\text{O}_2$  with phosphate buffer to a final volume 100 ml.

Blank	Sample
Phosphate buffer-2.9ml	1.9ml
$\text{H}_2\text{O}_2$	1ml
RBC lysate -25-100 $\mu\text{l}$ (Suitable diluted sample)	25-100 $\mu\text{l}$

The reaction commenced upon the addition of  $\text{H}_2\text{O}_2$ . The decomposition of  $\text{H}_2\text{O}_2$  was monitored by measuring the decrease in absorbance at 240 nm. The reduction in absorbance was recorded over a period of 65 seconds, and the variance between the absorbance at 5 seconds and 65 seconds was determined. Employing an extinction coefficient of 0.0394 ml

$\text{mM}^{-1} \text{cm}^{-1}$  the enzyme activity was computed and expressed as micro moles of  $\text{H}_2\text{O}_2$  consumed per minute per gram Hb in blood.

### **3.19.4 Estimation of total plasma antioxidant activity (FRAP)**

The total antioxidant activity was assessed through the ferric reducing antioxidant power (FRAP) assay as detailed by Benzie and Strain (1999). This method relies on antioxidants functioning as reductants in a redox-linked colorimetric process. It utilizes an oxidant system in stoichiometric excess that is readily reduced, thereby allowing for the measurement of antioxidant capacity.

#### **Principle**

When pH is low, the reduction of the ferric tripyridyl triazine ( $\text{Fe}^{\text{III}}$  TPTZ) complex to its ferrous form occurs characterized by an intense blue coloration. This reduction process can be effectively monitored by measuring the change in absorption at 593 nm. Importantly, the reaction is non specific, meaning that any half- reaction with a lower redox potential, than that of the ferric ferrous half reaction, under reaction conditions, facilitated the conversion of the ferric ions ( $\text{Fe}^{\text{III}}$ ) to ferrous ions ( $\text{Fe}^{\text{II}}$ ). Consequently, the alteration in absorbance directly correlates with the collective or “total” reducing power of the electron- donating antioxidants present within the reaction mixture.

#### **Reagents**

##### **1. FRAP Reagent**

- A) Acetate buffer 3.0 mM, pH 3.6:** First weigh 3.1 gm sodium acetate trihydrate. Then, add 16 ml of glacial acetic acid and make the volume to 1.0 liter with distilled water.
- B) Ferric chloride 2 mM in 40 mM HCl.**
- C) Tripyridyl triazine 10 mM**

The working FRAP reagent was prepared by mixing components A, B & C in the ratio of 10:1:1, immediately before use.

##### **2. Ferrous sulphate 1mM**

##### **3. Ascorbic Acid 100 $\mu\text{M}$**

**Procedure:**

A 100 µl plasma sample was mixed with 3 ml of working FRAP reagent and absorbance was immediately measured at 0 minute after vortexing. Subsequently, the samples were incubated at 37°C in water bath and absorbance readings were taken after 4 minutes. Ascorbic acids standards ranging from 100 µM to 1000 µM were prepared and processed using the same procedure. Results were calculated as follows.

$$FRAP \text{ value of sample}(\mu\text{mol/L}) = \frac{A}{B} \times FRAP \text{ value of standard}(100\mu\text{m})$$

Where, A (Change in absorbance of sample from 0 to 4 minute) and B (Change in absorbance of standard 0 to 4 minute)

$$FRAP \text{ value of sample}(\mu\text{mol/L}) = \frac{A - B}{X - Y} \times 100$$

Where A (Reading of sample at 0 minute), B (Reading of sample at 4 minute), X (Reading of standard at 0 minute), Y (Reading of standard at 4 minute) and 100(FRAP value of 100µM standard)

**3.20 Statistical analysis**

Collected raw data was stored in a Microsoft Excel database system used for data management. The collected data were analyzed using IBM SPSS version 20.

The results on the study “Prevalence of gastro-intestinal parasites of goats and comparative therapeutic efficacy of phyto-anthelmintics” are presented in this chapter.

#### 4.1 Prevalence study of gastrointestinal parasites in goats

Examinations of faecal samples revealed the prevalence of gastrointestinal parasitic infections in goats of Western Uttar Pradesh, India, throughout the year. A total of 1117 faecal samples were examined and out of these 788 samples were found positive for gastrointestinal parasites in goats. The overall prevalence of gastrointestinal parasites infections in goats were 70.55% (Table 4.1). The examinations of faecal samples of goats revealed the presence of several types of gastrointestinal parasites.


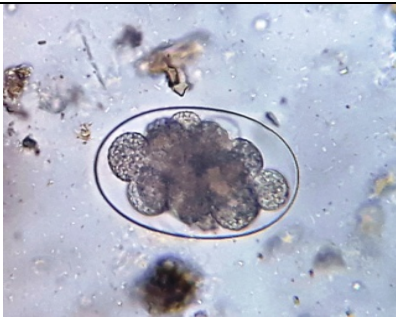




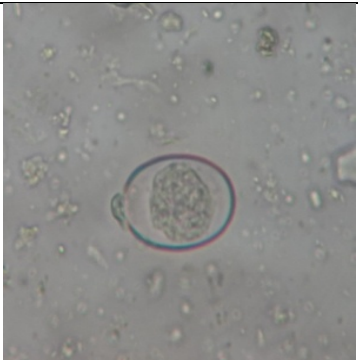
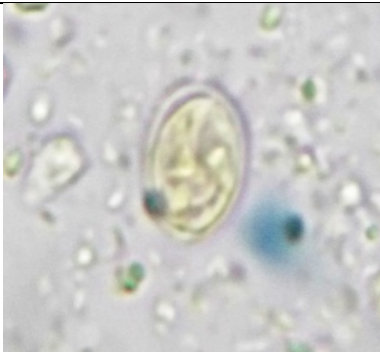
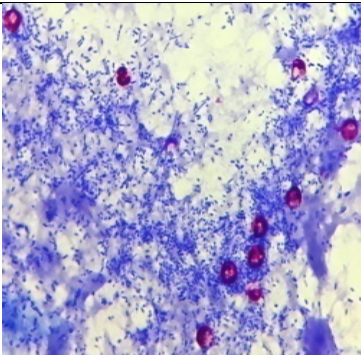
**Table 4.1: The overall prevalence of gastrointestinal parasites**

Total faecal samples examined	No. of positive faecal samples	No. of negative faecal samples	Prevalence (%)
1117	788	329	70.55%

The parasitic eggs were identified on the basis of their distinguishing characteristics. A total of nine gastrointestinal parasitic eggs (*Fasciola* spp., *Strongyle* spp., *Trichuris* spp., *Strongyloides* spp., *Amphistome* spp., *Moniezia* spp., *Eimeria* spp., *Giardia* spp. and *Cryptosporidium* spp.) were detected in goats during faecal examinations in current study (Figure 4.1).

##### 4.1.1 Breed wise prevalence of gastrointestinal parasites

The highest prevalence of gastrointestinal parasites was recorded in Barbari breed of goat (39.97%) followed by Jamunapari goat (36.93%), non descript goat (12.18%) and lowest in Sirohi goat (10.91%) (Figure 4.1.12). In the Barbari breed of goat the highest prevalence rate was recorded of *Strongyle* spp. (46.03%) followed by *Eimeria* spp. (40.0%), *Moniezia* spp. (3.49%), *Trichuris* spp. (2.86%), *Amphistome* spp. (2.86%), *Fasciola* spp. (2.54%), *Strongyloides* spp. (0.95%) and lowest in *Giardia* spp. (0.63 %) and *Cryptosporidium* spp. (0.63%) (Table 4.1.1.2).

		
<i>Fasciola</i> spp.	<i>Strongyle</i> spp.	<i>Trichuris</i> spp.
		
<i>Strongyloides</i> spp.	Amphistome spp.	<i>Moniezia</i> spp.
		
<i>Eimeria</i> spp.	<i>Giardia</i> spp.	<i>Cryptosporidium</i> spp.

**Figure 4.1: Parasitic eggs detected in the faecal samples of goats by microscopic examinations**

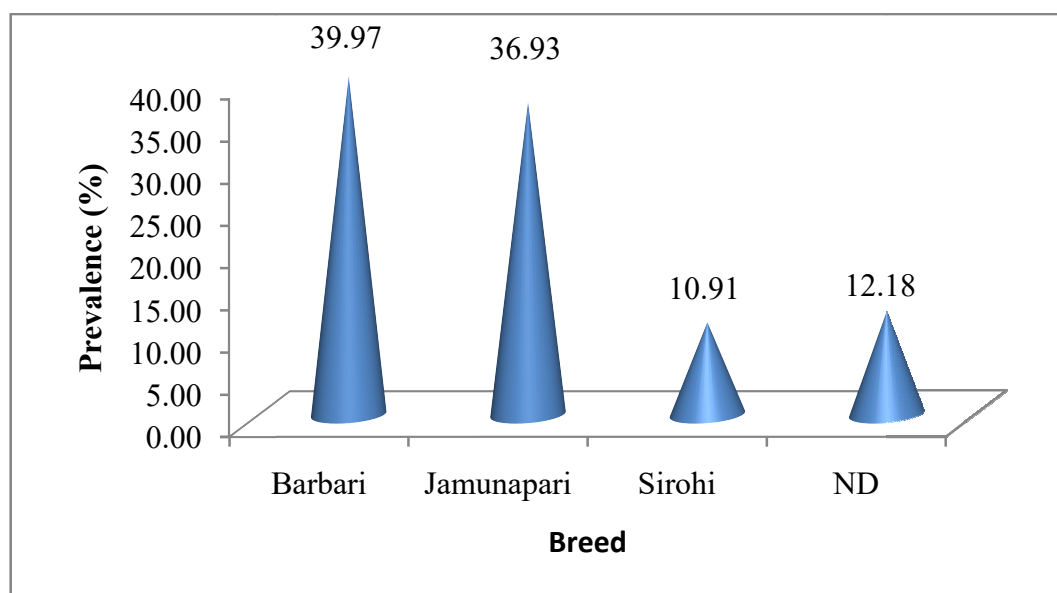
In Jamunapari breed of goat the highest prevalence rate was recorded of *Strongyle* spp. (48.11%) followed by *Eimeria* spp. (35.74 %), *Moniezia* spp. (4.47%), Amphistome spp. (4.12%), *Fasciola* spp. (3.09%), *Trichuris* spp. (2.75%), *Strongyloides* spp. (0.69%), *Cryptosporidium* spp. (0.69%) and lowest in (0.34%) (Table 4.1.1.2). In the Sirohi breed of goat the highest prevalence rate was recorded of *Eimeria* spp. (40.70%) followed by

Strongyle spp. (22.09%), Amphistome spp. (12.79%), *Fasciola* spp. (8.14%), *Trichuris* spp. (6.98%), *Moniezia* spp. (4.65%), *Cryptosporidium* spp. (2.33%) and lowest in *Strongyloides* spp. (1.16%) and *Giardia* spp. (1.16%) (Table 4.1.1.2).

**Table 4.1.1.2: Breed wise prevalence (%) of gastro-intestinal parasites in goats**

Parasites	Goat				$\chi^2$ Value
	Barbari (315)	Jamunapari (291)	Sirohi (86)	Non Descript (96)	
<i>Fasciola</i> spp.	2.54 (8)	3.09 (9)	8.14 (7)	3.09 (5)	6.90
<i>Strongyle</i> spp.	46.03 (145)	48.11 (140)	22.09 (19)	38.54 (37)	19.77***
<i>Trichuris</i> spp.	2.86 (9)	2.75 (8)	6.98 (6)	7.29 (7)	7.21
<i>Strongyloides</i> spp.	0.95 (3)	0.69 (2)	1.16 (1)	1.04 (1)	0.25
Amphistome spp.	2.86 (9)	4.12 (12)	12.79 (11)	5.21 (5)	15.24**
<i>Moniezia</i> spp.	3.49 (11)	4.47 (13)	4.65 (4)	3.13 (3)	0.67
<i>Eimeria</i> spp.	40.0 (126)	35.74 (104)	40.70 (35)	35.42 (34)	1.70
<i>Giardia</i> spp.	0.63 (2)	0.34 (1)	1.16 (1)	3.13 (3)	6.72
<i>Cryptosporidium</i> spp.	0.63 (2)	0.69 (2)	2.33 (2)	1.04 (1)	2.41

Bracket value indicate number of positive animals  
 $\chi^2$ , Chi square; \*\*\*p<0.001; \*\*p<0.01



**Figure 4.1.1.2: Breed wise prevalence (%) of gastrointestinal parasites in goats**

In the Non Descript breed of goat highest prevalence rate was recorded of Strongyle spp. (38.54 %) followed by *Eimeria* spp. (35.42 %), *Trichuris* spp. (7.29 %), Amphistome spp. (5.21 %), *Moniezia* spp. (3.13%), *Giardia* spp. (3.13%), *Fasciola* spp. (3.09%) and

lowest in *Strongyloides* spp. (1.04 %) and *Cryptosporidium* spp. (1.04 %) (Table 4.1.1.2). The prevalence of Strongyle spp. infections were highly significant ( $p < 0.001$ ) in Barbari, Jamunapari, Sirohi and Non Descript of goats. The prevalence of Amphistome spp. infections were significant ( $p < 0.01$ ) in Barbari, Jamunapari, Sirohi and Non Descript of goats.

#### 4.1.2 Age wise prevalence of gastro-intestinal parasites in goats

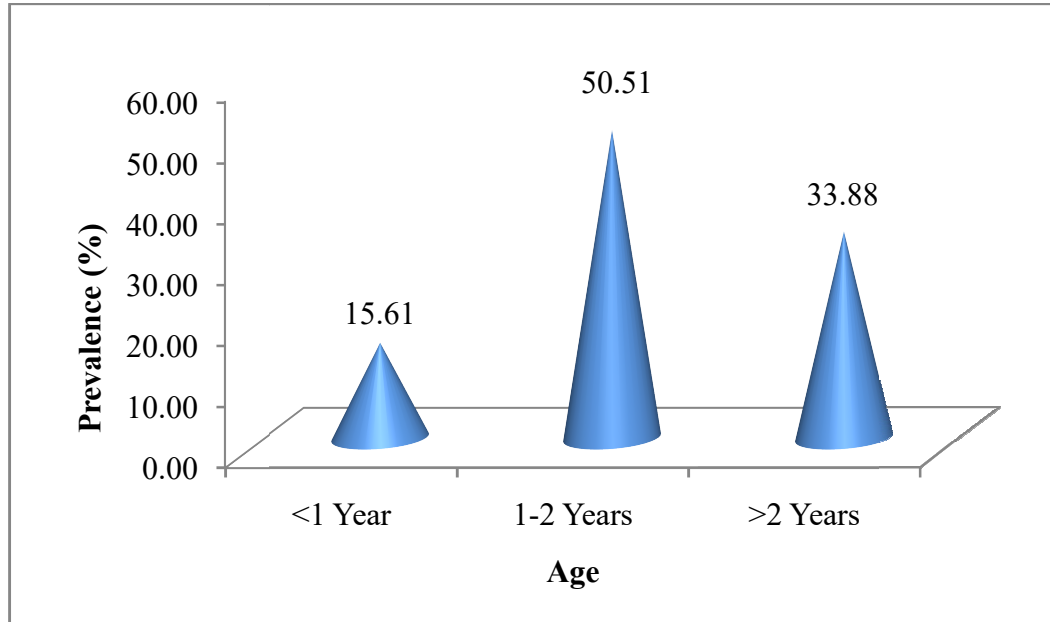
The highest prevalence of gastrointestinal parasites reported in age group of 1-2 years (50.51%) followed by in age group of > 2 years (33.88%) and the lowest was in < 1 year age group (15.61%) (Figure 4.1.2.3). In the < 1 year age group the highest prevalence rate was recorded of *Strongyle* spp. (37.40%) followed by *Eimeria* spp. (31.71%), *Trichuris* spp. (8.13%), *Giardia* spp. (7.32%), *Cryptosporidium* spp. (7.32%), *Moniezia* spp. (4.88%), *Strongyloides* spp. (2.44%), Amphistome spp. (0.81%) and lowest in *Fasciola* spp. (0.0%) (Table 4.1.2.3). In the 1-2 year age group the highest prevalence rate was recorded of *Strongyle* spp. (42.46%) followed by *Eimeria* spp. (31.91%), Amphistome spp. (8.29%), *Fasciola* spp. (5.78%), *Trichuris* spp. (5.53%), *Moniezia* spp. (3.52%) *Strongyloides* spp. (1.51%), *Cryptosporidium* spp. (0.75%) and lowest in *Giardia* spp. (0.25%) (Table 4.1.2.3). In the >2 year age group highest prevalence rate was recorded of Strongyle spp. (61.80%) followed by Amphistome spp. (8.24%), *Trichuris* spp. (7.12%), *Fasciola* spp. (5.78%), *Moniezia* spp. (4.12%), *Strongyloides* spp. (3.37%), *Eimeria* spp. (3.37%), *Cryptosporidium* spp. (0.37 %) and lowest in *Giardia* spp. (0.0%) (Table 4.1.2.3).

**Table 4.1.2.3: Age wise prevalence (%) of gastrointestinal parasites in goats**

Parasites	Goat			$\chi^2$ Value
	<1 Year (123)	1-2 Year (398)	>2 Year (267)	
<i>Fasciola</i> spp.	0.0 (0)	5.78 (23)	5.78 (31)	19.24***
<i>Strongyle</i> spp.	37.40 (46)	42.46 (169)	61.80 (165)	30.77***
<i>Trichuris</i> spp.	8.13 (10)	5.53 (22)	7.12 (19)	1.33
<i>Strongyloides</i> spp.	2.44 (3)	1.51 (6)	3.37 (9)	2.50
Amphistome spp.	0.81 (1)	8.29 (33)	8.24 (22)	8.75*
<i>Moniezia</i> spp.	4.88 (6)	3.52 (14)	4.12 (11)	0.50
<i>Eimeria</i> spp.	31.71 (39)	31.91 (127)	3.37 (9)	82.95***
<i>Giardia</i> spp.	7.32 (9)	0.25 (1)	0.0 (0)	42.63***
<i>Cryptosporidium</i> spp.	7.32 (9)	0.75 (3)	0.37 (1)	28.99***

Bracket value indicate number of positive animals

$\chi^2$ , Chi square; \*\*\*  $p < 0.001$ ; \*  $p < 0.05$



**Figure 4.1.2.3: Age wise prevalence (%) of gastro-intestinal parasites in goats**

The prevalence of *Fasciola* spp., *Strongyle* spp., *Eimeria* spp., *Giardia* spp. and *Cryptosporidium* spp. infections were highly significant ( $p < 0.001$ ) in <1 year, 1-2 year and in > 2 year age group of goats. The prevalence of Amphistome spp. infection was significant ( $p < 0.05$ ) in <1 year, 1-2 year and in > 2 year age group of goats.

#### 4.1.3 Sex wise prevalence

The prevalence of gastrointestinal parasites rate was higher in females (83.88%) in comparison to males (16.12%) (Figure 4.1.3.4). In the male the highest prevalence rate was recorded of *Strongyle* spp. (24.41%) followed by *Moniezia* spp.(17.32%), Amphistome spp.(14.96%), *Fasciola* spp.(12.60%), *Trichuris* spp.(11.81%), *Eimeria* spp.(8.66%), *Strongyloides* spp.(7.09%), *Cryptosporidium* spp.( 1.57 %) and lowest in *Giardia* spp.(1.00%) (Table 4.1.3.4). In the female the highest prevalence rate was recorded of *Strongyle* spp. (34.49%) followed by *Eimeria* spp. (29.80%), *Fasciola* spp. (12.60%), Amphistome spp. (8.17%), *Trichuris* spp. (7.72%), *Moniezia* spp. (7.41%), *Strongyloides* spp. (2.57%), *Cryptosporidium* spp. (1.97%) and lowest in *Giardia* spp. (1.36 %) (Table 4.1.3.4).

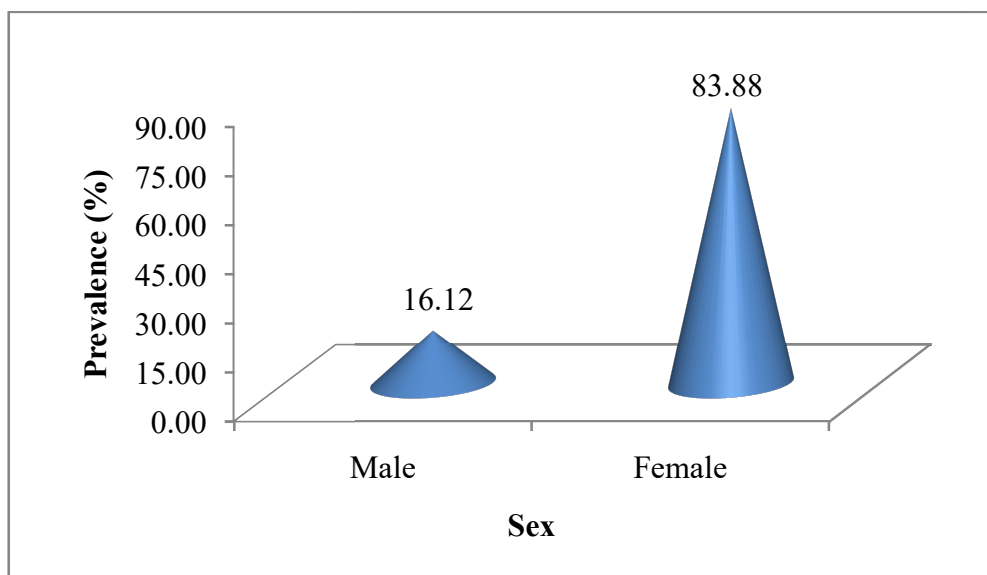
**Table 4.1.3.4: Sex wise prevalence (%) of gastrointestinal parasites in goats**

Parasites	Goat		$\chi^2$ Value
	Male (127)	Female (661)	
<i>Fasciola</i> spp.	12.60 (16)	12.60 (43)	5.71*
<i>Strongyle</i> spp.	24.41 (31)	34.49 (228)	3.74
<i>Trichuris</i> spp.	11.81 (15)	7.72 (51)	2.33
<i>Strongyloides</i> spp.	7.09 (9)	2.57 (17)	6.81**
Amphistome spp.	14.96 (19)	8.17 (54)	5.85*
<i>Moniezia</i> spp.	17.32 (22)	7.41 (49)	12.76***
<i>Eimeria</i> spp.	8.66 (11)	29.80 (197)	24.51***
<i>Giardia</i> spp.	1.0 (2)	1.36 (9)	0.04
<i>Cryptosporidium</i> spp.	1.57 (2)	1.97 (13)	0.09

Bracket value indicate number of positive animals

$\chi^2$ , Chi square; \*\*\* p<0.001; \*\* p<0.01; \* p<05

The prevalence of *Moniezia* spp. and *Eimeria* spp. infections were highly significant (p<0.001) in both male and female goat. The prevalence of *Strongyloides* spp. infection was significant (p<0.01) in both male and female. The prevalence of *Fasciola* spp. and Amphistome spp. infections were significant (p<05) in both male and female.



**Figure 4.1.3.4: Sex wise prevalence (%) of gastrointestinal parasites in goats**

#### 4.1.4 Season wise prevalence

The highest prevalence rate of gastrointestinal parasites were observed during rainy season (47.59%) followed by in summer season (33.63%) and the lowest during winter season (18.78%) (Figure 4.1.4.5). In the winter season the highest prevalence rate was recorded of *Strongyle* spp. (36.49 %) followed by *Eimeria* spp. (22.30%), *Fasciola* spp. (12.16%), *Trichuris* spp. (7.43%), *Moniezia* spp. (7.43%), *Cryptosporidium* spp. (4.73%), Amphistome spp. (4.05%), *Giardia* spp. (3.38%) and lowest in *Strongyloides* spp. (2.03 %) (Table 4.1.4.5).

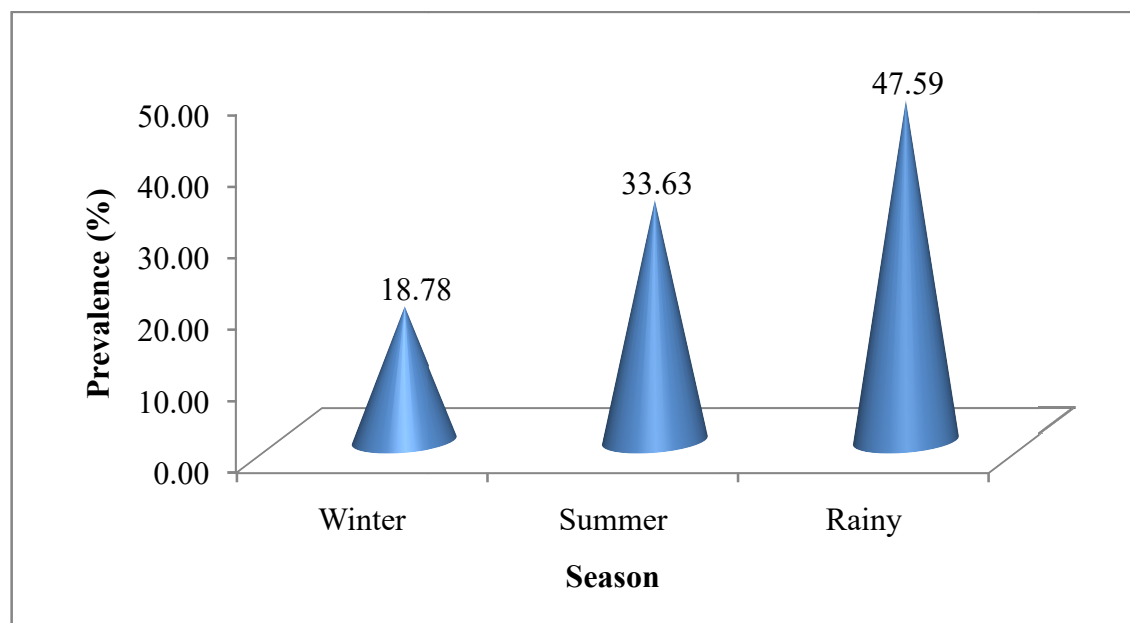
**Table 4.1.4.5: Season wise prevalence (%) of gastrointestinal parasites in goats**

Parasites	Goat			$\chi^2_{\text{value}}$
	Winter (148)	Summer (265)	Rainy (375)	
<i>Fasciola</i> spp.	12.16 (18)	8.30 (22)	8.26 (31)	2.21
<i>Strongyle</i> spp.	36.49 (54)	34.72 (92)	36.80 (138)	0.31
<i>Trichuris</i> spp.	7.43 (11)	5.28 (14)	4.80 (18)	1.45
<i>Strongyloides</i> spp.	2.03 (3)	0.75 (2)	3.20 (12)	4.41
Amphistome spp.	4.05 (6)	6.79 (18)	5.60 (21)	1.34
<i>Moniezia</i> spp.	7.43 (11)	21.89 (58)	7.73 (29)	32.75***
<i>Eimeria</i> spp.	22.30 (33)	20.38 (54)	26.13 (98)	3.01
<i>Giardia</i> spp.	3.38 (5)	1.13 (3)	3.20 (12)	3.20
<i>Cryptosporidium</i> spp.	4.73 (7)	0.75 (2)	4.27 (16)	7.67*

Bracket value indicate number of positive animals

$\chi^2$ , Chi square; \*\*\*p<0.001; \*p<05

In the winter season the highest prevalence rate was recorded of *Strongyle* spp. (34.72%) followed by *Moniezia* spp. (21.89%), *Eimeria* spp. (20.38%), *Fasciola* spp. (8.30%), Amphistome spp. (6.79%), *Trichuris* spp. (5.28%), *Giardia* spp. (1.13%) and lowest in *Strongyloides* spp. (0.75%) and *Cryptosporidium* spp. (0.75%) (Table 4.1.4.5). In the rainy season the highest prevalence rate was recorded of *Strongyle* spp. (36.80%) followed by *Eimeria* spp. (26.13%), *Fasciola* spp. (8.26%), *Moniezia* spp. (7.73%), Amphistome spp. (5.60 %), *Trichuris* spp. (4.80 %), *Cryptosporidium* spp. (4.27%) and lowest in *Strongyloides* spp. (3.20%) and *Giardia* spp. (3.20%) (Table 4.1.4. 5). The prevalence of *Moniezia* spp. infection was highly significant (p<0.001) in the winter, summer and rainy season. The prevalence of *Cryptosporidium* spp. infection was significant (p<05) in the winter, summer and rainy season.



**Figure 4.1.4.5: Season wise prevalence (%) of gastro-intestinal parasites in goats**

#### **4.2 Molecular characterization of strongyle nematode infection in goats**

Total 1117 faecal samples were screened by direct faecal wet smears and flotation methods for the identification of strongyle types of eggs. Three hundred sixty (360) faecal samples were found positive for strongyle type of eggs from total 1117 screened faecal sample. The overall prevalence of strongyle infection in goat was 32.22% (Table 4.2.6).

**Table 4.2.6: The overall prevalence of Strongyle in goats**

S. No.	Animal	Total number of faecal samples	Positive faecal samples for Strongyle	Prevalence (%)
1.	Goats	1117	360	32.22

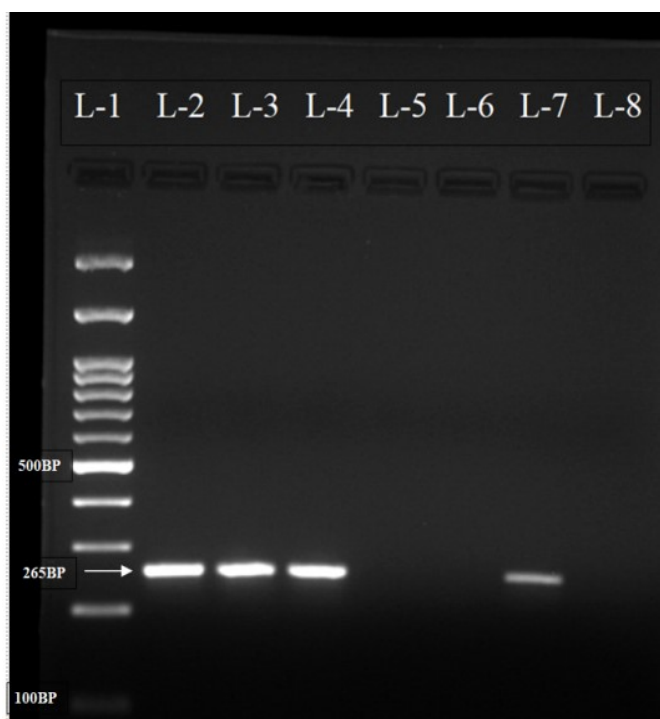
#### **4.3 Molecular characterization**

Polymerase chain reaction was carried out for the larvae isolated from faecal cultures for the confirmation of strongyle larvae at genus and species level. The PCR confirmed the presence of *Haemonchus contortus* and genus of *Trichostrongylus* and *Oesophagostomum* (Table 4.3.7).

**Table 4.3.7: Prevalence of *Haemonchus contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp.**

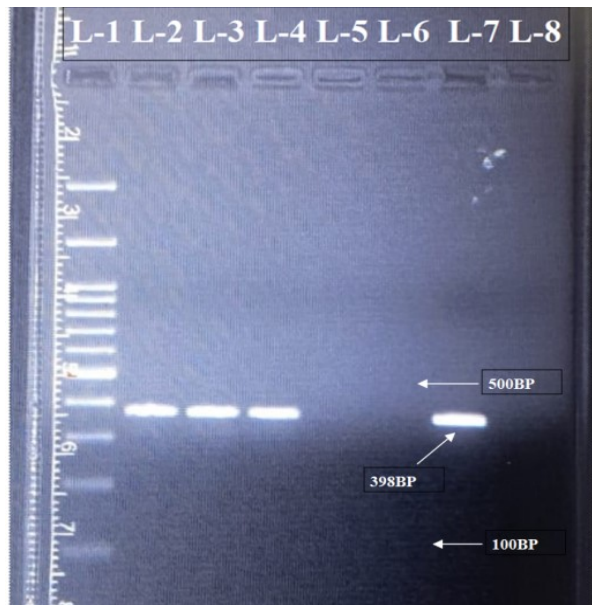
S.No.	Genus / Species	No. of positive faecal samples	Prevalence (%)
1.	<i>Haemonchus contortus</i>	195	54.16
2.	<i>Trichostrongylus</i> spp.	114	31.66
3.	<i>Oesophagostomum</i> spp.	51	14.16

In the present study prevalence rate was recorded highest in *Haemonchus contortus* (54.16%), followed by *Trichostrongylus* spp. (31.66%) and lowest in *Oesophagostomum* spp. (14.16%). The PCR confirmed the presence of *Haemonchus contortus* in 195 faecal samples. The species specific product of expected size 265bp was amplified (Figure 4.3.6).



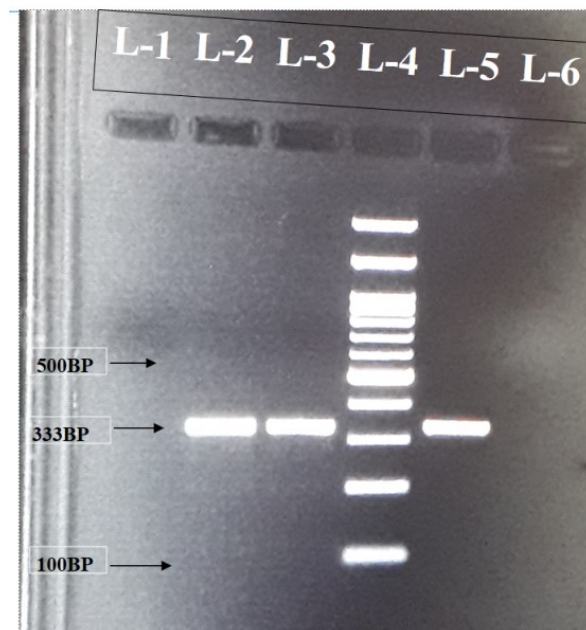
**Figure 4.3.6: Results of PCR amplification of ITS-2 of ribosomal DNA gene of *Haemonchus contortus* (265 bp). L1: DNA Ladder (100bp); L2, L3, L4: Positive Samples; L5, L6: Negative Sample; L7: Positive Control; L8: Negative Control**

The PCR confirmed the presence of *Trichostrongylus* spp. in 114 faecal samples. The genus specific product of expected size 398bp was amplified (Figure 4.3.7).



**Figure 4.3.7: Amplified product of ITS-2 of the ribosomal DNA gene of *Trichostrongylus* spp. (398bp). Lane L1: 100 bp plus DNA ladder, L2, L3, L4: positive samples, L5, L6: negative sample L7: positive control and L8: negative control**

The PCR confirmed the presence of *Oesophagostomum* spp. in 114 faecal samples. The genus specific product of expected size 333bp was amplified (Figure 4.3.8).



**Figure 4.3.8: Amplified product of ITS-2 of the ribosomal DNA gene of *Oesophagostomum* spp. (333bp). Lane L4: 100 bp plus DNA ladder; L2, L3,: positive samples, L1: negative sample L5: positive control and L6: negative control**

#### 4.4 Plants extraction yields

 <p>Coriandrum sativum (Seed extract)</p>	 <p>Allium sativum (Bulb extract)</p>
<p>Powder and aqueous extract of seeds of <i>Coriandrum sativum</i></p>	<p>Powder and aqueous extract of bulbs of <i>Allium sativum</i></p>
 <p>Carica papaya (Seed extract)</p>	 <p>Zingiber officinale (Rhizome extract)</p>
<p>Powder and aqueous extract of seeds of <i>Carica papaya</i></p>	<p>Powder and aqueous extract of rhizomes of <i>Zingiber officinale</i></p>
 <p>Azadirachta indica (Leaf extract)</p>	 <p>Swertia chirata (Whole plant extract)</p>
<p>Powder and aqueous extract of leaves of <i>Azadirachta indica</i></p>	<p>Powder and aqueous extract of whole plants of <i>Swertia chirata</i></p>
 <p>Prunus persica (Leaf extract)</p>	
<p>Powder and Aqueous Extract of leaves of <i>Prunus persica</i></p>	

**Figure 4.4.9: Powder and Aqueous extracts of Plants or their parts**

The powder and aqueous extract of selected medicinal plants or their parts were shown in Figure 4.4.9. The aqueous extract yield percentages of selected plants are presented in table 4.4.8. The aqueous extract yield percentage from *Coriandrum sativum* (Seeds), *Allium sativum* (Bulbs), *Carica papaya* (Seeds), *Zingiber officinale* (Rhizomes), *Azadirachta indica* (Leaves), *Swertia chirata* (Whole plants) and *Prunus persica* (Leaves) were 8.25%, 19.75%, 14.46%, 17.65%, 14.81%, 11.00% and 14.66% respectively.

**Table 4.4.8: Percentage yield of aqueous extract selected plants**

S.No.	Name of the plants	Extraction Yields (%)
1.	<i>Coriandrum sativum</i> (Seeds)	8.25
2.	<i>Allium sativum</i> (Bulbs)	19.75
3.	<i>Carica papaya</i> (Seeds)	14.46
4.	<i>Zingiber officinale</i> (Rhizomes)	17.65
5.	<i>Azadirachta indica</i> (Leaves)	14.81
6.	<i>Swertia chirata</i> (Whole plants)	11.00
7.	<i>Prunus persica</i> (Leaves)	14.66

#### 4.5 *In vitro* anthelmintic efficacy of some selected medicinal plants

The aim of this study was to assess the *in vitro* anthelmintic efficacy of aqueous extracts of seeds of *Coriandrum sativum*, bulb of *Allium sativum*, seeds of *Carica papaya*, rhizome of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plant of *Swertia chirata* and leaves of *Prunus persica*. This evaluation was conducted utilizing the following test:

- Egg hatch assay (EHA)
- Larval paralysis test
- Adult worm mortality assay (AWMA)

The effect of the aqueous extracts of aforementioned medicinal plants on egg, larvae, as well as on adult parasite was thoroughly investigated.

##### 4.5.1 Effect of different concentration of various plant extracts and closantel on EHA

###### (Egg hatch assay) inhibition

The egg hatch assay was conducted to assess the potential of plant extracts and closantel in inhibiting egg hatching. The percentage reduction in egg hatch rate was calculated in comparison to the negative controls (PBS) for each plant extract and dilution. The effectiveness of the extracts in inhibiting egg hatch was measured by the percentage of eggs that remained unhatched at the end of observation period, exhibited concentration dependent

trend. The egg hatch assay results of aqueous extracts from various plants including *Coriandrum sativum* seeds, *Allium sativum* bulbs, *Carica papaya* seeds, *Zingiber officinale* rhizomes, *Azadirachta indica* leaves, and *Swertia chirata* whole plants and *Prunus persica* leaves against goats strongyles (Table 4.5.1.9 and Figure 4.5.1.10). All the tested aqueous extracts of plants exhibited ovicidal activity the strongyle eggs.

**Table 4.5.1.9: The Percentage inhibition in egg hatching on strongyle eggs at different concentration of aqueous extracts, positive control (closantel, 0.5 mg/ml) and negative control (PBS) at 48 hours post treatment**

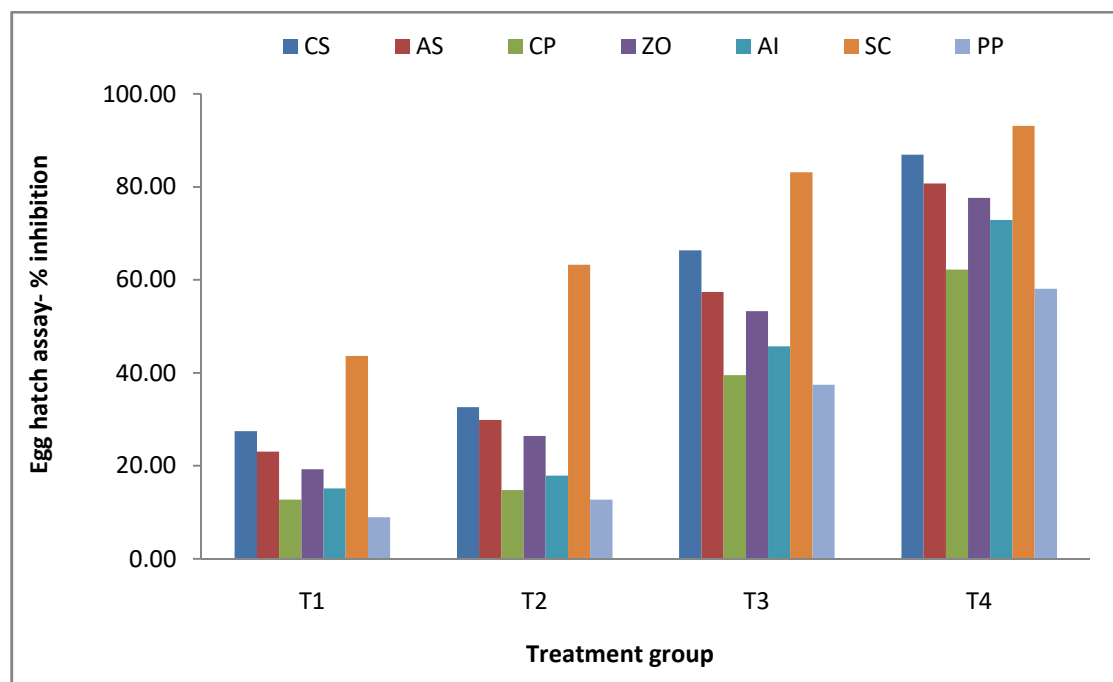
Group	CS	AS	CP	ZO	AI	SC	PP	NC	PC
T1	27.49	23.02	12.71	19.25	15.12	43.64	8.94	3.00	100.00
T2	32.65	29.90	14.77	26.46	17.87	63.23	12.71		
T3	66.32	57.39	39.51	53.26	45.70	83.16	37.45		
T4	86.94	80.75	62.20	77.66	72.85	93.12	58.07		

**Note:** NC, Negative control; PC, Positive control; T1, 12.5 mg/ml; T2, 25 mg/ml ; T3, 50 mg/ml; T4, 100 mg/ml, CS (*Coriandrum sativum* seeds), AS (*Allium sativum* bulbs), CP (*Carica papaya* seeds), ZO (*Zingiber officinale* rhizomes), AI (*Azadirachta indica* leaves), SC (*Swertia chirata* whole plants) and PP (*Prunus persica* leaves)

Closantel treated group at a concentration of 0.5 mg/ml demonstrated 100 % egg hatch inhibitions in strongyle eggs after 48 hours post treatments. Conversely, none of the selected medicinal plants aqueous extracts achieved 100 % inhibition of egg hatch in strongyle eggs after 48 hours post-treatment. In negative control group with PBS, only 3% strongyle eggs remained unhatched. The highest percent of egg hatch inhibition against strongyle eggs in goats was exhibited by aqueous extracts derived from the whole plant of *Swertia chirata* followed by Seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, seeds of *Carica papaya* and lowest was recorded in leaves of *Prunus persica*.

The aqueous extracts from the various plants @ 12.5 mg/ml, concentration demonstrated various level of inhibition against the hatching of strongyle eggs in goats. The inhibition percentage were as follows: *Coriandrum sativum* seeds showed 27.49 % inhibition, *Allium sativum* bulbs showed 23.02% inhibition, *Carica papaya* seeds showed 14.77% inhibition, *Zingiber officinale* rhizomes showed 26.46 % inhibition, *Azadirachta indica* leaves

showed 17.87% inhibition, *Swertia chirata* whole plants showed 63.23 % inhibition and *Prunus persica* leaves showed 12.71 % inhibition.



**Figure 4.5.1.10: Percent of egg hatch inhibition of aqueous extracts of CS (*Coriandrum sativum* seeds), AS (*Allium sativum* bulbs), CP (*Carica papaya* seeds), ZO (*Zingiber officinale* rhizomes), AI (*Azadirachta indica* leaves), SC (*Swertia chirata* whole plants) and PP (*Prunus persica* leaves) at the different concentrations (T1, 12.5 mg/ml; T2, 25 mg/ml ; T3, 50 mg/ml ; T4, 100 mg/ml) for 48 hours post treatment**

The aqueous extracts from the various plants @ 25 mg/ml, concentration demonstrated various level of inhibition against the hatching of strongyle eggs in goats. *Coriandrum sativum* seeds showed an inhibition rate 32.65%, *Allium sativum* bulbs showed an inhibition rate 29.90%, *Carica papaya* seeds showed an inhibition rate 14.77 %, *Zingiber officinale* rhizome showed an inhibition rate 26.46 %, *Azadirachta indica* leaves showed an inhibition rate 17.87 %, *Swertia chirata* whole plants showed an inhibition rate 63.23 % and *Prunus persica* leaves showed an inhibition rate 12.71 %.

The aqueous extracts from the various plants 50 mg/ml, concentration demonstrated various level of inhibition against the hatching of strongyle eggs in goats. *Coriandrum sativum* seeds exhibited an inhibition rate 66.32%, *Allium sativum* bulbs exhibited an inhibition rate 57.39%, *Carica papaya* seeds exhibited an inhibition rate 39.51%, *Zingiber*

*officinale* rhizomes exhibited an inhibition rate 53.26%, *Azadirachta indica* leaves exhibited an inhibition rate 45.70%, *Swertia chirata* whole plants exhibited an inhibition rate 83.16% and *Prunus persica* leaves exhibited an inhibition rate 37.45 %.

The aqueous extracts from the various plants 100 mg/ml, concentration demonstrated various level of inhibition against the hatching of strongyle eggs in goats. *Coriandrum sativum* seeds exhibited an 86.94% inhibition, *Allium sativum* bulbs exhibited an 80.75% inhibition, *Carica papaya* seeds exhibited a 62.20% inhibition, *Zingiber officinale* rhizomes exhibited a 77.66% inhibition, *Azadirachta indica* leaves exhibited a 72.85% inhibition, *Swertia chirata* whole plants exhibited a 93.12% inhibition and *Prunus persica* leaves exhibited a 58.07 % inhibition against the eggs of strongyle in goats.

#### **4.5.2. Effect of different concentration of various plants extracts and closantel on larval paralysis**

The larval paralysis test was performed to assess the efficacy of plant extracts and closantel in inhibition of larval motility against the strongyle larvae in goat. The percentage reduction in of larval motility rate was calculated in relative to the negative control (PBS) for each plant extract and dilution. The effectiveness of the extracts in inhibiting larval motility was expressed as percentage of inhibition. The inhibition exhibited a dose-dependent pattern with higher concentrations of plant extracts resulting in increased percentage larval motility inhibition.

The aqueous extracts from seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica* along with closantel were assessed for their effect on inhibiting the motility of strongyle larvae in goats after 24 hours of treatment (Table 4.5.2.10 & Figure 4.5.2.11). All the tested plant aqueous extracts exhibited inhibitory activity against larval motility.

In closantel treated group at concentration @ 0.5 mg/ml concentration exhibited 100% of inhibition of larval motility in strongyle larvae after 24 hours post treatments. None of the selected aqueous extracts of medicinal plants exhibited 100% inhibition of larval motility in strongyle larvae after 24 hours post-treatment. In negative control group with PBS, exhibit inhibition of larval motility 4% in strongyle larvae.

The highest inhibition of larval motility of was observed in aqueous extract of whole plants of *Swertia chirata* followed by the seeds of *Coriandrum sativum*, the bulbs of *Allium sativum*, the rhizomes of *Zingiber officinale*, the leaves of *Azadirachta indica*, the seeds of *Carica papaya* and lowest inhibition was found in the leaves of *Prunus persica* against the strongyle larvae in goats.

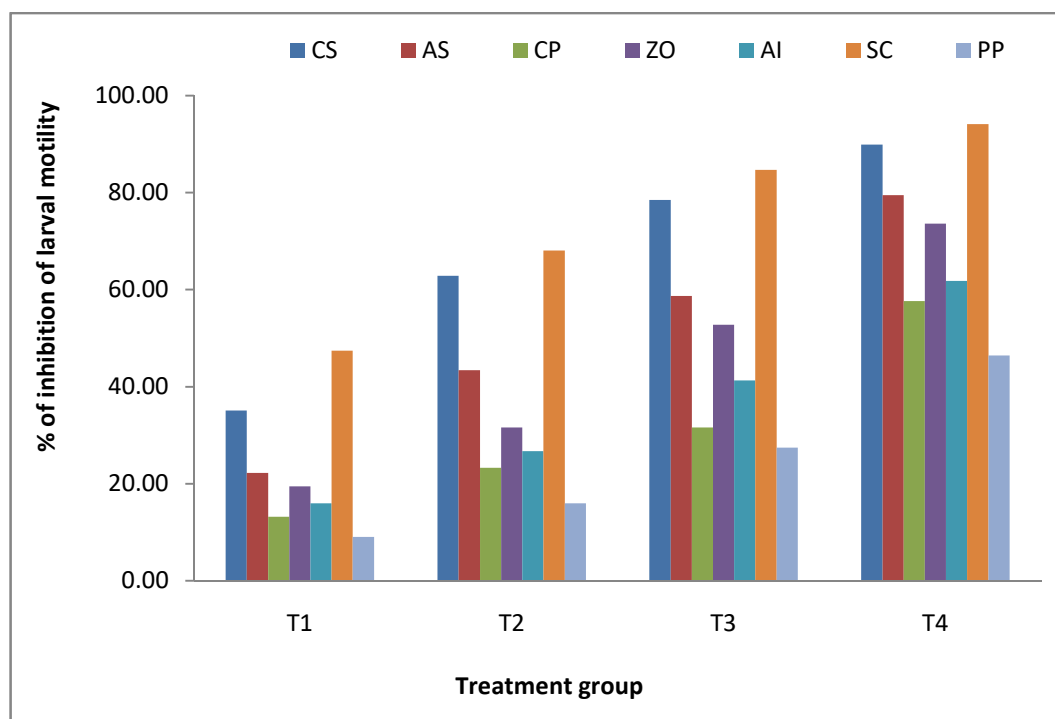
**Table 4.5.2.10: The Percentage (%) inhibition in larval motility at different concentration of aqueous extracts, positive control (closantel, 0.5mg/ml) and negative control (PBS) at 24 hours post treatment**

Group	CS	AS	CP	ZO	AI	SC	PP	NC	PC
T1	35.07	22.23	13.19	19.44	15.98	47.40	9.03	4	100
T2	62.85	43.40	23.27	31.60	26.73	68.06	15.98		
T3	78.48	58.68	31.60	52.78	41.32	84.71	27.43		
T4	89.93	79.48	57.64	73.61	61.81	94.10	46.47		

**Note:** NC, Negative control; PC, Positive control; T1, 12.5 mg/ml; T2, 25 mg/ml ; T3, 50 mg/ml; T4, 100 mg/ml, CS (*Coriandrum sativum* seeds), AS (*Allium sativum* bulbs), CP (*Carica papaya* seeds), ZO (*Zingiber officinale* rhizome), AI (*Azadirachta indica* leaves), SC (*Swertia chirata* whole plants) and PP (*Prunus persica* leaves)

The various plants extract @12.5 mg/ml, concentration exhibited different levels of inhibition against the strongyle larvae of the goat. *Coriandrum sativum* seeds showed 35.07 % inhibition of larval motility, *Allium sativum* bulbs showed 22.23 % inhibition of larval motility, *Carica papaya* seeds showed 13.19 % inhibition of larval motility, *Zingiber officinale* rhizomes exhibited 19.44% inhibition of larval motility, *Azadirachta indica* leaves exhibited 15.98%, *Swertia chirata* whole plants exhibited 47.40% inhibition of larval motility and *Prunus persica* leaves exhibited 9.03% inhibition of larval motility.

The various plants extract @ 25 mg/ml, concentration derived from *Coriandrum sativum* seeds exhibited 62.85% inhibition of larval motility, *Allium sativum* bulbs exhibited 43.40% inhibition of larval motility, *Carica papaya* seeds exhibited 23.27% inhibition of larval motility, *Zingiber officinale* rhizomes exhibited 31.60% inhibition of larval motility, *Azadirachta indica* leaves exhibited 26.73% inhibition of larval motility, *Swertia chirata* whole plants exhibited 68.06% inhibition of larval motility and *Prunus persica* leaves exhibited 15.98% inhibition of larval motility against the strongyle larvae of the goat.



**Figure 4.5.2.11: The inhibition percentage of larval motility of aqueous extracts of CS (*Coriandrum sativum* seeds), AS (*Allium sativum* bulbs), CP (*Carica papaya* seeds), ZO (*Zingiber officinale* rhizomes), AI (*Azadirachta indica* leaves), SC (*Swertia chirata* whole plants) and PP (*Prunus persica* leaves) at the different concentrations (T1, 12.5 mg/ml; T2, 25 mg/ml ; T3, 50 mg/ml ; T4, 100 mg/ml) for 24 hours post treatment**

At a concentration of 50 mg/ml various plant extracts exhibited different levels of inhibition of larval motility derived from *Coriandrum sativum* seeds exhibited 78.48% inhibition of larval motility, *Allium sativum* bulbs exhibited 58.68% inhibition of larval motility, *Carica papaya* seeds exhibited 31.60% inhibition of larval motility, *Zingiber officinale* rhizomes exhibited 52.78% inhibition of larval motility, *Azadirachta indica* leaves exhibited 41.32%, *Swertia chirata* whole plants exhibited 84.71% inhibition of larval motility and *Prunus persica* leaves exhibited 27.43 % inhibition of larval motility against the strongyle larvae of the goat.

At a concentration of 100 mg/ml of various plant extracts exhibited different levels of inhibition of larval motility derived from *Coriandrum sativum* seeds exhibited 89.93% inhibition of larval motility, *Allium sativum* bulbs exhibited 79.48% inhibition of larval motility, *Carica papaya* seeds exhibited 57.64% inhibition of larval motility, *Zingiber officinale* rhizomes exhibited 73.61% inhibition of larval motility, *Azadirachta indica* leaves

exhibited 61.81%, *Swertia chirata* whole plant exhibited 94.10% inhibition of larval motility and *Prunus persica* leaves exhibited 46.47 % inhibition of larval motility against the strongyle larvae of the goat.

#### **4.5.3 Effect of different concentration of various plants extracts and closantel on adult worm mortality assay (AWMA)**

The aqueous extract derived from the seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica* exhibit promising adulticidal effects on adult *Haemonchus contortus*. The highest adulticidal effects were observed with aqueous extracts from the whole plants of *Swertia chirata* followed by seeds of *Coriandrum sativum*, leaves of *Azadirachta indica*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale* and lowest in leaves of *Prunus persica* against the *Haemonchus contortus* in goats (Table 4.5.3.11).

The adulticidal efficacy of the extracts was assessed by measuring the percentage of adult parasites killed at the different time intervals and concentrations. Dose and time dependent anthelmintic activity responses were observed with across all concentrations of seven selected plant extracts. Complete mortality of worms was observed 8 hours post exposures to the 400 mg/ml concentration of aqueous extracts of *Swertia chirata*. In closantel treated group, all worms were found dead after 2 hours of post exposure. However, no worms were found dead in any of selected medicinal plants aqueous extracts groups after 2 hours of post exposure. In the negative control group, all worms remained alive during the first 8 hours of the test. Furthermore, none of the worms showed any signs of revival of motility after being placed in lukewarm PBS for 30 minutes.

**Table 4.5.3.11: Percentage of mortality of adult *Haemonchus contortus* at different concentrations of aqueous extract, positive control (Closantel, 1.25 mg/ml) and negative control (PBS) for 8 hours post treatments**

Treatment	Time (hr)	Treatment Group					
		NC	PC	T1	T2	T3	T4
<i>Coriandrum sativum</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	6.67	10.00
	4hr	0.00	100.00	16.67	23.33	50.00	56.67
	6hr	0.00	100.00	23.33	33.33	66.67	76.67
	8hr	0.00	100.00	26.67	43.33	76.67	90.00
	Mean± SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>13.33±5.68</b>	<b>20.00±8.76</b>	<b>40.00±15.60</b>	<b>46.67±17.89</b>
<i>Allium sativum</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	3.33	3.33
	4hr	0.00	100.00	6.67	16.67	30.00	50.00
	6hr	0.00	100.00	13.33	26.67	50.00	53.33
	8hr	0.00	100.00	20.00	30.00	60.00	66.67
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>8.00±3.89</b>	<b>14.67±6.38</b>	<b>28.67±12.05</b>	<b>34.67±13.77</b>
<i>Carica papaya</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	3.33	13.33	26.67	46.67
	6hr	0.00	100.00	10.00	20.00	36.67	43.33
	8hr	0.00	100.00	16.67	26.67	46.67	53.33
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>6.00±3.23</b>	<b>12.00±5.33</b>	<b>22.00±9.52</b>	<b>28.67±11.81</b>
<i>Zingiber officinale</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	0.00	10.00	20.00	33.33
	6hr	0.00	100.00	6.67	16.67	26.67	33.33
	8hr	0.00	100.00	13.33	23.33	36.67	43.33
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>4.00±2.67</b>	<b>10.00±4.59</b>	<b>16.67±7.30</b>	<b>22.00±9.17</b>
<i>Azadirachta indica</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	3.33	3.33
	4hr	0.00	100.00	10.00	20.00	40.00	46.67
	6hr	0.00	100.00	16.67	30.00	56.67	63.33
	8hr	0.00	100.00	23.33	33.33	66.67	76.67
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>10.00±4.59</b>	<b>16.67±7.15</b>	<b>33.33±13.62</b>	<b>38.00±15.58</b>
<i>Swertia chirata</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	10.00	13.33
	4hr	0.00	100.00	20.00	33.33	53.33	63.33
	6hr	0.00	100.00	26.67	43.33	76.67	86.67
	8hr	0.00	100.00	36.67	53.33	86.67	100.00
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>16.67±7.30</b>	<b>26.0±11.08</b>	<b>45.33±17.40</b>	<b>52.67±19.79</b>
<i>Prunus persica</i>	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	0.00	6.67	16.67	26.67
	6hr	0.00	100.00	3.33	13.33	20.00	23.33
	8hr	0.00	100.00	10.00	20.00	30.00	36.67
	Mean ±SE	<b>0.00±0.00</b>	<b>80.00±20.00</b>	<b>2.67±1.94</b>	<b>8.00±3.89</b>	<b>13.33±5.87</b>	<b>17.33±4.41</b>

**Note:** NC, Negative control; PC, Positive control; T1, 50 mg/ml; T2, 100 mg/ml; T3, 200 mg/ml; T4, 400 mg/ml

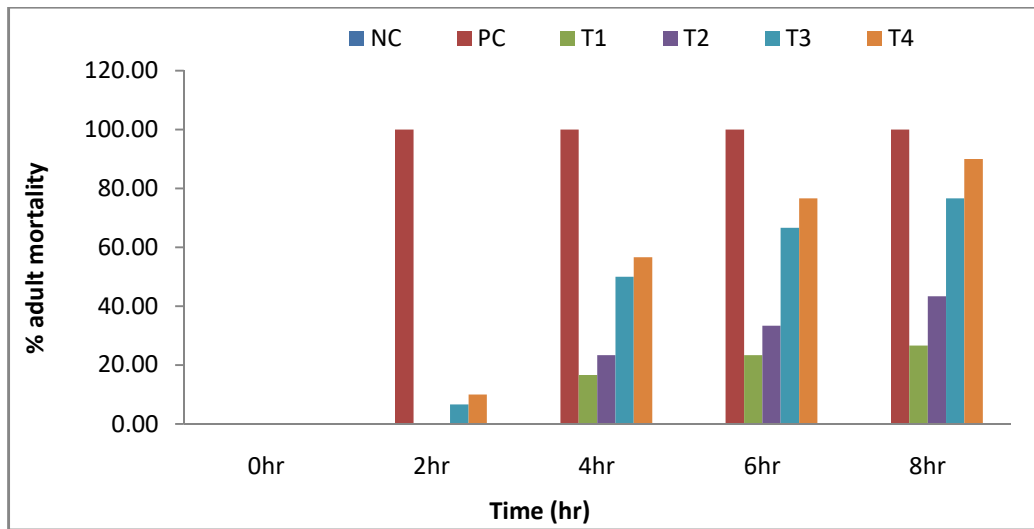


Figure 4.5.3.12: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of seeds of *Coriandrum sativum*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

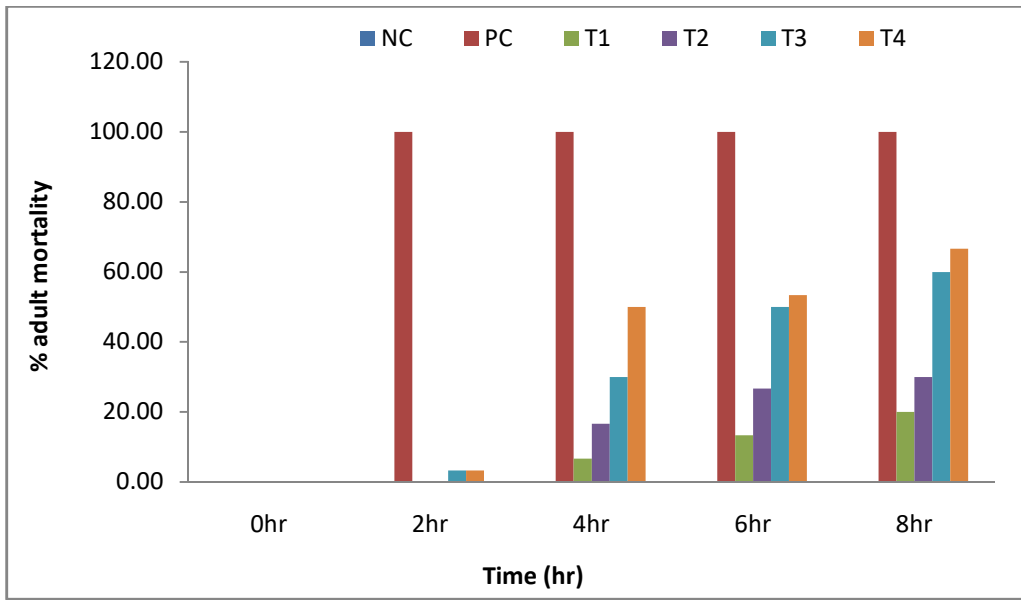
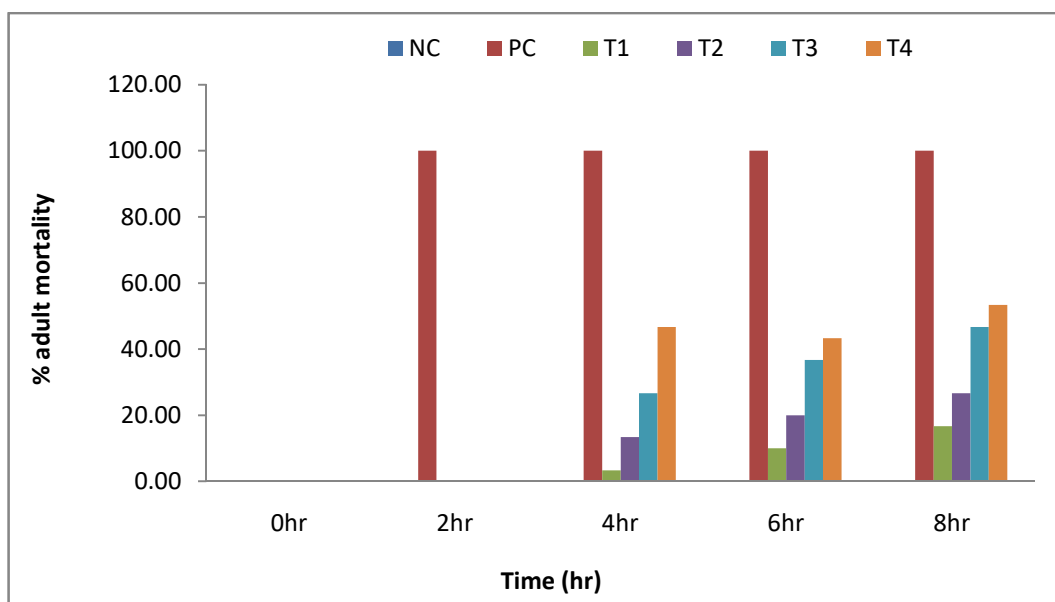
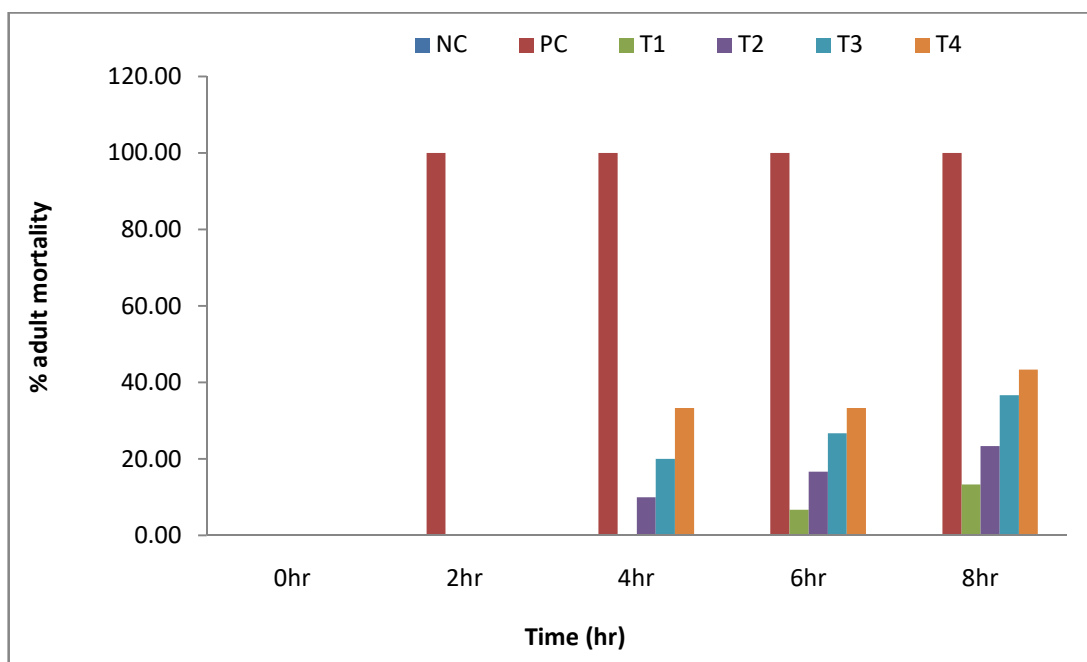


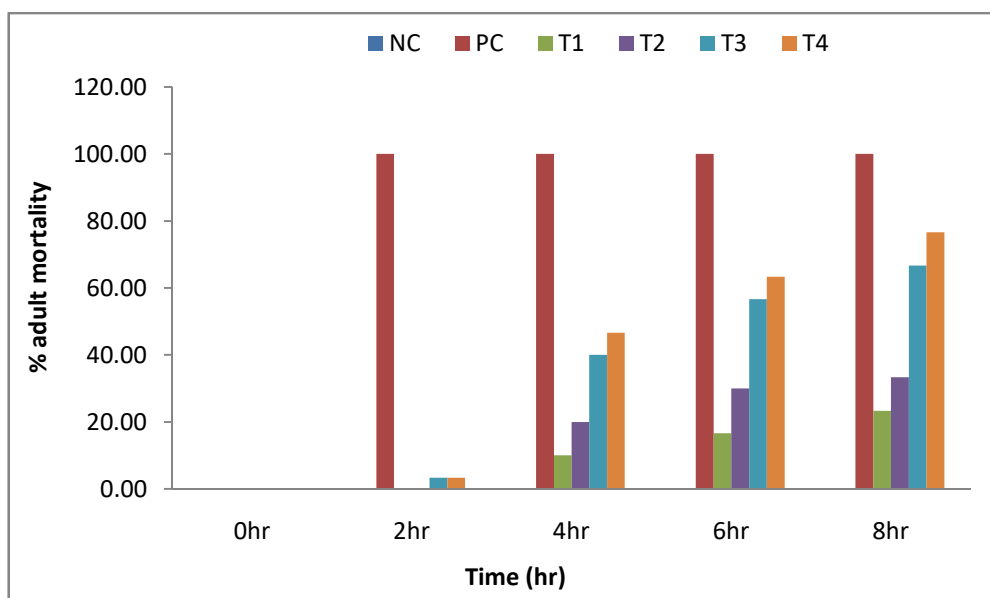
Figure 4.5.3.13: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of bulbs of *Allium sativum*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)



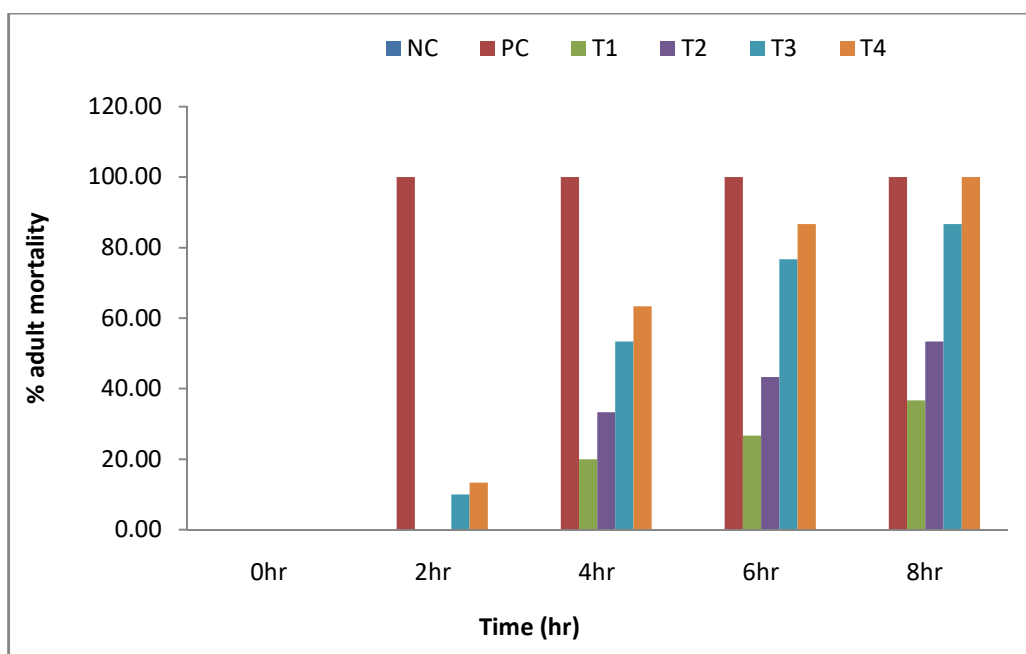
**Figure 4.5.3.14: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of seeds of *Carica papaya*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)**



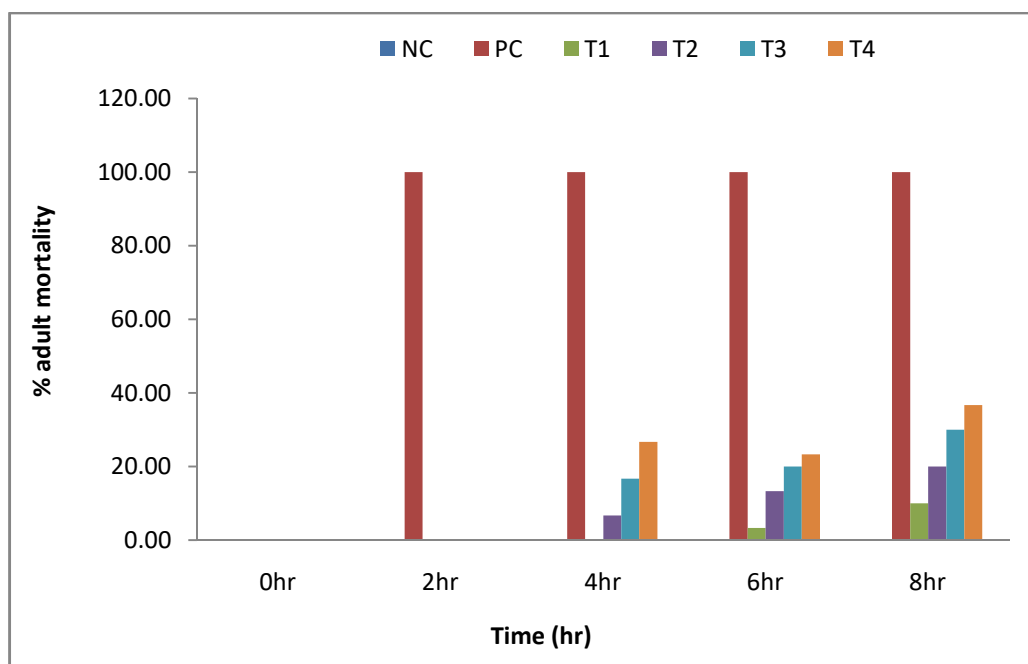
**Figure 4.5.3.15: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of rhizomes of *Zingiber officinale*, closantel, 1.25mg/ml (PC) and PBS, negative control (NC)**



**Figure 4.5.3.16: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of leaves of *Azadirachta indica*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)**



**Figure 4.5.3.17: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T1), 100 mg/ml (T2), 200 mg/ml (T3) and 400 mg/ml (T4) of aqueous extract of whole plants of *Swertia chirata*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)**



**Figure 4.5.3.18: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50mg/ml (T1), 100mg/ml (T2), 200mg/ml (T3) and 400mg/ml (T4) of aqueous extract of leaves of *Prunus persica*, closantel, 1.25mg/ml (PC) and PBS, negative control (NC)**

The aqueous extracts of *Coriandrum sativum* seeds at concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml exhibited varying percentage of mortality in *Haemonchus contortus*. After 2 hours post exposure the mortality rates were 0%, 0%, 6.67% and 10% respectively. After 4 hours, the rates increased to 16.67%, 23.33%, 50% and 56.67%. Subsequently, after 6 hours, the rates rose further to 23.33%, 33.33%, 66.67% and 76.67%, and after 8 hours, they reached 26.67%, 43.33%, 76.67% and 90% respectively (Figure 4.5.3.12).

The aqueous extracts of *Allium sativum* bulbs at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml exhibited varying percentage in mortality of *Haemonchus contortus*. After 2 hours post-exposure, the mortality rates were 0%, 0%, 3.33% and 3.33%, respectively. After 4 hours post-exposure, the rates increased to 6.67%, 16.67%, 30% and 50.00%. Subsequently, after 6 hours post-exposure, the rates further escalated to 13.33%, 26.67%, 50% and 53.33%. Finally, after 8 hours of exposure, the mortality rates were 20.0%, 43.33%, 30% and 66.67% respectively (Figure 4.5.3.13).

The aqueous extracts of *Carica papaya* seeds at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml exhibited varying percentage of mortality of *Haemonchus contortus*. After 2 hours post-exposure, the mortality rates were 0% in all the four concentrations. After 4 hours post-exposure, the rates increased to 3.33%, 13.33%, 26.67% and 46.67%. Subsequently, after 6 hours post-exposure, the rates further escalated to 10%, 20%, 36.67% and 43.33%. Finally, after 8 hours of exposure, the mortality rates were 16.67%, 26.67, 46.67% and 53.33% respectively (Figure 4.5.3.14)

The aqueous extracts of *Zingiber officinale* rhizomes at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml showed 0% mortality of *Haemonchus contortus* after 2 hours of exposure. After 4 hours, the mortalities were 0%, 10%, 20% and 33.33% respectively. This increased to 6.67%, 16.67%, 26.67%, and 33.33% after 6 hours, and further to 13.33%, 23.33%, 36.67% and 43.33% after 8 hours of exposure (Figure 4.5.3.15).

Aqueous extracts of *Azadirachta indica* leaves at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml, showed varying percentages of mortality in *Haemonchus contortus*. After 2 hours of exposure, the mortality rates were 0%, 0%, 3.33% and 3.33% respectively. After 4 hours, the rates increased to 10%, 20%, 40% and 46.67% respectively. Subsequently, after 6 hours, the rates enhanced to 16.67%, 30%, 56.67% and 63.33% and after 8 hours, they further increased to 23.33%, 33.33%, 67.67% and 76.67% respectively (Figure 4.5.3.16).

The aqueous extracts derived from whole plant of *Swertia chirata* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml varying percentages of mortality in *Haemonchus contortus*. After 2 hours of exposure, the mortality rates were 0%, 0%, 10% and 13.33% respectively. After 4 hours, these percentages increased to 20%, 33.3%, 53.33% and 63.33%, respectively. Subsequently, after 6 hours, the mortality rates enhanced to 26.67%, 43.33%, 76.67% and 86.67% and finally reached 36.67%, 53.33%, 86.67%, and 100% after 8 hours of exposure (Figure 4.5.3.17).

Aqueous extracts from *Prunus persica* leaves, at concentrations of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml showed varying degrees of effectiveness against *Haemonchus contortus* over different exposure durations. After 2 hours post exposures all

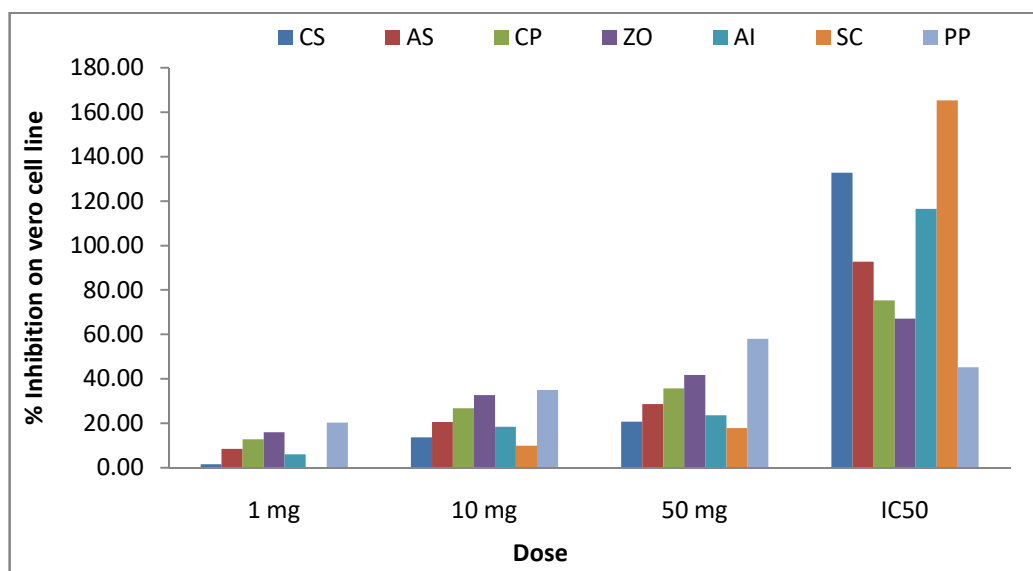
concentrated exhibited 0% mortality. However, after 4 hours post exposure the mortalities were 0%, 0%, 6.67%, 16.67% and 26.67% respectively. This trend continued with increased exposure time: at 6 hours, mortalities were 3.33%, 13.33%, 20% and 23.33%, at 8 hours they further increased to 10%, 20, 30% and 36.67% respectively (Figure 4.5.3.18).

#### 4.6 *In vitro* cytotoxicity study of medicinal plants

The cytotoxicity of selected medicinal plants extracts were evaluated *in vitro* with result presented in Table 4.6.12 & Figure 4.6.19.

**Table 4.6.12: *In vitro* cytotoxicity evaluation of aqueous extract of seven medicinal plants in vero cell line**

S. No.	Name of the plants	1mg	10mg	50 mg	IC <sub>50</sub> (mg/ml)
1.	<i>Coriandrum sativum</i> (Seeds)	1.62	13.66	20.76	132.78
2.	<i>Allium sativum</i> (Bulbs)	8.49	20.59	28.66	92.72
3.	<i>Carica papaya</i> (Seeds)	12.85	26.73	35.74	75.36
4.	<i>Zingiber officinale</i> (Rhizomes)	16.00	32.71	41.74	67.12
5.	<i>Azadirachta indica</i> (Leaves)	5.98	18.39	23.62	116.64
6.	<i>Swertia chirata</i> (Whole plants)	0.01	9.97	17.81	165.32
7.	<i>Prunus persica</i> (Leaves)	20.32	34.93	58.10	45.22



**Figure 4.6.19: Percentage (%) of inhibition on vero cell line**

The aqueous extract of leaves of *Prunus persica* exhibited the highest percentage of cell growth inhibition, followed by rhizomes of *Zingiber officinale*, seeds of *Carica papaya*,

bulbs of *Allium sativum*, leaves of *Azadirachta indica* and seeds of *Coriandrum sativum*. The lowest percentage of cell growth inhibition was observed in the aqueous extract of whole plants of *Swertia chirata*. In vero cell line, the aqueous extract of leaves of *Prunus persica* exhibited varying degrees of cell growth inhibition at concentration of 1 mg/ml, 10 mg/ml and 50 mg/ml resulting in inhibition rates of 20.32%, 34.93% and 58.10 % respectively. The half maximal inhibitory concentration (IC<sub>50</sub>) of aqueous extract of leaves of *Prunus persica* was recorded 45.22 mg/ml.

The aqueous extract of rhizomes of *Zingiber officinale* exhibited cell growth inhibition of 16.00%, 32.71% and 41.74% at concentration of 1 mg/ml, 10 mg/ml and 50 mg/ml respectively in vero cell line. The IC<sub>50</sub> value for aqueous extract of rhizome of *Zingiber officinale* was recorded 67.12 mg/ml. The aqueous extract of seeds of *Carica papaya* showed inhibition of cell growth in vero cell line by 12.85%, 26.73% and 35.74% at concentration of 1 mg/ml, 10 mg/ml and 50 mg/ml, respectively. The IC<sub>50</sub> values for the aqueous extract of seeds of *Carica papaya* was determined to be 75.36 mg/ml. The aqueous extract of bulbs of *Allium sativum* exhibited cell growth inhibition of 8.49%, 20.59% and 28.66% at concentration of 1 mg/ml, 10 mg/ml and 50 mg/ml respectively. The IC<sub>50</sub> value for the aqueous extract of bulbs of *Allium sativum* was determined to be 92.72 mg/ml.

The aqueous extract of leaves of *Azadirachta indica* exhibited cell growth inhibition rates of 5.98% 18.39% and 23.62%, at the concentration of 1 mg/ml, 10 mg/ml and 50 mg/ml respectively, in vero cell line. The IC<sub>50</sub> value of the aqueous extract of leaves of *Azadirachta indica* was recorded 116.46 mg/ml. The aqueous extract of seeds of *Coriandrum sativum* demonstrated inhibition of cell growth in the vero cell line of at the concentration of 1mg/ml, 10 mg/ml and 50 mg/ml with 1.62% 13.66% and 20.76%, respectively. The IC<sub>50</sub> value for the aqueous extract of seeds of *Coriandrum sativum* was recorded 132.78 mg/ml. The aqueous extract of whole plants of *Swertia chirata* demonstrated inhibition of cell growth in the vero cell line at the concentration of 1mg/ml, 10 mg/ml and 50 mg/ml resulting inhibition rates of 0.01%, 9.97% and 17.81%, respectively. The IC<sub>50</sub> of the aqueous extract from whole plant of *Swertia chirata* was determined to be 165.32 mg/ml.

#### **4.7 *In vivo* anthelmintic efficacy of aqueous extracts of *Coriandrum sativum* and *Swertia chirata***

To explore the anthelmintic potential of aqueous extracts from various plant sources including seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica* several *in vitro* tests were conducted. These tests included egg hatch assay, larval paralysis test and adult worm mortality assay. Additionally, cytotoxicity tests were performed on the aforementioned medicinal plants aqueous extract. Based on the results of *in vitro* tests and cytotoxicity evaluations, the aqueous extracts from *Swertia chirata* and *Coriandrum sativum* demonstrated high efficacy and comparatively low toxicity as phyto-anthelmintic in this study. Consequently, the aqueous extract of whole plants of *Swertia chirata* and aqueous extracts of seeds of *Coriandrum sativum* were selected for further *in vivo* study against the naturally strongyle infected Barbari breed of goat at the Instructional Livestock Farm Complex-II, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110.

The mean EPG values of different groups on day 0 (pre-treatment) and 7, 14, 21 and 28 post-treatment with aqueous extracts of seeds of *Coriandrum sativum* are presented in Table 4.7.13.

In control group (C), which did not receive any treatment, the egg per gram (EPG) values in faeces showed an increase from 7 day to day 28 post-treatment compared to day 0 (pre-treatment). The positive control (Group T1) treated with closantel @ 10 mg/kg body weight and exhibited the highest reduction in EPG counts on day 14 post-treatment which was sustained till day 28 post-treatment. Group T2, treated with aqueous extract of *Coriandrum sativum* seeds, @ 50 mg/kg, showed a decrease in EPG values on day 7 post-treatment compared to day 0 (pre-treatment). However, from day 14 to day 28 post-treatment, a gradual increase in EPG values was observed. The group T3, treated with aqueous extract of *Coriandrum sativum* seeds, @ 250 mg/kg, exhibited decreased EPG values on days 7,14 and 21 post-treatment compared to day 0 (pre-treatment). However, there was an increase in EPG values observed on the day 21 post-treatment.

**Table 4.7.13: Effect on egg per gram (EPG) in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post -treatment**

Days	C	T1	T2	T3	T4
0	1083.33±16.67	1300.00±73.60	1862.50±238.11	1695.83±107.90	1983.33±178.96
7	1108.33±16.67	62.50±10.70	1770.83±227.98	1541.67±98.04	1691.67±152.02
14	1133.33±16.67	0.00±0.00	1900.00±250.04	1345.83±85.49	1437.50±125.95
21	1175.00±19.36	0.00±0.00	1925.00±242.98	1554.17±98.41	1300.00±112.18
28	1233.33±16.67	0.00±0.00	2104.17±266.73	1812.50±117.57	1191.67±106.98
<b>Mean± SE</b>	<b>1146.67<sup>b</sup>±17.21</b>	<b>272.50<sup>a</sup>±16.86</b>	<b>1912.50<sup>d</sup>±245.17</b>	<b>1590.00<sup>c</sup>±101.48</b>	<b>1520.83<sup>c</sup>±135.22</b>

**Note:** C, control (not received treatment); T1, Positive control Closantel @ 10 mg/kg body weight; T2, *Coriandrum sativum* @ 50mg/kg body weight; T3, *Coriandrum sativum* @ 250mg/kg body weight; T4, *Coriandrum sativum* @ 500 mg/kg body weight

<sup>a, b, c, d</sup>Mean bearing different superscripts in a row showed a statistical difference at P<0.001

The group T4, treated with aqueous extract of *Coriandrum sativum* seeds, @ 500 mg/kg, showed gradual reduction in EPG values on the days 7, 14, 21 and 28 posts -treatment. On the 28<sup>th</sup> day post- treatment, the maximum reduction in EPG of faeces was observed in goat treated with closantel @ 10 mg/kg body weight (group T1), followed by those treated with aqueous extract of *Coriandrum sativum* seeds @ 500 mg/kg body weight (group T4) and then with aqueous extract of *Coriandrum sativum* seeds @ 250 mg/kg body weight. The least reduction was observed in group T2, treated with aqueous extract of *Coriandrum sativum* seeds @ 50 mg/kg body weight.

The mean percent faecal egg count reduction (FECR) in goats treated with aqueous extracts of seeds of *Coriandrum sativum* on days 7, 14, 21 and 28 post-treatment are presented in Table 4.7.14.

The positive control (Group T1), treated with closantel @ 10 mg/kg body weight achieved the maximum faecal egg count reduction (100±0.00) on day 14 post-treatment which was sustained till day 28 post-treatment. The group T2, treated with aqueous extract of *Coriandrum sativum* seeds @ 50 mg/kg, showed FECR percentage of 4.98±0.20,-2.00±0.16,-3.45±0.20 and -13.04± 0.36 on days 7,14,21 and 28 days post-treatment, respectively.

**Table 4.7.14: The effect on percent faecal egg count reduction (FECR) in goats treated with aqueous extracts of seeds of *Coriandrum sativum* and closantel in different groups at different days of pre and post-treatment**

Days	T1	T2	T3	T4
7	95.29±0.66	4.98±0.20	9.07±0.49	14.70±0.38
14	100.00±0.00	-2.00±0.16	20.63±0.31	27.46±0.33
21	100.00±0.00	-3.45±0.20	8.34±0.24	34.36±0.30
28	100.00±0.00	-13.04±0.36	-6.85±0.28	40.38±0.14
<b>Mean± SE</b>	<b>98.82<sup>d</sup>±0.16</b>	<b>-3.38<sup>a</sup>±0.23</b>	<b>7.80±0.33</b>	<b>29.23<sup>c</sup>±0.29</b>

T1, Positive control Closantel @ 10 mg/kg body weight; T2, *Coriandrum sativum* @ 50mg/kg body weight; T3, *Coriandrum sativum* @ 250mg/kg body weight; T4, *Coriandrum sativum* @ 500 mg/kg body weight

<sup>a, b, c, d</sup>Mean bearing different superscripts in a row showed a statistical difference at P<0.001

The group T3, treated with aqueous extract of *Coriandrum sativum* seeds @ 250 mg/kg, exhibited FECR percentage of 14.56±0.49, 20.63± 0.31, and 8.34±0.24 on days 7, 14, and 21 post-treatments respectively. However, FECR percentage of -6.85± 0.28 was observed on the day 28 post- treatment. The group T4, treated with aqueous extract of *Coriandrum sativum* seeds @ 500 mg/kg, showed FECR percentage of 14.70±0.38, 27.46±0.33, 34.36±0.30 and 40.38±0.14 on days 7, 14, 21 and 28 posts-treatments, respectively. On the 28<sup>th</sup> day post-treatment, the maximum FECR reduction of 100.00±0.00 was recorded in goat treated with closantel @ 10 mg/kg body weight, followed by those treated with aqueous extract of *Coriandrum sativum* seeds @ 500 mg/kg body weight (40.38±0.14). Aqueous extract of *Coriandrum sativum* seeds, @ 250 mg/kg body weight showed a negative FECR - 6.85±0.28. The least FECR was observed in goats treated with aqueous extract of *Coriandrum sativum* seeds @ 50 mg/kg body weight with a FECR of -13.04±0.36.

The mean EPG (egg per gram) of different groups on day 0 (pre-treatment) and 7, 14, 21, and 28 post-treatment with aqueous extracts of whole plant of *Swertia chirata* are presented in Table 4.7.15.

**Table 4.7.15: The effect on egg per gram (EPG) in goats treated with aqueous extracts of whole plants of *Swertia chirata* and closantel in different groups at different days of pre and post-treatment**

Days	C	T1	T2	T3	T4
0	1083.33±16.67	1300.00±73.60	1866.67±93.69	2033.33±198.08	1912.50±108.16
7	1108.33±16.67	62.50±10.70	1641.67±85.07	1745.83±171.32	1529.17±89.77
14	1133.33±16.67	0.00±0.00	1754.17±89.31	1554.17±150.06	1225.00±69.82
21	1175.00±19.36	0.00±0.00	1958.33±98.46	1462.50±143.72	929.17±51.00
28	1233.33±16.67	0.00±0.00	2075.00±103.28	1679.17±167.51	354.17±19.81
<b>Mean± SE</b>	<b>1146.67<sup>b</sup>±17.21</b>	<b>272.50<sup>a</sup>±16.86</b>	<b>1859.17<sup>d</sup>±93.96</b>	<b>1695.00<sup>c</sup>±166.14</b>	<b>1190.00<sup>b</sup>±67.71</b>

**Note:** C, Control (not received treatment); T1, Positive control Closantel @ 10 mg/kg body weight; T2, *Swertia chirata* @ 50mg/kg body weight; T3, *Swertia chirata* @ 250mg/kg body weight; T4, *Swertia chirata* @ 500mg/kg body weight

<sup>a, b, c, d</sup>Mean bearing different superscripts in a row showed a statistical difference at P<0.001

The control group (C), did not received any treatment, showed an increase in the egg per gram (EPG) values in faeces from day 7 to day 28 post-treatment to compared day 0 (pre-treatment). The positive control (Group T1), treated with closantel @ 10 mg/kg body weight, and showed the highest reduction in EPG on day 14 post-treatment, which was sustained through day 28 post-treatment. The group T2, treated with aqueous extract of whole plants of *Swertia chirata* @ 50 mg/kg body weight showed a decrease in EPG values on day 7 post-treatment compared to day 0 (pre-treatment). However, from day 14 to day 28 post-treatment, a gradual increase in EPG values was observed. The group T3, treated with aqueous extract of whole plants of *Swertia chirata* @ 250 mg/kg body weight exhibited decreased EPG values on days 7, 14 and 21 post-treatment, compared to day 0 (pre-treatment). However, there was a subsequent increase in EPG values observed on day 28 post-treatment. The group T4, treated with aqueous extract of whole plants of *Swertia chirata* @ 500 mg/kg body weight showed a gradual reduction in EPG values on the days 7, 14, 21 and 28 post-treatments. On the 28<sup>th</sup> day post-treatment, the maximum reduction in egg per gram (EPG) of faeces was recorded in goats treated with closantel @ 10 mg/kg body weight (group T1), followed by those treated with aqueous extract of whole plants of *Swertia chirata* @ 500 mg/kg body weight (group T4) and then @ 250 mg/kg body weight (group T3). The least reduction was observed in

group T2, treated with aqueous extract of whole plant of *Swertia chirata* @ 50 mg/kg body weight.

The mean percent faecal egg count reduction (FECR) in goats treated with aqueous extracts of whole plants of *Swertia chirata* on days 7, 14, 21, and 28 post-treatment are presented in Table 4.7.16.

**Table 4.7.16: The effect on percent faecal egg count reduction (FECR) in goats treated with aqueous extracts of whole plants of *Swertia chirata* and closantel in different groups at different days of pre and post treatment**

Days	T1	T2	T3	T4
7	95.29±0.66	12.10±0.23	14.16±0.24	20.08±0.50
14	100.00±0.00	6.04±0.20	23.53±0.28	35.96±0.18
21	100.00±0.00	-4.91±0.12	28.07±0.30	51.39±0.44
28	100.00±0.00	-10.97±0.22	17.51±0.36	60.65±0.44
<b>Mean± SE</b>	<b>98.82<sup>d</sup>±0.16</b>	<b>0.57<sup>a</sup>±0.19</b>	<b>20.82<sup>b</sup>±0.29</b>	<b>42.02<sup>c</sup>±0.39</b>

**Note:** T1, Positive control Closantel @ 10 mg/kg body weight; T2, *Swertia chirata* @ 50mg/kg body weight; T3, *Swertia chirata* @ 250mg/kg body weight; T4, *Swertia chirata* @ 500 mg/kg body weight

<sup>a, b, c, d</sup>Mean bearing different superscripts in a row showed a statistical difference at P<0.001

In the positive control (Group T1), treated with closantel @ 10 mg/kg body weight, achieved a maximum faecal egg count reduction of (100.00±0.00) on day 14 post-treatment which was maintained through day 28 post-treatment. The group T2, treated with aqueous extract of the whole plant of *Swertia chirata* @ 50 mg/kg body weight exhibited FECR percentage of 12.10±0.23, 6.04±0.20, -4.91± 0.12 and -10.97± 0.22 on days 7, 14, 21 and 28 days post-treatment, respectively. The group T3, treated with aqueous extract of the whole plants of *Swertia chirata* @ 250 mg/kg body weight, exhibited FECR percentage of 14.16±0.24, 23.53±0.28, 28.07±0.30 and 17.51±0.34 on days 7, 14, 21 and 28 days post-treatment, respectively. The group T4, treated with aqueous extract of the whole plant of *Swertia chirata* @ 500 mg/kg body weight, exhibited FECR percentage of 20.08±0.50, 35.96±0.18, 51.39±0.44 and 60.65± 0.44 on the days 7, 14, 21 and 28 post-treatments respectively.

On the 28<sup>th</sup> day post-treatment, the maximum FECR reduction of  $100.00 \pm 0.00$  was recorded in goats treated with closantel @ 10 mg/kg body weight, followed by those treated with aqueous extract of the whole plants of *Swertia chirata* @ 500 mg/kg body weight ( $60.65 \pm 0.44$ ). Aqueous extract of the whole plants of *Swertia chirata* @ 250 mg/kg body weight showed a FECR of  $17.51 \pm 0.36$ , while the least FECR was observed in goats treated with aqueous extract of the whole plants of *Swertia chirata* @ 50 mg/kg body weight, with a FECR of  $-10.97 \pm 0.22$ .

#### **4.8 Haematological alteration in goats treated with aqueous extracts of *Coriandrum sativum* seeds, whole plants of *Swertia chirata* and closantel**

Haematological parameters mean values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment are presented in Table 4.8.17. Haematological parameters mean values in goats treated with aqueous extracts of *Swertia chirata* and closantel in different groups at different days of pre and post treatment are presented in Table 4.8.18. The mean haemoglobin (Hb) values significantly decreased in the control group and T1 group from day 0 to day 28. Conversely, the mean haemoglobin value in the positive control and in group T3 showed a significant increase from day over the same period. Similarly, the mean packed cell volume (PCV) significantly decreased in the control group and T1 group from day 0 to day 28. In contrast, the mean PCV in the positive control group and group T3 significantly during this period. Additionally, the mean total erythrocyte count (TEC) significantly decreased in both the control group and T1 group from day 0 to day 28. Conversely, the mean TEC in the positive control group and in group T3 showed a significant increase from over the same time frame. Haematological analysis showed significant variations in total leukocyte count (TLC), neutrophil counts and lymphocyte counts between the untreated group and those treated with aqueous extract of *Swertia chirata* and closantel groups on days 0, 7, 14, 21 and 28. Additionally, the mean eosinophils counts in the control group and T1 group increased significantly from day 0 to day 28. Conversely, the mean eosinophils counts in the positive control group, T1 group and group T3 significantly decreased over the same period.

**Table 4.8.17: Haematological (Mean± SE) values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment**

Parameters	Days	C	PC	T1	T2	T3
Hb (gm/dl)	0	8.38±0.21	8.47±0.10	8.60±0.23	8.93±0.13	9.12±0.19
	7	8.20±0.16	8.87±0.10	8.50±0.23	9.03±0.13	9.28±0.17
	14	8.10±0.14	9.37±0.10	8.33±0.22	9.23±0.13	9.40±0.18
	21	8.03±0.16	10.37±0.09	8.20±0.18	8.83±0.13	9.52±0.17
	28	8.00±0.16	10.82±0.11	8.13±0.11	8.63±0.13	9.42±0.14
	<b>Mean ±SEM</b>		<b>8.14±0.17<sup>a</sup></b>	<b>9.58±0.10<sup>c</sup></b>	<b>8.35±0.19<sup>a</sup></b>	<b>8.93±0.13<sup>b</sup></b>
PCV (%)	0	26.50±0.92	26.67±0.49	27.50±1.02	28.83±0.79	28.50±0.89
	7	26.17±0.70	28.00±0.58	27.17±0.79	29.33±0.80	30.00±0.86
	14	25.67±0.61	29.67±0.49	26.33±0.76	30.33±0.95	30.50±0.92
	21	24.83±0.79	31.50±0.43	25.50±0.56	27.33±0.61	31.00±0.97
	28	24.67±0.80	33.50±0.43	24.50±0.76	26.67±0.76	30.50±0.85
	<b>Mean ±SEM</b>		<b>25.57±0.76<sup>a</sup></b>	<b>29.87±0.48<sup>c</sup></b>	<b>26.20±0.78<sup>a</sup></b>	<b>28.50±0.78<sup>b</sup></b>
TEC (× 10 <sup>6</sup> /μl)	0	8.72±0.21	8.30±0.17	8.40±0.18	8.73±0.13	8.92±0.18
	7	8.48±0.11	8.70±0.17	8.25±0.17	8.83±0.13	9.05±0.18
	14	8.38±0.14	9.20±0.17	8.15±0.13	9.03±0.13	9.13±0.17
	21	8.23±0.22	10.20±0.17	8.05±0.04	8.63±0.13	9.25±0.15
	28	8.10±0.19	10.73±0.17	7.95±0.06	8.43±0.13	9.13±0.14
	<b>Mean ±SEM</b>		<b>8.38±0.17<sup>a</sup></b>	<b>9.43±0.17<sup>d</sup></b>	<b>8.16±0.12<sup>a</sup></b>	<b>8.73±0.13<sup>b</sup></b>
TLC (×10 <sup>3</sup> /μl)	0	10.63±0.11	10.42±0.11	10.37±0.09	10.50±0.12	10.30±0.07
	7	10.58±0.10	10.82±0.11	10.25±0.09	10.58±0.11	10.40±0.07
	14	10.52±0.10	10.82±0.11	10.22±0.07	10.78±0.11	10.50±0.07
	21	10.48±0.07	11.00±0.10	10.13±0.06	10.47±0.12	10.60±0.07
	28	10.47±0.06	11.10±0.10	10.06±0.03	10.37±0.11	10.53±0.05
	<b>Mean ±SEM</b>		<b>10.54±0.09<sup>b</sup></b>	<b>10.83±0.11<sup>c</sup></b>	<b>10.21±0.07<sup>a</sup></b>	<b>10.54±0.11<sup>b</sup></b>
Neutrophils (%)	0	38.00±0.73	38.67±0.61	39.83±0.60	39.67±0.42	40.83±0.75
	7	38.17±0.79	38.17±0.60	39.33±0.49	39.33±0.42	40.50±0.72
	14	37.67±0.71	37.33±0.56	38.67±0.49	39.33±0.42	39.83±0.54
	21	37.33±0.80	37.33±0.56	37.67±0.42	38.50±0.22	39.50±0.34
	28	36.83±0.70	38.00±0.58	37.17±0.31	38.17±0.31	39.17±0.31
	<b>Mean ±SEM</b>		<b>37.60±0.75<sup>a</sup></b>	<b>37.90±0.58<sup>a</sup></b>	<b>38.53±0.46<sup>ab</sup></b>	<b>39.00±0.36<sup>bc</sup></b>
Lymphocytes (%)	0	47.83±1.08	49.17±0.70	49.17±0.54	48.17±0.70	46.83±0.65
	7	48.33±1.17	50.17±0.70	49.67±0.33	48.83±0.60	47.67±0.71
	14	50.83±1.85	51.50±0.43	50.00±0.37	49.33±0.33	48.50±0.43
	21	51.00±1.88	53.33±0.49	50.50±0.43	49.83±0.31	49.17±0.40
	28	51.33±1.84	54.00±0.52	50.67±0.33	50.00±0.45	49.33±0.49
	<b>Mean ±SEM</b>		<b>49.86±1.56<sup>b</sup></b>	<b>51.63±0.57<sup>c</sup></b>	<b>50.00±0.40<sup>b</sup></b>	<b>49.23±0.48<sup>ab</sup></b>
Eosinophils (%)	0	5.83±0.31	7.17±0.40	6.00±0.26	7.17±0.40	7.50±0.34
	7	6.17±0.31	6.67±0.33	6.17±0.40	6.83±0.31	7.00±0.26
	14	6.50±0.22	6.17±0.31	6.50±0.34	6.33±0.21	6.67±0.21
	21	6.67±0.33	4.33±0.21	6.83±0.54	6.67±0.33	6.33±0.33
	28	6.83±0.31	3.00±0.26	7.17±0.48	6.83±0.40	6.50±0.43
	<b>Mean ±SEM</b>		<b>6.40±0.30<sup>b</sup></b>	<b>5.47±0.30<sup>a</sup></b>	<b>6.53±0.40<sup>b</sup></b>	<b>6.77±0.33<sup>b</sup></b>

**Note:** C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Coriandrum sativum* @ 50mg/kg body weight; T2, *Coriandrum sativum* @ 250mg/kg body weight; T3, *Coriandrum sativum* @ 500 mg/kg body weight

<sup>a, b, c</sup> Mean bearing different superscripts in a row showed a statistical difference at P<0.05

**Table 4.8.18: Haematological (Mean± SE) values in goats treated with aqueous extracts of whole plant of *Swertia chirata* and closantel in different groups at different days of pre and post treatment**

Parameter	Days	C	PC	T1	T2	T3
Hb(gm/dl)	0	8.38±0.21	8.47±0.10	8.83±0.12	9.05±0.22	8.77±0.17
	7	8.20±0.16	8.87±0.10	8.92±0.12	9.25±0.22	8.95±0.17
	14	8.10±0.14	9.37±0.10	8.73±0.13	9.47±0.21	9.13±0.16
	21	8.03±0.16	10.37±0.09	8.45±0.12	9.58±0.20	9.27±0.15
	28	8.00±0.16	10.82±0.11	8.20±0.08	9.18±0.20	9.38±0.15
	<b>Mean ±SEM</b>		<b>8.14±0.17<sup>a</sup></b>	<b>9.58±0.10<sup>d</sup></b>	<b>8.35±0.11<sup>b</sup></b>	<b>8.93±0.21<sup>cd</sup></b>
PCV (%)	0	26.50±0.92	26.67±0.49	27.33±0.67	28.33±1.05	26.83±0.79
	7	26.17±0.70	28.00±0.58	27.83±0.60	29.50±1.31	27.83±0.79
	14	25.67±0.61	29.67±0.49	26.67±0.61	30.33±1.15	28.83±0.79
	21	24.83±0.79	31.50±0.43	26.17±0.48	31.33±1.26	29.67±0.84
	28	24.67±0.80	33.50±0.43	24.83±0.31	28.67±1.15	30.33±0.76
	<b>Mean ±SEM</b>		<b>25.57±0.76<sup>a</sup></b>	<b>29.87±0.48<sup>b</sup></b>	<b>26.20±0.53<sup>a</sup></b>	<b>28.50±1.18<sup>b</sup></b>
TEC(× 10 <sup>6</sup> /μl)	0	8.72±0.21	8.30±0.17	8.60±0.12	8.83±0.22	8.60±0.17
	7	8.48±0.11	8.70±0.17	8.70±0.12	9.02±0.22	8.75±0.17
	14	8.38±0.14	9.20±0.17	8.48±0.11	9.18±0.20	8.93±0.16
	21	8.23±0.22	10.20±0.17	8.22±0.09	9.38±0.20	9.03±0.16
	28	8.10±0.19	10.73±0.17	8.07±0.03	8.93±0.23	9.17±0.14
	<b>Mean ±SEM</b>		<b>8.38±0.17<sup>a</sup></b>	<b>9.43±0.17<sup>b</sup></b>	<b>8.16±0.09<sup>a</sup></b>	<b>8.73±0.21<sup>a</sup></b>
TLC(×10 <sup>3</sup> /μl)	0	10.63±0.11	10.42±0.11	10.55±0.12	10.50±0.12	10.40±0.10
	7	10.58±0.10	10.62±0.11	10.63±0.11	10.62±0.13	10.50±0.10
	14	10.52±0.10	10.82±0.11	10.48±0.09	10.70±0.13	10.60±0.10
	21	10.48±0.07	11.00±0.10	10.32±0.06	10.80±0.13	10.73±0.11
	28	10.47±0.06	11.10±0.10	10.18±0.05	10.68±0.10	10.78±0.09
	<b>Mean±SEM</b>		<b>10.54±0.09<sup>ab</sup></b>	<b>10.83±0.11<sup>c</sup></b>	<b>10.21±0.09<sup>a</sup></b>	<b>10.54±0.12<sup>bc</sup></b>
Neutrophils (%)	0	38.00±0.73	38.67±0.61	40.83±0.48	40.00±0.26	40.67±0.80
	7	38.17±0.79	38.17±0.60	40.17±0.40	40.00±0.26	40.17±0.79
	14	37.67±0.71	37.33±0.56	39.83±0.40	39.67±0.33	40.17±0.79
	21	37.33±0.80	37.33±0.56	39.50±0.56	39.50±0.34	39.33±0.61
	28	36.83±0.70	38.00±0.58	39.00±0.37	39.33±0.33	39.17±0.54
	<b>Mean ±SEM</b>		<b>37.60±0.75<sup>a</sup></b>	<b>37.90±0.58<sup>a</sup></b>	<b>38.53±0.44<sup>b</sup></b>	<b>39.00±0.30<sup>b</sup></b>
Lymphocytes (%)	0	47.83±1.08	49.17±0.70	46.50±0.56	47.17±0.31	46.33±0.88
	7	48.33±1.17	50.17±0.70	48.17±0.48	47.50±0.34	48.00±0.82
	14	50.83±1.85	51.50±0.43	48.33±0.42	48.50±0.56	48.67±0.92
	21	51.00±1.88	53.33±0.49	48.17±0.31	49.00±0.63	49.83±0.70
	28	51.33±1.84	54.00±0.52	48.33±0.42	48.67±0.76	50.33±0.76
	<b>Mean±SEM</b>		<b>49.86±1.56<sup>b</sup></b>	<b>51.63±0.57<sup>b</sup></b>	<b>50.00±0.44<sup>a</sup></b>	<b>49.23±0.52<sup>a</sup></b>
Eosinophils (%)	0	5.83±0.31	7.17±0.40	7.00±0.26	7.83±0.17	7.67±0.33
	7	6.17±0.31	6.67±0.33	6.67±0.21	7.50±0.22	6.83±0.31
	14	6.50±0.22	6.17±0.31	6.83±0.31	6.83±0.31	6.17±0.17
	21	6.67±0.33	4.33±0.21	7.33±0.49	6.50±0.34	5.83±0.31
	28	6.83±0.31	3.00±0.26	7.67±0.42	7.00±0.45	5.50±0.43
	<b>Mean±SEM</b>		<b>6.40±0.30<sup>b</sup></b>	<b>5.47±0.30<sup>a</sup></b>	<b>6.53±0.34<sup>c</sup></b>	<b>6.77±0.30<sup>c</sup></b>

**Note:** C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Swertia chirata* @ 50mg/kg body weight; T2, *Swertia chirata* @ 250mg/kg body weight; T3, *Swertia chirata* @ 500 mg/kg body weight

<sup>a, b, c</sup> Mean bearing different superscripts in a row showed a statistical difference at P<0.05

#### 4.9 Biochemical alteration in goats treated with aqueous extracts of *Coriandrum sativum* seeds, whole plants of *Swertia chirata* and closantel

Biochemical parameters mean values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post-treatment are presented in Table 4.9.19. Biochemical analysis showed no significant variations in AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) values between the untreated group and those treated with aqueous extract of *Coriandrum sativum* seeds extract and closantel groups on days 0,7,14,21 and 28. The values of ALT and AST remained with normal physiological range. The values of BUN (Blood urea nitrogen) showed a significant decrease from day 0 to 28, but they remained within the normal range. Serum creatinine values varied significantly among different groups on different days but their values were in normal range. There was a significant reduction in the value of TSP (Total serum protein), albumin and albumin globulin ratio. However, no significant variations were observed in the value of globulin.

**Table 4.9.19: Biochemical (Mean± SE) values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment**

Parameters	Days	C	PC	T1	T2	T3
AST(U/L)	0	57.97± 1.51	55.97±1.20	57.00±1.37	55.65±1.25	56.13±0.74
	7	58.27± 1.46	54.93±1.01	54.77±2.20	54.62±1.43	55.22±1.42
	14	60.13±1.60	54.80±0.85	54.95±0.97	54.37±0.70	55.47±0.94
	21	53.45±3.90	56.20±1.45	47.45±0.46	55.87±1.15	54.85±1.41
	28	53.67±1.18	54.58±1.48	55.18±1.64	53.58±1.35	54.43±0.85
	<b>Mean ±SEM</b>		<b>56.70±1.93</b>	<b>55.30±1.20</b>	<b>53.87±1.33</b>	<b>54.81±1.18</b>
ALT(U/L)	0	17.93±1.19	18.05±1.15	18.30±1.29	17.43±1.52	20.50±1.68
	7	18.05±1.20	19.67±1.22	20.62±1.47	20.85±0.97	20.13±0.70
	14	19.08±1.38	20.08±1.22	18.13±1.10	17.95±1.34	20.12±1.00
	21	18.17±0.84	18.42±1.83	19.23±1.43	19.48±1.49	17.00±1.51
	28	18.00±1.24	14.70±1.96	18.60±1.02	20.73±1.38	16.62±0.65
	<b>Mean ±SEM</b>		<b>18.25±1.17</b>	<b>18.18±1.48</b>	<b>18.98±1.26</b>	<b>18.29±1.34</b>
BUN(mg/dl)	0	24.13±1.50	21.85±0.96	21.28±1.43	21.57±0.99	20.07±0.84
	7	23.96±1.54	21.10±1.64	21.56±0.51	20.43±0.85	20.63±0.96
	14	23.92±0.95	24.27±0.67	21.23±0.92	22.65±0.45	21.12±0.94
	21	20.46±1.09	18.38±0.81	20.89±0.89	20.67±1.17	20.44±1.05
	28	21.89±1.27	21.57±1.22	21.77±1.03	20.59±1.19	18.22±1.19
	<b>Mean ±SEM</b>		<b>22.87±1.27<sup>b</sup></b>	<b>21.43±1.06<sup>ab</sup></b>	<b>21.35±0.96<sup>ab</sup></b>	<b>21.18±0.93<sup>ab</sup></b>
Creatinine(mg/dl)	0	1.48±0.03	1.54±0.05	1.54±0.09	1.58±0.06	1.45±0.10
	7	1.49±0.02	1.52±0.03	1.59±0.10	1.55±0.05	1.58±0.07
	14	1.45±0.03	1.61±0.03	1.70±0.04	1.56±0.05	1.58±0.06
	21	1.51±0.06	1.56±0.04	1.58±0.05	1.47±0.08	1.48±0.06
	28	1.54±0.04	1.37±0.09	1.60±0.03	1.50±0.03	1.37±0.05
	<b>Mean ±SEM</b>		<b>1.49±0.04<sup>a</sup></b>	<b>1.52±0.05<sup>ab</sup></b>	<b>1.60±0.06<sup>b</sup></b>	<b>1.53±0.05<sup>ab</sup></b>

TSP(gm/dl)	0	5.65±0.23	5.47±0.08	5.37±0.14	5.63±0.11	5.43±0.06
	7	5.57±0.22	5.48±0.12	5.35±0.11	5.40±0.05	5.53±0.07
	14	5.30±0.10	5.63±0.14	5.37±0.09	5.47±0.09	5.67±0.05
	21	5.22±0.09	5.67±0.07	5.32±0.07	5.48±0.12	5.72±0.05
	28	5.17±0.08	6.00±0.12	5.27±0.09	5.47±0.11	5.78±0.03
	<b>Mean ±SEM</b>	<b>5.38±0.14<sup>a</sup></b>	<b>5.65±0.11<sup>b</sup></b>	<b>5.34±0.10<sup>a</sup></b>	<b>5.49±0.10<sup>ab</sup></b>	<b>5.63±0.05<sup>b</sup></b>
Albumin(gm/dl)	0	3.48±0.14	3.18±0.09	3.15±0.08	3.33±0.10	3.23±0.07
	7	3.42±0.14	3.22±0.10	3.12±0.07	3.12±0.05	3.35±0.07
	14	3.05±0.06	3.48±0.14	3.27±0.10	3.18±0.05	3.52±0.03
	21	3.03±0.08	3.58±0.05	3.08±0.05	3.22±0.09	3.58±0.07
	28	3.02±0.06	3.88±0.14	3.07±0.06	3.23±0.08	3.67±0.02
	<b>Mean ±SEM</b>	<b>3.20±0.10<sup>a</sup></b>	<b>3.47±0.10<sup>b</sup></b>	<b>3.14±0.07<sup>a</sup></b>	<b>3.22±0.07<sup>a</sup></b>	<b>3.47±0.05<sup>b</sup></b>
Globulin(gm/dl)	0	2.17±0.11	2.30±0.09	2.22±0.11	2.27±0.05	2.20±0.06
	7	2.18±0.12	2.27±0.07	2.23±0.09	2.28±0.05	2.18±0.05
	14	2.25±0.07	2.15±0.07	2.10±0.09	2.28±0.08	2.15±0.07
	21	2.20±0.09	2.15±0.08	2.23±0.06	2.27±0.09	2.13±0.04
	28	2.13±0.05	2.08±0.07	2.20±0.06	2.25±0.06	2.12±0.03
	<b>Mean ±SEM</b>	<b>2.19±0.09</b>	<b>2.19±0.08</b>	<b>2.20±0.08</b>	<b>2.27±0.07</b>	<b>2.16±0.05</b>
A:G ratio	0	1.62±0.06	1.39±0.09	1.44±0.09	1.48±0.06	1.48±0.06
	7	1.60±0.07	1.48±0.07	1.41±0.06	1.37±0.05	1.54±0.06
	14	1.36±0.04	1.63±0.09	1.57±0.09	1.40±0.06	1.65±0.06
	21	1.39±0.08	1.68±0.07	1.39±0.04	1.43±0.08	1.69±0.06
	28	1.42±0.04	1.88±0.10	1.40±0.04	1.44±0.05	1.73±0.03
	<b>Mean ±SEM</b>	<b>1.48±0.06<sup>a</sup></b>	<b>1.61±0.08<sup>b</sup></b>	<b>1.44±0.06<sup>a</sup></b>	<b>1.42±0.06<sup>a</sup></b>	<b>1.62±0.05<sup>b</sup></b>

**Note:** C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Coriandrum sativum* @ 50mg/kg body weight; T2, *Coriandrum sativum* @ 250mg/kg body weight; T3, *Coriandrum sativum* @ 500 mg/kg body weight

<sup>a, b, c</sup> Mean bearing different superscripts in a row showed a statistical difference at P<0.05

Biochemical parameters mean values in goats treated with aqueous extracts of *Swertia chirata* and closantel in different groups at different days of pre and post-treatment are presented in Table 4.9.20. Biochemical analysis showed no significant variations in AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) values between untreated group and treated with aqueous extracts of whole plants of *Swertia chirata* and closantel groups on days 0,7,14,21 and 28. The values of ALT and AST were within the normal physiological range. The values of blood urea nitrogen showed significant decrease from day 0 to 28, but they remained within the normal range. But there was no significant variations were observed in the values of serum creatinine in different groups at different days. There was significant reduction in the value of TSP (Total serum protein), albumin and albumin globulin ratio. However, no significant variations were observed in the value of globulin.

**Table 4.9.20: Biochemical (Mean± SE) values in goats treated with aqueous extracts of whole plants of *Swertia chirata* and closantel in different groups at different days of pre and post treatment**

Parameter	Days	C	PC	T1	T2	T3
AST(U/L)	0	57.97±1.51	55.97±1.20	55.25±1.37	56.45±1.05	54.93±0.94
	7	58.27±1.46	54.93±1.01	53.73±1.29	55.42±1.06	54.78±1.09
	14	60.13±1.60	54.80±0.85	54.18±1.40	53.60±1.01	55.43±1.38
	21	53.45±3.90	56.20±1.45	54.53±1.16	54.05±0.82	55.30±1.26
	28	53.67±1.18	54.58±1.48	55.38±1.24	56.43±1.22	54.15±1.50
	<b>Mean ±SEM</b>	<b>56.70±1.93</b>	<b>55.30±1.20</b>	<b>54.62±1.29</b>	<b>55.19±1.03</b>	<b>54.92±1.23</b>
ALT(U/L)	0	17.93±1.19	18.05±1.15	18.72±1.17	18.47±0.59	18.58±0.89
	7	18.05±1.20	19.67±1.22	18.92±1.45	18.50±1.19	16.13±0.84
	14	19.08±1.38	20.08±1.22	17.90±1.20	19.52±0.93	16.38±1.28
	21	18.17±0.84	18.42±1.83	16.42±0.91	17.97±0.75	17.95±1.06
	28	18.00±1.24	14.70±1.96	16.98±1.24	18.15±0.83	18.02±1.27
	<b>Mean ±SEM</b>	<b>18.25±1.17</b>	<b>18.18±1.48</b>	<b>17.79±1.19</b>	<b>18.52±0.86</b>	<b>17.41±1.07</b>
BUN(mg/dl)	0	24.13±1.50	21.85±0.96	21.48±0.98	17.17±1.47	20.18±0.94
	7	23.96±1.54	21.10±1.64	20.48±0.56	19.51±1.07	21.13±0.70
	14	23.92±0.95	24.27±0.67	19.62±1.09	20.21±1.72	18.59±0.93
	21	20.46±1.09	18.38±0.81	21.88±0.97	19.39±1.07	20.25±0.87
	28	21.89±1.27	21.57±1.22	19.85±1.51	20.17±1.12	19.37±1.04
	<b>Mean ±SEM</b>	<b>22.87±1.27<sup>c</sup></b>	<b>21.43±1.06<sup>bc</sup></b>	<b>20.66±1.02<sup>ab</sup></b>	<b>19.29±1.29<sup>a</sup></b>	<b>19.90±0.90<sup>ab</sup></b>
Creatinine(mg/dl)	0	1.48±0.03	1.54±0.05	1.43±0.09	1.50±0.07	1.53±0.08
	7	1.49±0.02	1.52±0.03	1.53±0.09	1.55±0.05	1.47±0.08
	14	1.45±0.03	1.61±0.03	1.36±0.08	1.45±0.07	1.48±0.08
	21	1.51±0.06	1.56±0.04	1.49±0.08	1.49±0.07	1.51±0.06
	28	1.54±0.04	1.37±0.09	1.45±0.08	1.52±0.07	1.38±0.05
	<b>Mean ±SEM</b>	<b>1.49±0.04</b>	<b>1.52±0.05</b>	<b>1.45±0.08</b>	<b>1.50±0.07</b>	<b>1.47±0.07</b>
TSP(gm/dl)	0	5.65±0.23	5.47±0.08	5.55±0.08	5.68±0.06	5.32±0.12
	7	5.57±0.22	5.48±0.12	5.42±0.06	5.48±0.07	5.45±0.08
	14	5.30±0.10	5.63±0.14	5.30±0.06	5.48±0.09	5.55±0.09
	21	5.22±0.09	5.67±0.07	5.27±0.08	5.32±0.07	5.57±0.08
	28	5.17±0.08	6.00±0.12	5.20±0.06	5.18±0.05	5.60±0.07
	<b>Mean ±SEM</b>	<b>5.38±0.14<sup>a</sup></b>	<b>5.65±0.11<sup>b</sup></b>	<b>5.35±0.07<sup>a</sup></b>	<b>5.43±0.07<sup>a</sup></b>	<b>5.50±0.09<sup>ab</sup></b>
Albumin(gm/dl)	0	3.48±0.14	3.18±0.09	3.27±0.07	3.23±0.09	3.15±0.08
	7	3.42±0.14	3.22±0.10	3.22±0.08	3.20±0.05	3.23±0.12
	14	3.05±0.06	3.48±0.14	3.10±0.04	3.27±0.05	3.30±0.10
	21	3.03±0.08	3.58±0.05	3.13±0.06	3.12±0.06	3.32±0.08
	28	3.02±0.06	3.88±0.14	3.08±0.05	3.05±0.02	3.38±0.09
	<b>Mean ±SEM</b>	<b>3.20±0.10<sup>a</sup></b>	<b>3.47±0.10<sup>b</sup></b>	<b>3.16±0.06<sup>a</sup></b>	<b>3.17±0.05<sup>a</sup></b>	<b>3.28±0.09<sup>a</sup></b>
Globulin(gm/dl)	0	2.17±0.11	2.30±0.09	2.28±0.09	2.45±0.09	2.17±0.06
	7	2.18±0.12	2.27±0.07	2.20±0.06	2.27±0.07	2.22±0.08
	14	2.25±0.07	2.15±0.07	2.20±0.07	2.22±0.06	2.27±0.05
	21	2.20±0.09	2.15±0.08	2.13±0.06	2.20±0.07	2.25±0.05
	28	2.13±0.05	2.08±0.07	2.10±0.04	2.13±0.07	2.22±0.07
	<b>Mean ±SEM</b>	<b>2.19±0.09</b>	<b>2.19±0.08</b>	<b>2.18±0.06</b>	<b>2.25±0.07</b>	<b>2.23±0.06</b>
A:G ratio	0	1.62±0.06	1.39±0.09	1.45±0.07	1.34±0.08	1.46±0.05
	7	1.60±0.07	1.48±0.07	1.47±0.07	1.44±0.04	1.47±0.10
	14	1.36±0.04	1.63±0.09	1.42±0.05	1.48±0.03	1.46±0.06
	21	1.39±0.08	1.68±0.07	1.48±0.06	1.42±0.06	1.48±0.05
	28	1.42±0.04	1.88±0.10	1.47±0.03	1.44±0.06	1.54±0.08
	<b>Mean ±SEM</b>	<b>1.48±0.06<sup>a</sup></b>	<b>1.61±0.08<sup>b</sup></b>	<b>1.46±0.06<sup>a</sup></b>	<b>1.42±0.05<sup>a</sup></b>	<b>1.48±0.07<sup>a</sup></b>

**Note:** C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Swertia chirata* @ 50mg/kg body weight; T2, *Swertia chirata* @ 250mg/kg body weight; T3, *Swertia chirata* @ 500 mg/kg body weight

<sup>a, b, c</sup> Mean bearing different superscripts in a row showed a statistical difference at  $P < 0.05$

#### **4.10 Effect on Catalase, SOD and FRAP (Antioxidant) levels in goats treated with aqueous extracts of *Coriandrum sativum* seeds, whole plants of *Swertia chirata* and closantel**

Catalase, SOD and FRAP mean values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment are presented in Table 4.10.21. In control group C (not received treatment) and the T1 group (*Coriandrum sativum* @ 50mg/kg body weight), the mean value of catalase (CAT), SOD and FRAP showed non-significant decrease at day 28 (post-treatment) compared to day 0 (pre-treatment). In the positive control group (Closantel @ 10 mg/kg body weight), T2 group (*Coriandrum sativum* @ 250mg/kg body weight) and T3 group (*Coriandrum sativum* @ 500 mg/kg body weight), the mean levels of catalase (CAT), SOD and FRAP showed a non-significant increase at day 28 (post-treatment) compared to day 0 (pre-treatment).

Catalase, SOD and FRAP mean values in goats treated with aqueous extracts of *Swertia chirata* and closantel in different groups at different days of pre and post treatment are presented in Table 4.10. 22. In the control group (not received treatment) and in T1 group (*Swertia chirata* @ 50 mg/kg body weight), the mean value of catalase (CAT) and SOD showed a non significant decrease at day 28 (post-treatment) compared to day 0 (pre-treatment). Conversely, in the positive control group (Closantel @ 10 mg/kg body weight), T2 group (*Swertia chirata* @ 250 mg/kg body weight) and T3 group (*Swertia chirata* @ 500 mg/kg body weight) mean value of catalase (CAT) and SOD exhibited a non significant increase at day 28 (post-treatment) compared to day 0 (pre-treatment). The FRAP values significantly decreased in control group and in the T1 group at day 28 (post-treatment) compared to day 0 (pre-treatment). In contrast, the FRAP values showed a significant increase in the positive control group (PC), T2 group and T3 group at day 28 (post-treatment) compared to day 0 (pre-treatment).

**Table 4.10.21: Catalase, SOD and FRAP (Mean± SE) values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment**

Parameters	Days	C	PC	T1	T2	T3
Catalase ( $\mu\text{M}/\text{min}/\text{g}$ Hb)	0	47.38 $\pm$ 3.57	35.53 $\pm$ 3.28	45.26 $\pm$ 6.66	43.15 $\pm$ 4.14	36.38 $\pm$ 4.28
	28	41.03 $\pm$ 3.90	46.95 $\pm$ 3.57	40.61 $\pm$ 4.49	46.53 $\pm$ 6.07	42.30 $\pm$ 3.63
	<b>Mean <math>\pm</math>SEM</b>	<b>44.21<math>\pm</math>3.74</b>	<b>41.24<math>\pm</math>3.43</b>	<b>42.94<math>\pm</math>5.58</b>	<b>44.84 <math>\pm</math> 5.11</b>	<b>39.34<math>\pm</math>3.96</b>
SOD (U/g Hb)	0	3066.67 $\pm$ 688.32	2000.00 $\pm$ 647.04	3033.33 $\pm$ 912.75	2133.33 $\pm$ 345.12	2166.67 $\pm$ 307.32
	28	2300.00 $\pm$ 690.41	2433.33 $\pm$ 340.26	2466.67 $\pm$ 266.67	2266.67 $\pm$ 341.24	2400.00 $\pm$ 338.62
	<b>Mean <math>\pm</math>SEM</b>	<b>2683.34<math>\pm</math>689.37</b>	<b>2216.67<math>\pm</math>493.65</b>	<b>2750.00<math>\pm</math>589.71</b>	<b>2200.00<math>\pm</math>343.18</b>	<b>2283.34<math>\pm</math>322.97</b>
FRAP ( $\mu\text{mol}/\text{L}$ )	0	114.29 $\pm$ 27.23	77.89 $\pm$ 8.19	142.86 $\pm$ 21.46	79.93 $\pm$ 10.83	118.03 $\pm$ 7.25
	28	105.44 $\pm$ 19.28	168.03 $\pm$ 24.39	134.35 $\pm$ 21.08	114.63 $\pm$ 9.22	174.83 $\pm$ 25.24
	<b>Mean <math>\pm</math>SEM</b>	<b>109.87<math>\pm</math>23.26</b>	<b>122.96<math>\pm</math>16.29</b>	<b>138.61<math>\pm</math>21.27</b>	<b>97.28<math>\pm</math>10.03</b>	<b>146.43<math>\pm</math>16.25</b>

C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Coriandrum sativum* @ 50mg/kg body weight; T2, *Coriandrum sativum* @ 250mg/kg body weight; T3, *Coriandrum sativum* @ 500 mg/kg body weight

<sup>a, b</sup>, Mean bearing different superscripts in a row showed a statistical difference at P<0.05

**Table 4.10.22: Catalase, SOD and FRAP (Mean± SE) values in goats treated with aqueous extracts of whole plants of *Swertia chirata* and closantel in different groups at different days of pre and post treatment**

Parameter	Days	C	PC	T1	T2	T3
Catalase ( $\mu\text{M}/\text{min}/\text{g}$ Hb)	0	47.38 $\pm$ 3.57	35.53 $\pm$ 3.28	51.18 $\pm$ 5.73	37.65 $\pm$ 3.79	34.69 $\pm$ 3.51
	28	41.03 $\pm$ 3.90	46.95 $\pm$ 3.57	45.69 $\pm$ 4.25	42.30 $\pm$ 4.76	42.72 $\pm$ 5.73
	<b>Mean <math>\pm</math>SEM</b>	<b>44.21<math>\pm</math>3.74</b>	<b>41.24<math>\pm</math>3.43</b>	<b>48.44<math>\pm</math>4.99</b>	<b>39.98<math>\pm</math>4.28</b>	<b>38.71<math>\pm</math>4.62</b>
SOD (U/g Hb)	0	3066.67 $\pm$ 688.32	2000.00 $\pm$ 647.04	3000.00 $\pm$ 764.20	2366.67 $\pm$ 468.80	1733.33 $\pm$ 388.73
	28	2300.00 $\pm$ 690.41	2433.33 $\pm$ 340.26	2566.67 $\pm$ 380.93	2600.00 $\pm$ 450.19	2033.33 $\pm$ 660.13
	<b>Mean <math>\pm</math>SEM</b>	<b>2683.34<math>\pm</math>689.37</b>	<b>2216.67<math>\pm</math>493.65</b>	<b>2783.34<math>\pm</math>572.57</b>	<b>2483.34<math>\pm</math>459.50</b>	<b>1883.33<math>\pm</math>524.43</b>
FRAP ( $\mu\text{mol}/\text{L}$ )	0	114.29 $\pm$ 27.23	77.89 $\pm$ 8.19	222.79 $\pm$ 30.12	188.78 $\pm$ 13.40	127.89 $\pm$ 22.84
	28	105.44 $\pm$ 19.28	168.03 $\pm$ 24.39	210.54 $\pm$ 28.80	230.27 $\pm$ 26.43	189.46 $\pm$ 5.64
	<b>Mean <math>\pm</math>SEM</b>	<b>109.87<math>\pm</math>23.26<sup>a</sup></b>	<b>122.96<math>\pm</math>16.29<sup>a</sup></b>	<b>216.67<math>\pm</math>29.46<sup>b</sup></b>	<b>209.53<math>\pm</math>19.92<sup>b</sup></b>	<b>158.68<math>\pm</math>14.24<sup>ab</sup></b>

C, Control (Untreated); PC, Positive control Closantel @ 10 mg/kg body weight; T1, *Swertia chirata* @ 50mg/kg body weight; T2, *Swertia chirata* @ 250mg/kg body weight; T3, *Swertia chirata* @ 500 mg/kg body weight

<sup>a, b</sup>, Mean bearing different superscripts in a row showed a statistical difference at P<0.05

**5.1 Prevalence of gastrointestinal parasitic infections in goats**

In the present study, the prevalence of GI parasitic infections in goats was observed throughout the year. Occurrence of parasitic gastroenteritis is a common problem in sheep and goat in major part of the world including India. The present study revealed overall prevalence of gastrointestinal parasites infection was 70.55% in goats. Similar prevalence was recorded in sheep and goats by the earlier researches (Paul *et al.*, 2020; Dahourou *et al.*, 2021). The overall prevalence of gastrointestinal parasites was recorded 74.66% by Kalwaghe *et al.* (2022). Several studies had previously reported the high prevalence of gastrointestinal parasites in goats from various regions of India. Singh *et al.* (2015) observed that overall prevalence of gastrointestinal parasite was 94.48% in goats from Madhya Pradesh, India. Pathak and Pal (2008) observed that the overall prevalence of gastrointestinal parasite was 85.22% in goats from Durg District, Chhattisgarh. The probable reasons for variation in the prevalence of gastrointestinal parasites in goats may be due to the variations in number of goats faecal samples were examined, study period and climatic conditions such as humidity, rainfall temperature etc. of the area that boon the viability of infective stages of the parasites. The managerial practices, deworming schedule and accessibility of susceptible host (Vohra *et al.*, 2018), also the variations in goat husbandry practices and breed composition of goats from various parts have important role in regional disparity in occurrence of gastrointestinal parasites infection in goats (Amran *et al.*, 2018).

**5.1.1 Breed wise prevalence of gastrointestinal parasites**

The highest prevalence of gastrointestinal parasites was in Barbari breed of goat (39.97%) followed by Jamunapari (36.93%), Non Descript (12.18%) and lowest in Sirohi (10.91%). Overall Barbari goats were found comparatively more prone to the infection in comparison to Jamunapari, Sirohi and Non Descript in the current study. Amran *et al.* (2018) also reported that various goat breeds exhibit differences in the occurrence of gastrointestinal parasites infection. Dappawar *et al.* (2018) reported that overall prevalence of gastrointestinal parasite in goats was 48.93% in ND (Non-descript) and 52.89% in Osmanabadi goats from Marathwada region of Maharashtra. The susceptibility of any breed to the gastrointestinal parasitic infection mostly is based on the genetic resistance of a specific breed and its

adaptability with the existing environmental circumstances of the area (Kalwaghe *et al.*, 2022). The probable reasons in differences in prevalence of gastrointestinal parasitic infections in goats may be describes on the basis of breeds genetic differences and owing to variations in various management practices such as housing, feeding, watering, stocking rate, rearing and adopted health control measures. Variations in climatic surroundings in a particular geographical area also play an important role (Verma *et al.*, 2018).

### **5.1.2 Age wise prevalence of gastrointestinal parasites in goats**

The highest prevalence of gastrointestinal parasites was reported in age group of 1-2 years (50.51%), followed by in age group of > 2 years (33.88%) and the lowest one was in < 1 year age group (15.61%). The current study revealed that the parasitic infections were highest in 1-2 years age group of goats in comparison to other age group. Similar finding was also observed by Yadav *et al.* (2006) and Emiru *et al.* (2013), who reported a higher prevalence of gastrointestinal parasites infection in adult goats as compared to young goats. The probable reasons for higher prevalence of gastrointestinal parasites infection in adult goats may be due to big areas of grazing pastures accompanied by stress conditions such as transport, immune status and pregnancy. Young goats are less susceptible to parasitic infections for the reason that kids suckle their mothers till they attain adulthood, which check their exposure for grazing. This is in accordance with results reported by Vieira *et al.* (2014) and Singh *et al.* (2017).

### **5.1.3 Sex wise prevalence of gastrointestinal parasites in goats**

In the present study, a higher prevalence of gastrointestinal parasites was observed in females (83.88%) in comparison to males (16.12%). Various researchers have reported prevalence of gastrointestinal parasites in goats in various breed and sex. Similar findings were reported various earlier researchers that prevalence of gastrointestinal parasites were higher in female goat in comparison to male goats (Bautista-Garfias *et al.*, 2022; Pilarczyk *et al.*, 2021; Singh *et al.*, 2017; Mir *et al.*, 2013; Bashir *et al.*, 2012). The probable reasons for variations in susceptibility to infection of gastrointestinal parasites in goats between males and females may be due to several physio-biochemical factors like gestation and lactation which may results into stress and reduction in immunity of body. Physiological conditions of female goats and lactating goat makes them deficient in nutrients, it makes them more

susceptible against the infections due to lack of proper availability of sufficient and balanced nutrition against higher requirements. Mpofu *et al.*, (2020b), reported that prevalence of gastrointestinal parasites same in both sex including males and females. The probable reasons for this may be due provide same management systems for male and female goat. Some studies reported that there was no any effect of sex on the prevalence of gastrointestinal parasites in goats (Sivajothi and Reddy, 2018).

#### **5.1.4 Season wise prevalence of gastrointestinal parasites in goats**

The highest prevalence rate of gastrointestinal parasites was observed during rainy season (47.59%) followed by in summer season (33.63%), and the lowest one during winter season (18.78 %). Seasonal changes was highly affected the occurrence of intensity of parasites (Zouyed *et al.*, 2016).The present finding was in line with Das *et al.*(2017), who observed season wise prevalence of gastrointestinal parasitism in goats were highest during rainy season (34.92%) followed by cool (26.86%), hot (26.62%), and lowest in cold (20.39%) seasons. Pathak and Pal (2008) observed that the highest prevalence of gastrointestinal parasites in goat was in monsoon (94.60%), moderate in summer (87.50%) and minimum in winter (63.15%). Similar results were observed by Sutar *et al.* (2010) and Singh *et al.* (2015).

Varadharajan and Vijayalakshmi (2015) and Dhara *et al.* (2015) have observed the highest prevalence gastrointestinal parasites in goats in monsoon season and minimum prevalence in summer season in their respective study area. The probable reason for high occurrence of gastrointestinal parasites in goats during rainy season may be due to usefulness of this season for viability, growth and spreading of nematode larvae in the pasture, which results into occurrence of higher infection in the pasture during the grazing (Singh *et al.*, 2015). Incidence of intestinal parasitic infection was highest during rainy season can be correlated with the seasonal or climatic pattern and situations. This season gives the most favourable situations for herbage development in this area and the needed moisture is sustained on the grasslands. This may be reasons for higher occurrence of infections of gastrointestinal parasite in goat during the months of monsoon. The probable reason for the relatively decrease the prevalence of gastrointestinal parasites in goats during winter months may be due to low temperature and dry situations hamper the development and growth of eggs and larvae. Besides weather conditions, the self cure phenomenon was also responsible

for this (Stewart, 1953; Stewart, 1955). Adverse environmental situations in winter seasons force mostly gastrointestinal parasites, like strongyles, to go through hypobiosis. Additionally, decreased grazing durations decrease the possibility of contact hours between the host and parasites, happen in a lower occurrence of parasites during winter seasons (Verma *et al.*, 2018).

## 5.2 Prevalence of different gastrointestinal parasites in goats

Current study observed that prevalence of Strongyle spp. was highest in comparison to other gastrointestinal parasites in goats. Various earlier researchers have found similar results (Zanzani *et al.* 2014; Babják *et al.* 2017; Bedada *et al.*, 2017; Sivajothi and Reddy, 2018). The probable reasons for higher occurrence of Strongyle spp. may be due there are enormous number of nematode species such as *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Oesophagostomum*, *Chabertia*, *Nematodirus* and *Cooperia*. The higher occurrence of strongyles, mostly *H. contortus*, can be due to its short generation interval and its capacity to reproduce rapidly if environmental situations are suitable.

Coccidiosis is caused by *Eimeria* spp. is one of the very significant parasitic diseases in sheep and goats globally (Alcala-Canto *et al.*, 2020). In the current study the prevalence of *Eimeria* spp. was higher. The similar findings were also reported by various previous researchers (Hashim and Mat Yusof, 2016). The reasons for higher prevalence of *Eimeria* spp. infections in goats may be owing to the distinct feature of the oocysts of *Eimeria* that may survive in utmost environmental situations in comparisons to helminth eggs (Hisamuddin *et al.*, 2016). This can be related with household, landholdings, which directly determine the level of management of livestock like hygiene proper available living space, and nutritional requirements (Sharma and Mandal, 2013).

In this study within cestode only *Moniezia* spp. was identified. The finding of this study was supported by earlier worker (Hurisa *et al.*, 2021), who reported that *Moniezia* spp. was only cestodes observed in the goats. The utilization of oribated mites contaminated with *Moniezia* spp. blastocysts is related to the occurrence of this parasite in the tropical regions (Diop *et al.*, 2015). The prevalence of this parasites varies 0.1- 40.34% in the goats reported by various earlier researchers (Brahma *et al.*, 2015; Tan *et al.*, 2017; Hassan *et al.*, 2019). The prevalence of *Moniezia* spp. was recorded as high as 53 % in goats from Ethiopia (Sissay *et*

*al.*, 2008). Attindehou and Salifou, (2012) from Benin reported the prevalence of *Moniezia* in all season but higher infections rates was recorded in rainy seasons (more than 50%) in goats.

In this study, only two species of trematode were identified and these were *Amphistome* spp. and *Fasciola* spp. in goats. Fasciolosis in goat was reported 9.25% in Maharashtra (Sutar *et al.*, 2010), 3.88-14.8 % in Jammu and Kashmir (Khajuria *et al.*, 2013). Islam *et al.* (2016), reported prevalence of fasciolosis in goats was highest during rainy season in Bangladesh. They also observed that prevalence significantly higher in female goats in comparisons to males. The probable reasons for higher prevalence of *Fasciola* spp. prevalence in rainy season related to weather situations. This is due to that hatching of eggs of fluke and multiplication of snail, the intermediate host, needed suitable high rainfall and temperature (>10°C) (Taylor, 2012).

The incidence of *Amphistome* spp. observed in several studies in India as high as 18.26 - 91 % in goats (Lone *et al.*, 2012; Singh *et al.*, 2015). Highest prevalence was reported during monsoon seasons and lowest during winter season in goats (Choubisa, 2010; Singh *et al.*, 2015) and related it with most likely accessibility of intermediate host, the snails. One researchers reported higher prevalence of *Amphistome* spp. during summer in comparison to rainy season (Yadav *et al.*, 2010). Ibrahim *et al.* (2014) reported significantly higher prevalence *Amphistome* spp. in young and female goats.

The prevalence of *Trichuris* spp. reported by various earlier researchers varies 0.6-68.6% in goats (Almalaiik *et al.*, 2008; Sutar *et al.*, 2010; Futagbi *et al.*, 2015; Zvinorova *et al.*, 2016; Hassan *et al.*, 2019). Dagnachew *et al.* (2011) described the prevalence of *Trichuris* spp. was significantly higher in young animals in comparisons to adult's goat. *Strongyloides* spp. prevalence of in goats was observed 3.75-47 % in India (Sutar *et al.*, 2010; Lone *et al.*, 2012; Brahma *et al.*, 2015).

The prevalence of *Cryptosporidium* spp. in kids was recorded 3.5% (Maurya *et al.*, 2013). In the present study, higher prevalence of *Cryptosporidium* spp. was recorded in females than males. The probable reason for higher prevalence of *Cryptosporidium* in kids may be due to the lower tolerance levels in kids due to their inexperienced immunological level. The low prevalence of infection with *Cryptosporidium* was probably due to that majority of samples was collected from adult goats (Maurya *et al.*, 2013). The prevalence of

*Giardia* spp. in goats globally ranges between 10 and 40%. These differences due to various inherent in every area, but also to variations between research conducted, particularly regarding goat age and type of techniques of diagnosis utilized (Robertson, 2009).

### **5.3 Molecular characterization of strongyle infection in goats**

The occurrences of gastrointestinal parasites are usually common in the ruminant and responsible for huge economic losses (El-Alfy *et al.*, 2019). Owing to their diversity and dissimilar susceptibility against the anthelmintic (Han *et al.*, 2017; Rashid *et al.*, 2018), it is necessary to recognize their hosts, species, and infection place to make clear which animals may be simply recognized in particular region. The eggs of various species of gastrointestinal parasites are identical in shape and size. Additionally, the recognition for 3<sup>rd</sup> stage of infective larvae has need of the support of expert, which makes some times inaccurate and difficult in recognition from each other (El-Alfy *et al.*, 2019; Redman *et al.*, 2019). The features of molecular techniques have quickness and correctness, which have major benefits for particular recognition for various species of nematodes (Callaghan and Beh, 1994; Roeber *et al.*, 2011).

The molecular methods established to be sensitive for the identification of amplified DNA amongst pools of eggs, larvae, or adult worms (Santos *et al.*, 2020). Second internal transcribed spacer region -2 (ITS-2) genes was targeted for the recognition of the species. ITS-2 has been extensively used and has become an applicable tool for species recognition because it is highly specific for species, has more conservative regions and has universal primers binding to the 5.8S and 28S ribosomal DNA genes against the various nematodes (Heise *et al.*, 1999).

In present study the overall prevalence of strongyle infection in goat was recorded 32.22%. The similar findings were also observed by various previous researchers. Zvinorova *et al.* (2016) was reported the prevalence of strongyles (31%) in five agro ecological regions of Zimbabwe in the dry and wet seasons. Das *et al.* (2017) reported the prevalence of Strongyle spp. (32.63%) in two districts of Meghalaya. Sebro *et al.* (2022) observed the prevalence of Strongyles (26.63%) gastrointestinal parasites of sheep and goats in AnLemo, Hadiya Zone of Southern Ethiopia. Contrary some researchers observed high prevalence of Strongyle in comparison to present result. Kalule *et al.* (2023), reported prevalence of

strongyle was 97.5% in Uganda. Singh *et al.* (2014) reported the prevalence of strongyles was 69.27% in Balaghat, Narsinghpur and Chhindwara in Madhya Pradesh, India. Rajpoot *et al.* (2017) reported the prevalence rate of strongyle was 65% in goats from Malwa region of Madhya Pradesh. Dissimilarity in the prevalence of Strongyle nematodes in goats are based on differences in various factors such as environmental and host factors. Environmental factors consist of climatic situations, practices of animal husbandry like system of housing, feeding and watering, management schedule of deworming, levels of sanitation and management of pasture (Ratanapob *et al.*, 2012). These factors in large extent decide the prevalence, kind and seriousness of several diseases of parasites (Badran *et al.*, 2012). Other risk factors were included the species of host, sex of the animal, age, body conditions, breed and genetic constitutions (Badaso and Addis, 2015). Season also influence the occurrence and severity of the parasitic disease in goats. Levels of low infection rate may be ascribed to the solitary host's potential to prevent infection or tolerant a certain extent of infections without exhibiting the susceptibility (Zvinorova *et al.*, 2016).

In present study the highest prevalence rate was recorded in *Haemonchus contortus* 54.16%, followed by *Trichostrongylus* spp. 31.66% and lowest in *Oesophagostomum* spp. 14.16%. This result was different from Hassan *et al.*, 2017 who reported the prevalence of *Haemonchus contortus* 47%, *Trichostrongylus* spp. 4% and *Oesophagostomum columbianum* zero % in goats from Nyala Area South Darfur State of Sudan. On the other hand the prevalence of *Trichostrongylus axei* was 87.5% in the goat in Dakahlia governorate, Egypt by employing molecular methods (Elseadawy *et al.*, 2021). However, another study reported that the prevalence of *H. contortus* 41 to 54%, *Trichostrongylus* spp. 9 to 13% and *Oesophagostomum* spp. 7 to 14% in goats of Uttarakhand (Sankar *et al.*, 2020).

The major prevalent nematodes were Strongyles, in which occurrence of *Haemonchus* was higher in comparison to others (Zvinorova *et al.*, 2016). Various earlier research on gastrointestinal helminths epidemiology were also observed that presence of *Haemonchus* was predominant among the other nematode (Tsetetsi and Mbatia, 2003; Ntonifor *et al.*, 2013; Bakunzi *et al.*, 2013). The higher prevalence of *Haemonchus* can be attributable that capacity of adult females are high regarding the productions of thousands of eggs in each

day that can result into fast contamination pasture land by larvae. This may be correlated haemonchosis epidemic (Roeber *et al.*, 2013b).

The climatic situations play an important role due to that the parasites have excessive biotic capability and pathogenicity which increase rapidly the issue in humid tropics and subtropics parts of the world (Waller and Chandrawathani, 2005). Additionally, one disadvantage of *Haemonchus contortus* that it is having considerable potential to evolve resistance against the anthelmintic drugs (Kotze and Prichard, 2016), which give rise to a problem regarding the prevention and control. It has been suggested that the prevalence of different species documented in literature can be describe in various geographical distribution, factors of the host and situations of climate needed for the growth of free-living stages in various nematodes (Zvinorova *et al.*, 2016).

#### **5.4 Aqueous extraction yield percentage of medicinal plants**

In present study the highest aqueous extract yield percentage was obtained from the bulbs of *Allium sativum* of 19.75%, while lowest was obtained from the seeds of *Coriandrum sativum* of 8.25%. Eguale *et al.* (2007) reported 7.75% yield of aqueous extract from the seeds of *Coriandrum sativum*. Orengo *et al.* (2016) reported a yield of 18.25% aqueous extract from the bulbs of *Allium sativum*. Muundaa *et al.* (2020) recorded an aqueous extraction yield of 19.8% from the seeds of *Carica papaya* while Adeneye *et al.* (2009) reported aqueous extraction yield of 22.5% from the seeds of *Carica papaya*. These percentage yields were compared to present study. Dixit *et al.* (2019) reported a yield of 21.96% aqueous leaves extract from *Azadirachta indica*.

This study observed variations in the yield percentages of extracts among the different plants. These variations in the percentage yield may be due to various factors, such as the inherent nature of the plant species, disparities in the chemical composition of the extracts, distinct environmental conditions leading to variations in phytochemical constitution and differences in harvest time. Moreover, the choice of solvents and test protocols during the extraction could also contribute to differences in the concentrations and classes of secondary bioactive found in extracts (Marie-Magdeleine *et al.*, 2009).

## 5.5 *In vitro* anthelmintic efficacy of medicinal plants

### 5.5.1 Egg hatch assay (EHA)

The heightened recognition of the potential health risks associated with anthelmintic drug residues in food, coupled with the escalating prevalence of anthelmintic resistance in domestic animals has spurred a greater interest in exploring the therapeutic potential in medicinal plants and their secondary metabolites. This pursuit aims to develop safer alternatives to conventional treatments (Buza *et al.*, 2020). This study is aimed to assess *in vitro* anthelmintic efficacy of aqueous extracts derived from *Coriandrum sativum* seeds, *Allium sativum* bulbs, *Carica papaya* seeds, *Zingiber officinale* rhizomes, *Azadirachta indica* leaves, *Swertia chirata* whole plants and *Prunus persica* leaves against strongyle in goats.

Increasing the concentration of the plant extracts led to a greater inhibition of egg hatching indicating a dose-dependent activity as observed in current study. Similar findings were reported by Ahmed *et al.* (2020) where a significant inhibition in egg hatching effect from plant extracts in a concentration dependent trend.

Laribi *et al.* (2015) demonstrated pharmacological effects of *Coriandrum sativum* including antioxidants, antimutagenic, antidiabetic, anthelmintic, anxiolytic, hepatoprotective properties and anticonvulsant. Debella *et al.* (2007) reported that both aqueous and hydro-alcoholic extracts of *Coriandrum sativum* exhibited anthelmintic activity. In present study highest percentage of aqueous extract of seeds of *Coriandrum sativum* exhibited in egg hatching on strongyle egg was 86.94% at concentration of 100 mg/ml and lowest was 27.49% at concentration of 12.5 mg/ml. Eguale *et al.*, 2007 reported that aqueous extracts of *Coriandrum sativum* showed significant inhibition of egg hatching, with complete (100%) inhibition of egg occurring at a concentration below 0.5 mg/ml. The estimated effective dose (ED<sub>50</sub>) was found to be 0.12 mg/ml.

Allicin predominantly found in aqueous garlic extract and raw garlic products, is considered the primary bioactive compound (Elosta *et al.*, 2017). These compounds have potentially to form disulfide bonds with free thiol groups, thereby inhibiting enzymes or other proteins crucial for parasites survival (Krstin *et al.*, 2018). In present study the percentage inhibition of aqueous extract of bulb of *Allium sativum* exhibited in egg hatching on strongyle eggs were 23.02%, 29.90%, 57.39% and 80.75% at the concentration of 12.5 mg/ml, 25

mg/ml, 50 mg/ml and 100 mg/ml respectively. A higher percentage of egg inhibition was observed by Kanojiya *et al.*, (2015), recorded that aqueous extract of *Allium sativum* bulbs exhibited 100% inhibition of egg hatching in naturally occurring ovine gastrointestinal nematodiosis at 200, 100, 50, 25 and 12.5 mg/ml concentrations.

Papaya seeds possess various pharmacological activities, including anthelmintic, contraceptive, anti-fertility, anti-analgesic, inflammatory and antimicrobial properties (Agarwal *et al.*, 2016). Kermanshai *et al.* (2001) conducted a study where benzyl isothiocyanate isolated from *C. papaya* Linn. seeds extracts was investigated for its anthelmintic activity using a viability assay with *Caenorhabditis elegans*. This study identified benzyl isothiocyanate as the primary anthelmintic agent. In present study the aqueous extract of seeds of *Carica papaya* inhibited the egg hatching of strongyle were 12.71%, 14.77%, 39.51% and 62.20% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. A higher percentage of inhibition was reported by Odhong *et al.* (2014) where a reduction of more than 95.8% in egg hatch at concentration of 500 mg/ml for aqueous extract *C. papaya* seeds. The probable reason for higher inhibition may be higher concentration of aqueous extract.

In present study the aqueous extract of rhizomes of *Zingiber officinale* inhibited the egg hatching of strongyle were 19.25%, 26.46%, 53.26% and 77.66% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. Abdullahi *et al.* (2017) identified phytochemical constituents in *Zingiber officinale* including flavonoids, tannins, saponins, phenols and terpenoids which exhibits an anthelmintic property. Van *et al.* (2021) conducted *in vitro* tests and observed that ginger exhibited a dose-dependent antiparasitic effect against *Dactylogyrus* spp.

In present study the percentage inhibition of egg hatching of aqueous extract of leaves of *Azadirachta indica* on strongyle eggs were 15.12%, 17.87%, 45.70% and 72.85% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. As concentration of extract was increased percent of inhibition was also increased. Similar findings were reported by Okwudiri *et al.* (2006) who reported that the highest inhibition was observed at the concentration of 100 mg/ml of the extracts reaching 51% and 50% for the leaf and stem bark extracts respectively. The anthelmintic properties of neem are likely due to the

presence of an active alkaloid, azadirachtin, which disrupts the CNS of the parasites by inhibition excitatory cholinergic transmission and partially blocking the calcium channel leading to the expulsion of parasites from the host body (Qiao *et al.*, 2013; Veerakumari and Priya, 2006).

Aqueous extract of whole plant of *Swertia chirata* exhibited highest percentage of inhibition in egg hatching on strongyle was 93.12% and lowest 43.64% at the concentration of 100mg/ml and 12.5mg/ml respectively. Aleem and Kabir (2018) documented the therapeutic significance across all components of the chirata plant. Sinha *et al.* (2023) further elucidated that chemical compounds within *Swertia chirata*, notably amarogentin and sweroside play a pivotal role in the anthelmintic activity along with other observed biological effects.

Aqueous extract of leaves of *Prunus persica* exhibited highest percentage of inhibition in egg hatching on strongyle was 58.07% and lowest 8.94% at the concentration of 100 mg/ml and 12.5 mg/ml respectively in the current study. Kazan *et al.* (2014) found that *Prunus persica* leaves exhibited pharmaceutical potential and are traditionally used as antihelminthics, sedative and laxative. Kumar *et al.* (2015) tested the efficacy of leaves of *P. persica* against helminths, particularly, targeting *Pheretima posthuma*, which similar in anatomy and physiology to intestinal roundworms found in humans, and *Ascaridia galli*, a roundworm that presents significant challenges in the poultry industry.

The variations observed between the results of the egg hatch assay and larval paralysis assay for plant extracts have prompted to hypothesize that certain plants may show pronounced ovicidal effects while others exhibit potent larvicidal effects. To attain potent anthelmintic activity, encompassing both ovicidal and larvicidal properties, it is imperative to grasp the underlying mechanisms action of the secondary metabolites within these plants. Once a plant has demonstrated efficacy *in vitro*, it becomes crucial to conduct further *in vivo* testing to validate the findings and assess potential risks, side effects, and prospects for future applications (Chantawannakul *et al.*, 2005).

### **5.5.2 Larval paralysis test**

Demonstrating anthelmintic activity in the various life cycle stages of the parasite is highly valuable. This is because it hypothetically reduces the likelihood of the parasite

developing resistance when using extract as routine therapy, due to its anthelmintic effects on multiple stages of development (Hounzangbe-Adote *et al.*, 2005).

*In vitro* findings have revealed the anthelmintic potential of aqueous extracts derived from various plant sources, including *Coriandrum sativum* seeds, *Allium sativum* bulbs, *Carica papaya* seeds, *Zingiber officinale* rhizome, *Azadirachta indica* leaves, *Swertia chirata* whole plants and *Prunus persica* leaves, exhibited a dose-dependent efficacy against *strongyle* larvae. The effect was comparable to that of noted drug closantel. Davuluri *et al.* (2019) elucidated that paralysis of larvae was dose dependent of hydro alcoholic extracts of 3 plants.

In the percent study aqueous extract of seeds of *Coriandrum sativum* inhibited the larval motility of 35.07%, 62.85%, 78.48% and 89.93% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. As concentration of extract increased percent of inhibition of larval motility was also increased. Jaiswal *et al.*, (2020b) conducted an experiment where in aqueous extracts of *Coriandrum sativum* seeds were tested on *Haemonchus* spp. at a concentration of 1 mg/ml, 2.5 mg/ml, 5 mg/ml, and 10 mg/ml. Reported shortest time ( $388.3 \pm 3.8$ ) of paralysis was at the highest concentration of 10 mg/ml of aqueous extracts.

In the present study aqueous extract of bulbs of *Allium sativum* inhibited the larval motility of 22.23%, 43.40%, 58.68% and 79.48% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. As concentration of aqueous extract of *Allium sativum* was increased percent of inhibition of larval motility was also increased. Similar dose- dependent finding was reported by Kanojiya *et al.* (2015). They also found that the aqueous extract of bulb of *Allium sativum* induced 100% paralysis of L3 stage larvae at concentrations of 200 and 100 mg/ml in naturally occurring gastrointestinal nematodiosis in ovine.

Aqueous extract of seeds of *Carica papaya* exhibited the inhibition in larval motility were 13.19%, 23.27%, 31.60% and 57.64% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively in the percent study. A higher percentage was reported by Sen *et al.* (2020) of aqueous extract of *C. papaya* against deshelled larvae of *A. galli* that was 93.33% at the concentration of 60 mg/ml. The probable reason of higher percentage may be

due to species variations. The aqueous extract *C. papaya* achieved 100% effectiveness in killing adult *A. galli* worms at a concentration of 20 mg/ml in poultry (Sen *et al.*, 2020). Notably, this anthelmintic effect exhibited by the extract was found to be contingent on both concentration and duration of exposure.

Aqueous extract of rhizomes of *Zingiber officinale* exhibited the inhibition in larval motility were 19.44%, 31.60%, 52.78% and 73.61% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively in the present study. Percentage inhibition was observed dose-dependent. Similar finding was reported by Van *et al.* (2021) *in vitro* tests of anti-parasitic effect of ginger's against *Dactylogyrus* spp. Marwa *et al.* (2024) observed that aqueous extract of *Zingiber officinale* exhibited paralysis times of 1.22 hours, 1.08 hours and 1.0 hours at concentrations of 20 mg/ml, 40 mg/ml and 60 mg/ml respectively. The time of death was recorded as 1.5 hours at all concentrations against the earthworms. The efficacy of the extracts inducing paralysis and death depended on increase concentration.

Aqueous extract of leaves of *Azadirachta indica* exhibited the inhibition in larval motility were 15.98%, 26.73%, 41.32% and 61.81% at the concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml respectively in the present study. Percentage inhibition was observed dose-dependent. Similar finding was also reported by Nwosu *et al.* (2006). Nwosu *et al.* (2006) also found that the highest inhibition observed at a concentration of 100 mg/ml of the extracts resulting in 51% and 50% reduction for the leaf and stem bark extracts of *Azadirachta indica* respectively against the nematode larvae. Ibekwe (2019) reported that the anthelmintic activity of *Azadirachta indica* was dependent on both time and concentration.

In the current study highest percentage of inhibition of larval motility was recorded in aqueous extract of whole plant of *Swertia chirata* at all the tested concentration. The highest percent of inhibition was 94.10% at the concentration of 100 mg/ml and lowest percentage of inhibition was 47.40% at the concentration of 12.5 mg/ml. *Swertia chirata* is employed as a bitter tonic to alleviate gastrointestinal disorders and is also known for its effectiveness against intestinal worms, bronchial asthma, body burns, and bowel regulation (Khanal *et al.*, 2014).

In the current study the lowest percentage of inhibition of larval motility was recorded in aqueous extract of leaves of *Prunus persica* at all the tested concentration. The highest

percent of inhibition was 46.47% at the concentration of 100 mg/ml and lowest was 9.03% at the concentration of 12.5 mg/ml.

Hydro alcoholic plant extracts demonstrate significantly greater anthelmintic efficacy compared to aqueous extracts (Kamaraj *et al.*, 2010). This superiority arises from the higher concentration of the alcohol soluble active molecules present in them, facilitating easier transcuticular absorption of extracts into the parasite's body. The anthelmintic properties of these medicinal plants are primarily stem from the presence of various secondary metabolites including tannins, alkaloids, flavonoids, and triterpenoides (Niezen *et al.*, 1995). These secondary metabolites exert their effects by disrupting the energy-generating - pathways, of the parasites ultimately resulting in paralysis and subsequent death (Veerakumari and Munuswamy, 2000). Significant anthelmintic effect was observed in seven selected plant extracts; however variations were noticed depending on the stage of a parasite. The study findings suggested that these plants could be serving as promising alternative anthelmintic for controlling of strongyle infections in goats. Nevertheless, further steps such as identifying the active compounds in these plants extracts, estimating *in vivo* bioavailability and studying their toxicity are essential before establishing their use in therapy.

### **5.5.3. Adult worm mortality assay**

The challenge of anthelmintic resistance, toxicity and growing apprehension about drug residue in animal products has results into a renewal of interest in the use of plant based remedies (Zenebe *et al.*, 2017). *In vitro* assays employing the free living stages of parasitic nematodes provide a valuable method for assessing the anthelmintic activity of new plant compounds (Asase *et al.*, 2005). Furthermore, *Haemonchus contortus* has been proved as an excellent test worm for *in vitro* studies due to its prolonged survival in PBS and its widespread prevalence worldwide. This abomasal helminth has recently been utilized *in vitro* by other workers (Zenebe *et al.*, 2017). Various extracts derived from plants exhibited capabilities for development as anthelmintic remedies (Ndlela *et al.*, 2021; Soren *et al.*, 2020).

*In vitro* findings demonstrated significant anthelmintic effect from *Coriandrum sativum* seeds, *Allium sativum* bulbs, *Carica papaya* seeds, *Zingiber officinale* rhizomes, *Azadirachta indica* leaves and *Swertia chirata* whole plants and *Prunus persica* leaves on *H. contortus* showing a dose-dependent pattern and the effect was comparable to that of

closantel. Moreover, the study indicated that mortality of adult *Haemonchus contortus* with escalating dosages and exposure duration following treatment with tested plant extracts. These results align with prior researchers by Avinash *et al.* (2017), in which mortality effect of plant materials was depended on the dosage and exposure durations.

The aqueous extracts of various plant extracts exhibited differing percentage of mortality in *Haemonchus contortus* in the current study. The variance in mortality percentage among could be attributed to variations in the concentrations of secondary metabolites present in their extracts (Mumed *et al.*, 2022).

Aqueous extract of seeds of *Coriandrum sativum* exhibited the 90% of mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments in the present study. Eguale *et al.* (2007) reported that aqueous extracts of *C. sativum* exhibited 45% mortality of *Haemonchus contortus* collected from infected sheep at the concentration of 8 mg/ml after 24 hours post treatments. The probable reasons for variation in results may be due to variation in species and duration of treatments.

Aqueous extract of bulbs of *Allium sativum* showed 66.67%, of mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments in the present study. As concentration of extracts and exposure time was increased percent of mortality of *Haemonchus contortus* was also increased. *In vitro* result exhibited that a significant anthelmintic activity of aqueous extracts of *Allium sativum* was found at 16 hours with lowest concentration (10%) and at 10 hours with the highest concentration (50%) against *Ascaridia galli* (Singh, 2014). Krstin *et al.* (2018) reported that antiparasitic activity of *A. sativum* can be due to the sulfur containing compounds like ajoene and allicin. These compounds have potentially form disulfide bonds with free thiol groups, thereby inhibiting enzymes or other proteins crucial for parasites survival. Lee (1996) observed that ethanolic extract of *A. sativum* hampers the motility of *H. contortus* by exerting a destructive and inhibitory effect on the enzyme acetyl cholinesterase. This enzyme plays a vital role in hydrolyzing acetylcholine a neurotransmitter involved in cholinergic synaptic transmission.

The aqueous extract of seeds of *Carica papaya* showed 53.33%, of mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments in the current study. Benzyl isothiocyanate is main constituents found in papaya seeds has exhibits

anthelmintic properties as reported by Rumiya (2006). Yadav and Tangpu (2006) conducted a study where they examined the efficacy of an aqueous extract derived from *Carica papaya* seeds against *Ascaris lumbricoides* and *Ascaridia galli*. Their findings demonstrated significant anthelmintic properties in the extract, suggesting its effectiveness in combating these parasitic infections.

In present study aqueous extract of *Zingiber officinale* exhibited 43.33% mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments. Mumed *et al.* (2022) reported that methanolic extract of rhizomes of *Zingiber officinale* exhibited 50% mortality and 90% mortality of *Haemonchus contortus* at concentration of 125 mg/ml after 4 and 6 hours exposure. Abdullahi *et al.* (2017) reported several phytochemical constituents in *Zingiber officinale* that exhibit anthelmintic properties including flavonoids, tannins, saponins, phenols and terpenoids. Dubey *et al.* (2010) observed that the aqueous extract of the rhizomes of *Zingiber officinale* exhibited paralysis against *Pheretima posthuma* occurred within 32 to 34 minutes, 28 to 31 minutes, and 25 to 29 minutes, respectively, at concentrations of 25, 50, and 100 mg/ml. Subsequent death occurred at 86 minutes, 78 minutes, and 65 minutes, respectively, at the same concentrations.

Aqueous extract of leaves of *Azadirachta indica* exhibited 76.67% mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments. Qiao *et al.* (2013) suggested that the anthelmintic properties of neem may be attributed to the presence of an active alkaloid, azadirachtin. This compound is thought to disrupt the central nervous system of parasites by inhibiting excitatory cholinergic transmission and partially blocking calcium channels, ultimately leading to the expulsion of parasites from the host body.

In the present study aqueous extract of whole plant of *Swertia chirata* exhibited highest (100%) mortality of *Haemonchus contortus* among the all test plant extract at the concentration of 400 mg/ml after 8 hours post treatments. Khanal *et al.* (2014) reported that *Swertia chirata* is also effective against intestinal worms. Iqbal *et al.* (2006) reported that *in vitro* studies on anthelmintic properties of *Swertia chirata* have demonstrated that at a concentration of 25 mg/ml the crude aqueous extract (CAE) derived from *S. chirata* whole plants exhibited an anthelmintic effect against live *Haemonchus contortus*.

In the present study aqueous extract of leaves of *Prunus persica* exhibited 36.67% mortality of *Haemonchus contortus* at the concentration of 400 mg/ml after 8 hours post treatments. The leaves of *Prunus persica* possess anthelmintic was reported by Aziz and Rahman (2013). Among the tested plant extract leaves of *Prunus persica* exhibited lowest percentage of mortality against the *Haemonchus contortus*.

The variations observed among these studies could stem from difference in the solvents utilized for extraction. Research has demonstrated that organic solvent extracts exhibit higher biological activity compared to aqueous extracts (Chanda and Parekh, 2007). Hydro-alcoholic plant extracts demonstrate significant anthelmintic efficacy compared to aqueous extracts (Kamaraj *et al.*, 2010) because they contain a high concentration of the alcohol-soluble active molecules. These molecules facilitate easier transcuticular absorption of extracts into parasite's body.

Due to the biotransformation of drugs potential interactions with food materials, and variations in absorption, the results obtained through *in vitro* methods may not accurately reflects *in vivo* activity. Therefore, it is essential to confirm these results through *in vivo* evaluation (Zenebe *et al.*, 2017). After demonstrating efficiency *in vitro*, additional *in vivo* testing becomes imperative to validate the findings, assess potential risks and side effects and gauge the plant's viability for future applications (Chantawannakul *et al.*, 2005).

## **5.6 Cytotoxicity study of selected medicinal plants**

Medicinal plants are distinguished by their capacity to synthesize a diverse array of bioactive chemical substances or their precursors, which can carry out specific physiological functions (Sofowora *et al.* 2013). Cellular cytotoxicity refers to a disruption of fundamental cellular processes those results in observable cell damage at a microscopic level (Cureño *et al.*, 2017). The cytotoxic effects of traditional extracts or remedies can be evaluated using various cell types (Misra *et al.*, 2013; Ajaiyeoba *et al.*, 2006). Tamokou and Kuete (2014) noticed that over 40% of the medicinal plants examined in their study were identified as potentially toxic. Many factors contribute to the toxicity of plant compounds, including the concentration used, duration of use, and route of exposure, among others (McGaw *et al.*, 2014). Differences in results may also be attributed to variability in their geographic growing area, growth stage, and collection season.

In present study, the half maximal inhibitory concentration ( $IC_{50}$ ) of the aqueous extract of leaves of *Prunus persica* was recorded 45.22 mg/ml. In a previous study by Koyu *et al.* (2020), the  $IC_{50}$  of *Prunus persica* leaves in HeLa cell line was reported greater than 50 $\mu$ g/ml. The  $IC_{50}$  of aqueous extract of rhizome of *Zingiber officinale* was found to be 67.12 mg/ml. Sharma *et al.* (2015), reported that the  $IC_{50}$  of aqueous extract *Zingiber officinale* in the vero cell line was 348.8 $\mu$ g/ml and further demonstrated that this concentration had no significant effects on the cell viability. This concentration was therefore utilized as the maximum concentration for all subsequent studies. In present study the aqueous extract of *Azadirachta indica* leaves showed cell growth inhibition in vero cell line 23.62%, 18.39% and 05.98% in the concentration of 50 mg/ml, 10 mg/ml and 1 mg/ml respectively and the  $IC_{50}$  was 116.46 mg/ml. The  $CC_{50}$  (50% cytotoxicity concentration) value of the methanolic extract of *Azadirachta indica* in the vero cell line was reported as 8000  $\mu$ g/ml (Joel *et al.*, 2019). The aqueous extracts of *Azadirachta indica* leaves were found to be non - cytotoxic (more than 80% viability) to cells at the highest tested concentration (1200 $\mu$ g/ml). This evaluation was conducted on WI38 cells, which are human noncancerous fibroblast cell lines (Tchetan *et al.*, 2022).

The  $IC_{50}$  value of the aqueous extract derived from the bulbs of *Allium sativum* was measured 92.72 mg/ml. Meanwhile, the toxicity of the aqueous extract from garlic leaves was assessed on Vero cells within a concentration range of 0.391-25.000 mg/ml. The observed toxicity ranged from 10.56%  $\pm$  1.67% to 97.08%  $\pm$  8.49%, with a 50% cytotoxic dose recorded at 9.628  $\pm$  0.036 mg/ml (Sirisa-ard *et al.*, 2023). The crude extract from seeds of *Carica papaya* was observed non-toxic to vero cells as indicated by Kateihwa *et al.* (2022). In fact, 1 mg/ml, the extract increased cell viability by approximately 43 % compared to the control value after 4 hours of incubation. Furthermore, Panzarini *et al.* (2014), suggest that aqueous extract of *C. papaya* seeds is not toxic and is more effective than effective than vitamin C in mitigating oxidative damage caused by  $H_2O_2$ .

The hexane and aqueous extracts of *Coriandrum sativum* was showed no cytotoxicity towards vero cells, as their values more than 20  $\mu$ g/ml. According to the MTT assay, both hexane and aqueous extracts were exhibited inhibition of vero cells with  $CC_{50}$  values of 600 $\mu$ g/ml and 700 $\mu$ g/ml respectively. The minimum inhibition of HepG2 (hepatocarcinoma)

cells was observed at a concentration of 350 µg/ml for all three extracts (Fayyad *et al.*, 2017). The aqueous extract of *Swertia chirata* whole plant depicted cell growth inhibition in vero cell line of 17.81%, 09.97% and 0.01% in the concentration of 50 mg/ml, 10 mg/ml and 1 mg/ml respectively and the IC<sub>50</sub> was 165.32 mg/ml. The methanolic extract of *Swertia chirata* exhibited significant activity in the Brine Shrimp Bioassay with a concentration of 199 ppm, as reported by Shrestha *et al.* (2015). However, *in vitro* cytotoxicity does not conclusively determine overall toxicity. Therefore, caution is necessary when administering even the least toxic plant extracts to animal models, with close monitoring of their performance (Abd'quadri-Abojukoro *et al.*, 2022).

### **5.7 *In vivo* anthelmintic efficacy of aqueous extracts of *Swertia chirata* and *Coriandrum sativum***

The effectiveness of synthetic anthelmintic drugs has declined due to their widespread and indiscriminate use, resulting in the emergence of anthelmintic resistance (AR). Consequently, it is crucial to identify alternative, safer anthelmintic agents to manage gastrointestinal parasites. Many medicinal plants have been traditionally employed to treat intestinal parasites in both humans and animals (Githiori *et al.*, 2005; Liu, 2020). Parasitic gastroenteritis caused by strongylosis presents a significant health threat and hampers the productivity of goats due to the high morbidity and mortality (Nwosu *et al.*, 2007). Therapeutic trials were conducted to assess the comparative efficacy of closantel and aqueous extract from seeds of *Coriandrum sativum* and whole plants of *Swertia chirata* against the naturally strongyle infected Barbari breed of goats.

The efficacy of the aqueous extract showed a dose-dependent response, with increased efficacy was observed as dosage was raised. This finding aligns with previous research by Prichard *et al.* (1978), suggested that the efficacy could be enhanced through higher doses or repeated treatment over several days. Based on the EPG (Eggs per gram) values and FECR (Faecal egg count reduction), closantel showed more efficacy compared to aqueous extracts of seeds of *Coriandrum sativum* and whole plants of *Swertia chirata*. Treatment with closantel at a dosage of 10 mg/kg body weight orally in goats against strongyle infection significantly reduced EPG counts by 100 % and achieved maximum faecal egg count reduction (100.00±0.00) on day 14 post -treatment in current study.

The anthelmintic efficacy of closantel is widely recognized, particularly for its rapid action compared to aqueous extract. Closantel, a salicylanilide drug, targets haematophagus parasite like *Haemonchus* species. Its mechanism of action involves disrupting oxidative phosphorylation in the mitochondria of cells, thereby hindering ATP production. This disruption occurs by inhibiting the activity of succinate dehydrogenase and fumarate reductase crucial enzymes involved in ATP synthesis, leading to impaired parasites motility. Additionally, closantel also interferes with transport mechanisms of liquids and ion across the parasite's membranes (Westers *et al.*, 2016). Singh *et al.* (2022) observed a significant decrease in EPG (91.12 %) following treatment with closantel at 10 mg/kg body weight orally against strongyle infection in goats. Dixit *et al.* (2019) reported an overall efficacy of 95.64% for closantel in goats against fenbendazole-resistant *Haemonchus contortus* using faecal egg count reduction test (FECRT). Parmar *et al.* (2020) highlighted high efficacy of closantel specifically against *Haemonchus*, suggesting its use as an alternative to benzimidazoles could reduce pasture contamination. The combined use of closantel and benzimidazoles may offer comprehensive control of gastrointestinal nematodiasis.

The efficacy of the aqueous extracts from whole plants of *Swertia chirata* at a dosage of 500 mg/kg body weight was found to be superior to that of the aqueous extract from seeds of *Coriandrum sativum* at the same dosage. The efficacy of both aqueous extract of whole plants of *Swertia chirata* and seeds of *Coriandrum sativum* at a dosage of 500 mg/kg body weight showed better response from day 7 to day 28. No physical or clinical signs of toxicity were observed in the goats across the various doses administered during the experimental periods using the aqueous extract of seeds of *Coriandrum sativum* and whole plants of *Swertia chirata*. The present study indicated that higher concentrations of these extracts were associated with greater efficacy compared to lower concentrations against the naturally occurring strongyle infection in goats of Barbari breed.

The reduction in EPG indicates the effectiveness of both aqueous extract against strongyle infections in Barbari breed of goats. Negative FECR results were recorded @ 50 mg/kg body weight of the aqueous extract of *Coriandrum sativum* seeds on days 14, 21 and 28 post-treatments. Similarly, negative FECR was observed @ 250 mg/kg body weight of aqueous extract of *Coriandrum sativum* seeds on day 28 post- treatments. Negative FECR

results were observed of aqueous extract of the whole plant of *Swertia chirata* @ 50 mg/kg body weight on days 21 and 28 post-treatment. A negative FECR indicates an increase rather than a decrease in egg counts following treatment in naturally infected group. This outcome suggests that the plant extract administered was ineffective in reducing the parasites egg counts.

*Coriandrum sativum* has demonstrated pharmacological effects such as antioxidants, antimutagenic, antidiabetic, anthelmintic, anxiolytic, anticonvulsant and hepatoprotective properties (Laribi *et al.*, 2015). These effects are likely mediated by potent antioxidant activity of this plants and its primary constituents, linalool (Sobhani *et al.*, 2022). Both aqueous and hydro-alcoholic extracts of *Coriandrum sativum* demonstrate anthelmintic activity (Debella *et al.*, 2007). The antiparasitic action of *Coriandrum sativum* can be attributed to its main bioactive compounds, namely terpenoids, which are presents in its essential oils (Laribi *et al.*, 2015; Hosseinzadeh *et al.*, 2016; Kumar and Subrahmanyam, 2013). Coriander is known for its low toxicity in both humans and animals, even when consumed in large amounts (Singletary, 2016). In their study, Eguale *et al.* (2007) administered crude aqueous extracts of *Coriandrum sativum* seeds at doses of 0.45g/kg body weight and 0.9 g/kg body weight to sheep infected with *Haemonchus contortus*. They observed no physical or clinical sign of toxicity in any groups of sheep treated with the aqueous extract of *Coriandrum sativum* seeds.

In the present study, it was found that a higher dose of the aqueous extract of *Coriandrum sativum* seeds exhibited greater efficacy *in vivo* compared to lower dose. Similar finding was reported by Eguale *et al.* (2007), who observed that *in vivo* efficacy of aqueous extract of a higher dose of *Coriandrum sativum* aqueous extract against *Haemonchus contortus* infection in sheep as assessed by FECRT on day 2 post- treatment did not persist over time. This effect may be attributed to the impact of extract on fecundity of female parasites during the initial days when the extract concentration was high in the animals (Athanasiadou *et al.*, 2001).

Orally administered aqueous extract of seeds of *Coriandrum sativum* at the dose rate of 0.9 g/kg body weight to sheep infected with *Haemonchus contortus* there was significant reduction in faecal egg count with coriander extract compared to the positive control group

(Ahmed *et al.*, 2019). Hosseinzadeh *et al.* (2016) administered various doses of alcoholic seed extracts from *Coriandrum sativum* @ 250, 500, and 750 mg/kg body weight to White Balb/c mice. They observed that efficacy of 500 and 750 mg/kg body weight of *Coriandrum sativum* achieved 100 % effectiveness against the *Hymenolepis nana* infection 15 days after treatment.

*Swertia chirata* exhibits significant medicinal properties including anti-inflammatory, hepatoprotective, hypoglycemic, antibacterial and wound healing effect (Laxmi *et al.*, 2011). It also demonstrates antioxidant, antispasmodic, antipyretic anti-diabetic, and antitussive activities (Tabassum *et al.*, 2012; Saha *et al.*, 2004). According to Chakravarty and Mukhopadhaya (1992), *S. chirata* has been suggested to possess anthelmintic properties. It is also effective against intestinal worms, body burns, bronchial asthma and bowel regulating (Khanal *et al.*, 2014). All parts of the chirata plant hold therapeutic value (Aleem and Kabir, 2018). Chemical constituents of *Swertia chirata* such as amarogentin and sweroside contribute to its anthelmintic activity along with other biological effects (Sinha *et al.*, 2023).

Iqbal *et al.* (2006) administered crude aqueous extract of *Swertia chirata* @ 3 g/kg body weight to sheep naturally infected with a various gastrointestinal nematodes. They observed 34 % reduction in egg count per gram of faeces on day 14 post-treatment. Arora *et al.* (2009) reported that oral administration of aqueous extract of *Swertia chirata* seeds @ 550 mg/kg body weight for 10 consecutive days resulted in 62.5% anthelmintic efficacy against gastrointestinal nematodes in goats by day 12 of the experiment. Jain and Sahni, (2009) reported that crude powdered and cold aqueous extracts of *S. chirata*, administered orally at a dosage of 500 mg/kg, body weight administered for seven consecutive days, exhibited anthelmintic efficacy ranging from 70 % to 90 % against various gastrointestinal nematodes. Among the treatments tested, *Swertia chirata* demonstrated the highest effectiveness against *Trichostrongyle* spp., achieving an efficacy of 86.88 %.

In the present study, on day 28 post-treatment, FECR reduction was recorded in goats treated with aqueous extract of *Coriandrum sativum* seeds and the whole plants of *Swertia chirata* @ 500 mg/kg body weight and these were  $40.38 \pm 0.14$  and  $60.65 \pm 0.44$  respectively. The variation in reduction of faecal egg count observed in various studies may be attributed to differences in solvents used, host species, dosage and timing of assessment post-treatment. Kumar *et al.* (2023) observed variability in mean EPG values for Haemonchosis in goats in

their study. This variability might stem from the genetic makeup of individual animals and the environment conditions to which they are exposed (Gadahi *et al.*, 2009).

High faecal egg count indicates an elevated presence of adult parasites in reproductive status within the digestive system of host (Chiejina *et al.*, 2002; Pralomkarn *et al.*, 1997). The extraction procedure can significantly influence the concentration of active substance. In traditional medicine, plants preparation is commonly extracted using water (Githiori *et al.*, 2004). The reported anthelmintic properties have shown variability due to differences in plant material sources, seasonal variations, environmental factors; extraction methods and host species (Athanasiadou *et al.*, 2007; Chandrawathani *et al.*, 2006; Costa *et al.*, 2006).

### **5.8 Haematological observations in goats treated with aqueous extracts of *Coriandrum sativum* seeds and whole plants of *Swertia chirata***

Gastrointestinal parasites not only cause morphological changes in the gut lumen and tissue injury but also lead to reduction in feed digestibility, along with range of haematological and biochemical alterations (Jesse *et al.*, 2019; Mpofu, *et al.*, 2020a). Haematological studies are crucial for diagnosing various diseases and can serve as a valuable tool for detecting early sign of toxicity affecting the cellular components of blood due to natural or chemical agents (Etim *et al.*, 2014). Certain gastrointestinal parasites, particularly strongyles, are blood-suckers. In case of heavy parasitic infestations, they can induce anaemia (Ahmed *et al.*, 2015) along with other hematological and biochemical disturbances (Moudgil, *et al.*, 2017; Wamboi, *et al.*, 2020).

After 28 days of treatment with closantel @ 10 mg/kg body weight there was a significant increase in mean values of Hb (from  $8.47 \pm 0.10$  to  $10.82 \pm 0.11$ ), PCV (from  $26.67 \pm 0.49$  to  $33.50 \pm 0.43$ ) and TEC ( $8.30 \pm 0.17$  to  $10.73 \pm 0.17$ ). Additionally, there was significant decrease in eosinophil counts from  $7.17 \pm 0.40$  to  $3.00 \pm 0.26$ . After 28 days of treatment with aqueous extract of seeds of *Coriandrum sativum* at dosage of 500 mg/kg body weight there was significant increase in mean values of Hb (from  $9.12 \pm 0.19$  to  $9.42 \pm 0.14$ ), PCV (from  $28.50 \pm 0.89$  to  $30.50 \pm 0.85$ ) and TEC (from  $8.92 \pm 0.18$  to  $9.13 \pm 0.14$ ). Additionally, there was significant decrease in eosinophil counts from  $7.50 \pm 0.34$  to  $6.50 \pm 0.43$ . After 28 days of treatment with aqueous extract of seeds of *Swertia chirata* @ 500 mg/kg body weight there was significant increase in mean values of Hb (from  $8.77 \pm 0.17$  to  $9.38 \pm 0.15$ ), PCV (from

26.83±0.79 to 30.33±0.76) and TEC (from 8.60±0.17 to 9.17±0.14). Additionally, there was significant decrease in eosinophil counts from 7.67±0.33 to 5.50±0.43.

The present study revealed that goats suffering from strongyle infection had significantly lower levels of Hb, PCV and TEC ( $p < 0.05$ ). These findings align with previous researchers (Sarkar *et al.*, 2024; Moudgil *et al.*, 2017; Alam *et al.*, 2020). Ashfaque *et al.* (2016) similarly reported a significant decrease in TEC, PCV and Hb in goat infested with helminth parasites. The reduction in these haematological parameters among goats infested with nematodes attributed to the parasite's blood sucking ability and a consequent losses due to gastrointestinal mucosal damage (Urquhart *et al.*, 1996).

Rapid blood loss in affected goats with haemonchosis is primarily caused by the blood-sucking nature of the worms (Besier *et al.*, 2016). Significant increase in Hb, PCV and TEC in both positive control group and group T3, indicating the efficacy of aqueous extract of *Coriandrum sativum* seeds, whole plants of *Swertia chirata* at a dosage of 500 mg/kg body weight, as well as treatment with closantel in this study. Sunder *et al.* (2022) suggested that lower values of Hb, TEC, PCV in goats infested with gastrointestinal parasites are due to acute blood loss from the parasites blood-sucking activity and leakage through the damaged gastrointestinal tract caused by the parasites. The decrease in haemoglobin levels is attributed to blood-sucking helminths like strongyles. Heavy worm infestations typically lead to anaemia, caused by both migrating larvae and adult worms. The reduced PCV values may result from direct blood loss due to blood-sucking activities of the helminth and concurrent haemorrhages caused by the parasites.

Haematological analysis revealed significant variations in total leukocyte count (TLC), neutrophils and lymphocytes. The increase in TLC value has been documented by Ashfaque *et al.* (2016); Ahmed *et al.* (2015) and Jas *et al.* (2008). The elevated TLC levels may be attributed to heightened local immune response involving eosinophils and monocytes (Ahmed *et al.*, 2015). Estabén (1968) suggested that possible reasons for decrease in lymphocyte count could result from factors such as malnutrition, stress condition and subsequent lymphoid atrophy which impair lymphopoiesis. Ahmed *et al.* (2015) further suggested that the increase in TLC levels might stem from enhanced local immune response against the parasites involving eosinophils.

The current study observed an increase in eosinophil count before treatment, consistent with finding from previous researchers by Aziz and Mahmoud (2021) and Al-Bayati *et al.* (2023). Ashfaque *et al.* (2016) also reported a significant rise in total eosinophil counts in goats suffering from helminth parasites infestations. Eosinophilia is associated with cell-mediated immunity and phagocytic activity, which aid in breaking down particulate matter and parasite debris during persistent infections (Awad *et al.*, 2016). Eosinophils are considered pivotal in responding nematodes infestations (Balic *et al.*, 2000). Animal's infested with nematodes exhibited a significant degree of blood eosinophilia, compared to non-infested animals. Eosinophils mobilized in response to specific parasites have been observed to induce immobility and death of larvae from homologous or heterologous parasites, often in conjunction with antibodies or other factors (Rainbird *et al.*, 1998). Increase levels of IgE in parasitized individuals trigger mast cell degranulation, which stimulate release of eosinophil chemotactic factor of anaphylaxis, thereby, mobilizes the body eosinophil pool and leading to an increased circulation of eosinophil (Tizzard, 1982). Eosinophils phagocytose the antigen- antibody complexes and possess antihistaminic properties.

The improvement in haematological parameters in goats was likely due to the reduction of blood-sucking parasites and other gastrointestinal infections over a time. Parameters such as Hb, PCV and TEC showed a progressive increase day by day. The effectiveness of aqueous extracts of medicinal plants indicated a stimulatory effect on the hemopoietic system. The increase in mean PCV after treatment may be linked to the rise of Hb %, as these parameters are closely interconnected. Goat treated with lower doses of plant extracts did not maintain or improved their haematological values as effectively as those treated with higher doses, likely due to a lesser reduction in worm burden.

### **5.9 Biochemical observations in goats treated with aqueous extracts of *Coriandrum sativum* seeds and whole plants of *Swertia chirata***

Parasitic infections can lead to a range of biochemical changes, which can vary depending on the parasites species, the site of invasion and the intensity of the infection (Esmailnejad *et al.*, 2012; De Oliveira *et al.*, 2013; Ortolani *et al.*, 2013). Infestations of strongyle significantly impact the body weight gain of sheep and goats which is reflected in their haemato-biochemical profile (Dhara *et al.*, 2020b). Shashank *et al.* (2019) suggested that

decreases in hemoglobin, total erythrocyte counts, total serum proteins, and albumin were crucial indicators of gastrointestinal nematodiasis infestations in goats.

In the current study, no significant variations were observed in the levels of AST and ALT. Similarly, Dhara *et al.* (2020b) reported no significant change in serum the activity of in black Bengal goats with varying gastrointestinal parasitic load. However, Moudgil *et al.* (2017) reported markedly increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the in Gaddi breed of goat suffering from gastrointestinal parasitic infections. The elevation of serum ALT and AST level indicates potential organ disruption, altered membrane permeability, or impaired excretion, as suggested by Bordoloi *et al.* (2012).

The values of blood urea nitrogen and serum creatinine showed variations. But the values of blood urea nitrogen and serum creatine were within normal range. Amulya *et al.* (2014) reported that creatinine levels were within the normal physiological limits and did not exhibit significant differences in gastrointestinal parasitism. No significant variation was observed in AST and ALT values, which remained within the normal range, suggesting no adverse effects on the liver. Creatinine and BUN values were also within normal physiological range, indicating that kidney function was not affected. Furthermore, this suggests that treatments with aqueous extracts were not toxic to the liver and kidneys.

Biochemical analysis showed a significant reduction in the level of total serum protein, albumin as well as the albumin globulin ratio. The decrease in the total serum protein, albumin aligns with the earlier reports by Ahmed *et al.* (2015); Ashfaque *et al.* (2016) and Shashank *et al.* (2019). Similar findings were also reported by Faran *et al.* (2024) where total protein and albumin showed significant decreases, while globulin exhibited a slight decrease in the helminth- infected goats. After 28 days of treatment with closantel @ 10mg/kg, *Coriandrum sativum* @ 500mg/kg body weight and *Swertia chirata* @ 500 mg/kg body weight there was a significant increase in mean values of TSP (from  $5.47 \pm 0.08$  to  $6.00 \pm 0.12$ ), (from  $5.43 \pm 0.06$  to  $5.78 \pm 0.03$ ) and ( $5.32 \pm 0.12$  to  $5.60 \pm 0.07$ ) respectively.

Moudgil *et al.* (2017) also reported a significant decrease in total serum proteins in goats with gastrointestinal parasitic infections. Jesse *et al.* (2019) observed intense hypoproteinemia and hypoalbuminemia in goats suffering from severe parasitic gastroenteritis. In the present study, non significant variations were observed in serum

globulin levels. However, Diogenes *et al.* (2010) reported an increase in globulins values. This increase in a globulin levels is thought to be due to an inflammatory responses or immune reactions against the parasites, as suggested by Ashfaque *et al.* (2016). The decreased ratio of A: G recorded in current study aligns with earlier findings reported by Ashfaque *et al.*, (2016) and Ahmed *et al.* (2015). The probable reason for this decrease in A: G ratio might be attributed to an increase in globulin levels and a decrease in albumin during parasitic infections suggested by Anumol *et al.* (2012).

Hypoproteinemia and hypoalbuminemia in gastrointestinal parasitism can be attributed to protein-losing enteropathy (Soulsby, 1982) and the malabsorption of proteins due to damaged intestinal mucosa (Ahmed *et al.*, 2015). Damage to the gastrointestinal mucosa leads to the leakage of proteins in to the gastric lumen, resulting in protein-losing enteropathy and decreased concentrations of blood serum proteins (Baker *et al.*, 2003).

Bandhaiya *et al.* (2019) also reported lower concentrations of albumin in goats with high worm burden, and they found strong correlation between the egg count in infected animals and concentration of albumin. The decreased total serum protein in infected goats may be attributed to haemodilution, which serve as compensatory mechanism for abomasal hemorrhages. The significantly lower mean albumin levels could be due to abomasal damage and selective loss of albumin, which is smaller in size and more sensitive to osmotic fluid movement (Tanwar and Mishra, 2001).

#### **5.10 Effect on Catalase, SOD and FRAP (Antioxidant) levels in goats treated with aqueous extracts of *Coriandrum sativum* seeds and whole plants of *Swertia chirata***

Oxidative stress is characterized by an imbalance between heightened sources of oxidation and diminished or impaired antioxidant mechanisms. This condition arises when there is an imbalance between free radicals and antioxidants (Jain and Shakkarpude, 2024). Various studies have documented the presence of oxidative stress both in humans and animals infected with parasites, indicating that parasitic infections can be a significant cause of oxidative stress (Upcroft and Upcroft, 2001). The susceptibility to nematode infection varies significantly among ruminant species, with goats showing notably higher susceptibility and less ability to develop immune resistance to compare to sheep (Huntley *et al.*, 1995; Wollaston and Baker, 1996; Hoste *et al.*, 2008). Reactive oxygen species (ROS)

concentrations increased significantly in the cells of hosts infected with various parasite species in various studies (Abd Allah, 2010).

In the present study the values of catalase (CAT), SOD and FRAP were non significantly lower at 28 days in compared to 0 days in both the control group and T1 group. This decrease in antioxidants concentrations is attributed to parasitic infection. The infected animals exhibited depletion in their antioxidant status, as evidenced by reduced serum SOD levels. This reduction may result from the increased production of hydrogen peroxide ( $H_2O_2$ ) due to enzymatic oxidation of the superoxide anion radical, leading to exhaustion of antioxidant enzymes responsible for scavenging free radicals (Halliwell and Gutteridge, 1999).

In the present study the values of catalase (CAT), SOD and FRAP were found to be non significantly higher on day 28 in compared to day 0 in the positive control group (Closantel @ 10 mg/kg body weight), T2 group (*Coriandrum sativum* @ 250 mg/kg body weight) and T3 group (*Coriandrum sativum* @ 500 mg/kg body weight). This increase could potentially be attributed to reduced stress in animals due to parasitism or other factors, possibly linked to antioxidant property of *Coriandrum sativum*. Samojlik *et al.* (2010) reported that oral administration of aqueous extracts of coriander seeds led to elevated levels of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while simultaneously reducing lipid peroxidation. Aqueous extracts of coriander seeds have demonstrated antioxidant activity both *in vitro* and *in vivo* (Satyanarayana *et al.*, 2004). This antioxidant effect of coriander seeds is attributed to their rich content of tocopherols, carotenoids and phospholipids which operate through various mechanisms (Ramadan and Morsel, 2004).

In the present study the FRAP values showed a significant increase in the group of closantel treated group (from  $77.89 \pm 8.19$  to  $168.03 \pm 24.39$ ), *Swertia chirata* treated group @ 250 (from  $188.78 \pm 13.40$  to  $230.27 \pm 26.43$ ) and 500 mg/kg body weight (from  $127.89 \pm 22.84$  to  $189.46 \pm 5.64$ ). The values of catalase (CAT) and SOD were non- significantly lower at 28 days compared to day 0 in the control group (not received treatment) and in the T1 group (*Swertia chirata* @ 50mg/kg body weight). The likely reason for the decrease in CAT and SOD levels was parasitism. Conversely, in the positive control group (Closantel @ 10 mg/kg

body weight), T2 group (*Swertia chirata* @ 250 mg/kg body weight) and T3 group (*Swertia chirata* @ 500 mg/kg body weight) mean values of catalase (CAT) and SOD showed a non significant increase on day 28 (post-treatment) compared to day 0 (pre-treatment). This numerical increase in CAT and SOD levels could be attributed to a reduction in the stress caused by parasitic infections or antioxidant property of *Swertia chirata*. Du *et al.* (2018) reported that mangiferin, a bioactive compound found in chirayita species, is well known for its antioxidant properties and various therapeutic potential. Phoboo *et al.* (2010) also documented the antioxidant activity of *S. chirayita*.

The host responds to parasites through various mechanisms, one of which involves the production of reactive oxygen species (ROS). These ROS are generated during normal aerobic metabolism, of the host and are also produced by activated leukocytes through oxidative burst (Chiumiento and Bruschi, 2009). The production of ROS by immune effector cells significantly increases during parasitic infections and is believed to contribute to the elimination or expulsion of parasites from the host, thereby preventing infection establishment (Batra *et al.*, 1993; Ben-Smith *et al.*, 2002).

Goat farming is primary source of income for small and marginal farmers in India. Approximately 56% of the global goat population is bred and reared in Asian countries. However, gastrointestinal parasitism, especially nematode infections, poses a significant health challenge for goats worldwide, leading to clinical diseases and reduced productivity.

The present study aimed to assess the prevalence of gastrointestinal parasites among goats in Western Uttar Pradesh. A total of 1117 faecal samples were collected and examined for parasitic infestations. The examination of faecal samples involved using compound microscopes with 10× and 40× objectives lenses. Techniques employed included direct faecal wet smears, flotation, sedimentation and modified Ziehl-Neelsen staining method to detect the eggs, ova, larvae, cysts and trophozoites of the parasites. The overall prevalence of gastrointestinal parasites infections in goats was found 70.55%. A total of nine types of gastrointestinal parasitic eggs were detected during faecal examinations in present study. Among the breeds examined, the highest prevalence of gastrointestinal parasites was found in Barbari goat (39.97%), followed by Jamunapari goat (36.93%), Non descript goat (12.18%) and lowest in Sirohi goat (10.91%). In case of age group, the highest prevalence of gastrointestinal parasites was found goats in aged 1-2 years (50.51%), followed by those older than 2 years (33.88%) and the lowest one goats younger than one year (15.61%). In terms of gender, females showed a higher prevalence rate of gastrointestinal parasites (83.88%) compared to males (16.12%). Seasonally, the prevalence of gastrointestinal parasites was highest during rainy season (47.59%), followed by summer season (33.63%), and the lowest during winter season (18.78%).

In present study molecular-based techniques were employed for identification of strongyle type of eggs in naturally infected goats from districts Meerut, Baghpat, Saharanpur and Muzaffarnagar of Western Uttar Pradesh. Faecal samples were examined via direct faecal wet smears and flotation methods to specifically identify of strongyle eggs types. Total 1117 faecal samples were examined and out of these 360 were found positive for strongyle eggs, resulting in an overall prevalence of strongyle type eggs of 32.22% in goats. The positive faecal samples were subjected for larval culture, followed by DNA extraction from the larvae. PCR amplification of the ITS-2 region of ribosomal DNA confirmed the presence of *Haemonchus*

*contortus* and as well as genus of *Trichostrongylus* and *Oesophagostomum*. The prevalence rate of *Haemonchus contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. were found as (54.16%), (31.66%) and (14.16%) respectively. These findings indicate that *Haemonchus contortus* is predominant among the strongyle species identified in the study.

The continuous and indiscriminate use of anthelmintic drugs resulted in development of anthelmintic resistance. As a result, there is a growing need for alternative therapies such as anthelmintic drugs derived from herbal sources. Screening of medicinal plants for their anthelmintic activity has become increasingly important. This approach aims to identify herbs that possess effective anthelmintic properties while minimizing side effects and mitigating the problem of anthelmintic resistance. In the present study, seven medicinal plants were selected including seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plants of *Swertia chirata* and leaves of *Prunus persica*. Adequate quantities of these plant materials were collected and transported to the laboratory for further processing. After collection, plant materials were properly cleaned and washed to remove any impurities. Subsequently, the cleaned plant materials were dried to remove moisture content. After drying, the plant materials were powdered using an electric grinder machine. The highest yield percentage of aqueous extract was obtained from the bulbs of *Allium sativum* (19.75%), while the lowest was from the seeds of *Coriandrum sativum* (8.25%). Aqueous extracts of each plant were prepared by using standard protocols. These extracts were concentrated using a rotary evaporator.

*In vitro* anthelmintic efficacy of selected medicinal plants was assessed using the egg hatch assay, larval paralysis test and adult worm mortality assay. Among the tested extracts, the highest percent of egg hatch inhibition against strongyle eggs in goats was observed by aqueous extracts of the whole plants of *Swertia chirata* (93.12%), while the lowest inhibition was recorded in aqueous extract of leaves of *Prunus persica* (58.07%) after 48 hours post-treatment. In the closantel treated groups after 48 hours post-treatment, egg hatch inhibition was found 100%. Regarding larval motility, the highest inhibition was noted with aqueous extract of whole plants of *Swertia chirata* (94.10%) against strongyle larvae in goats, whereas the lowest inhibition occurred with the extract from the leaves of *Prunus persica* (46.47%) at 24 hours post-treatment. In the closantel treated group, inhibition of strongyle larvae was found

100% after 24 hours post-treatments. In term of adulticidal effects the highest adulticidal effects was observed with the aqueous extracts of whole plants of *Swertia chirata* (100%) against the *Haemonchus contortus* in goats, where as the least effect was observed with the extracts from the leaves of *Prunus persica* (36.67%) after 8 hours post-treatments. Treatment with closantel 100% mortality was found after 2 hours post-treatments.

Cytotoxicity of seven medicinal plants was conducted by using the SRB assay protocols. The aqueous extract from the leaves of *Prunus persica* showed the highest percentage of inhibition of cell growth 20.32%, 34.93% and 58.10% at concentrations of 1 mg, 10 mg and 50 mg respectively. In contrast, aqueous extract from whole plants of *Swertia chirata* exhibited the lowest percentage of inhibition of cell growth 0.01%, 9.97% and 17.81% at concentration of 1mg, 10 mg and 50 mg respectively.

Based on the results of *in vitro* anthelmintic evaluation and *in vitro* cytotoxicity assessments, selected the two most effective and non toxic plants aqueous extracts consist of seeds of *Coriandrum sativum* and whole plants of *Swertia chirata* for *in vivo* evaluation against strongyle infections in naturally infected Barbari goats in the current study. On the 28<sup>th</sup> day post- treatment, the highest reduction in EPG of faeces was observed in goat treated with closantel @ 10 mg/kg body weight (group T1). Among the groups treated with aqueous extract of whole plants of and *Swertia chirata* and *Coriandrum sativum* seeds at doses rate of 50, 250 and 500 mg/kg body weight. In which maximum reduction of EPG was recorded in aqueous extract of *Swertia chirata* @ 500 mg/ kg body weight and lowest was recorded in aqueous extract of seeds of *Coriandrum sativum* @ 50 mg/kg body weight. Treated with closantel @ 10 mg/kg body weight achieved the maximum faecal egg count reduction (100±0.00) on day 14 post-treatment which was maintained till day 28 post-treatment in goats. In plant extract treated group whole plants of and *Swertia chirata* observed maximum faecal egg count reduction (60.65±0.44) on day 28<sup>th</sup> day post-treatment. The lowest faecal egg count reduction was observed in *Coriandrum sativum* seeds @ 50 mg/kg that was - 13.04±0.36 on day 28<sup>th</sup> day post-treatment.

In goats naturally infected with strongyle infection there were significantly reduction in the mean Hb, PCV and TEC values, accompanied by a significant increase in mean eosinophils counts. After 28 days of treatment with closantel @ 10mg/kg body weight there

was a significant increase in mean values of Hb (from  $8.47 \pm 0.10$  to  $10.82 \pm 0.11$ ), PCV (from  $26.67 \pm 0.49$  to  $33.50 \pm 0.43$ ) and TEC ( $8.30 \pm 0.17$  to  $10.73 \pm 0.17$ ). Additionally, there was significant decrease in eosinophil counts from  $7.17 \pm 0.40$  to  $3.00 \pm 0.26$ . After 28 days of treatment with aqueous extract of seeds of *Coriandrum sativum* at dosage of 500 mg/kg body weight there was significant increase in mean values of Hb (from  $9.12 \pm 0.19$  to  $9.42 \pm 0.14$ ), PCV (from  $28.50 \pm 0.89$  to  $30.50 \pm 0.85$ ) and TEC (from  $8.92 \pm 0.18$  to  $9.13 \pm 0.14$ ). Additionally, there was significant decrease in eosinophil counts from  $7.50 \pm 0.34$  to  $6.50 \pm 0.43$ . After 28 days of treatment with aqueous extract of whole plants of *Swertia chirata* @ 500 mg/kg body weight there was significant increase in mean values of Hb (from  $8.77 \pm 0.17$  to  $9.38 \pm 0.15$ ), PCV (from  $26.83 \pm 0.79$  to  $30.33 \pm 0.76$ ) and TEC (from  $8.60 \pm 0.17$  to  $9.17 \pm 0.14$ ). Additionally, there was significant decrease in eosinophil counts from  $7.67 \pm 0.33$  to  $5.50 \pm 0.43$ .

However, biochemical analysis revealed no significant differences in AST and ALT values between treated and untreated group. The mean values of BUN and serum creatinine varied significantly among different groups on different days but their values were within normal ranges. There was a significant reduction in the value of TSP, albumin and albumin globulin ratio in strongyle infected goats. However, no significant variations were observed in the value of globulin. After 28 days of treatment with closantel @ 10mg/kg, *Coriandrum sativum* @ 500mg/kg body weight and *Swertia chirata* @ 500 mg/kg body weight there was a significant increase in mean values of TSP (from  $5.47 \pm 0.08$  to  $6.00 \pm 0.12$ ), (from  $5.43 \pm 0.06$  to  $5.78 \pm 0.03$ ) and ( $5.32 \pm 0.12$  to  $5.60 \pm 0.07$ ) respectively. After the treatment with closantel @ 10 mg/kg body weight and aqueous extract of *Swertia chirata* whole plants and *Coriandrum sativum* seeds @ 250 and 500 mg/kg body weight showed improvement the haemato biochemical parameters.

Catalase, SOD and FRAP mean values in goats treated with aqueous extracts of *Coriandrum sativum* seeds and closantel in different groups at different days of pre and post treatment showed non significant changes. But in PC, T2 and T3 group values were increase. The FRAP values showed a significant increase in the group of closantel treated group (from  $77.89 \pm 8.19$  to  $168.03 \pm 24.39$ ), *Swertia chirata* treated group @ 250 (from  $188.78 \pm 13.40$  to  $230.27 \pm 26.43$ ) and 500mg/kg body weight from  $127.89 \pm 22.84$  to  $189.46 \pm 5.64$ ).

In conclusion, the current study demonstrated that all examined plants extracts possess varying degrees of anthelmintic activity. Aqueous extracts of whole plants of *Swertia chirata* and *Coriandrum sativum* seeds were having capacity to improve the altered haemato-biochemical parameters in naturally strongyle infected goats bringing them closer to normal level. Notably, goats treated with selected plants extracts did not exhibit any adverse effects, as evidenced by clinical observations and haemato-biochemical assessments. Further research is required to elucidate the active compounds responsible for their anthelmintic effect, evaluate their *in vivo* bioavailability and investigate their toxicity and establish optimal dosage regimens. The herbal anthelmintic approach shows alternative therapy for effective managing parasitic infestations and addressing resistance issues associated with chemical anthelmintics.

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### ABSTRACT

The present study was carried out to determine the prevalence of gastrointestinal parasites in goats and evaluate the anthelmintic efficacy of aqueous extract of seven medicinal plants namely seeds of *C. sativum*, bulbs of *A. sativum*, seeds of *C. papaya*, rhizomes of *Z. officinale*, leaves of *A. indica*, whole plants of *S. chirata* and leaves of *P. persica*. A total of 1117 faecal samples of goats were collected and examined from 4 districts of Western U.P. The overall prevalence of gastrointestinal parasites infections in goats were recorded 70.55%. Aqueous extracts were prepared from selected medicinal plants to conduct both *in vitro* and *in vivo* test. *In vitro* egg hatch assay, larval paralysis and adult motility assay were conducted to determine anthelmintic effects of aqueous extracts of the 7 selected medicinal plants on the eggs and larvae of strongyle as well as on the adult parasite of *H. contortus*. Based on the results of *in vitro* tests and cytotoxicity evaluations, the aqueous extracts of *S. chirata* and *C. sativum* demonstrated high efficacy and comparatively low toxicity as phyto-anthelmintic in present study. Forty eight female goats naturally infected with strongyle having more than 1000 EPG were selected for the *in vivo* study. Efficacy was assessed on the basis of faecal egg count reduction (FECR) test. Treatment with closantel @ 10 mg/kg BW resulted in highest percent faecal egg count reduction was 100±0.00 on day 14 post-treatment. Among plants extracts treated group the whole plant of aqueous extract of *S. chirata* @ 500 mg/kg BW, exhibited maximum percent faecal egg count reduction was 60.65±0.44 on day 28 post-treatment. After the treatment with aqueous extracts of *S. chirata* and *C. sativum* there were improve in the altered haemato-biochemical parameters in naturally strongyle infected goats returned these parameters closer to normal level. Goats treated with selected plant extracts did not exhibit any adverse effects. Thus herbal plant extract may be used as an alternative therapy to control the gastrointestinal parasites in goats.

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शोध शीर्षक : बकरियों के उदर - आंत्र परजीवी की व्यापकता और पादप कृमिनाशक की तुलनात्मक चिकित्सीय प्रभावकारिता

### सारांश

वर्तमान अध्ययन बकरियों में उदर - आंत्र परजीवियों की व्यापकता और सात औषधीय पौधों जैसे कि कोरियनड्रम सैटिवम के बीज, एलियम सैटिवम के बल्ब, कैरिका पपाया के बीज, जिंजिबर ऑफिसिनेल के प्रकंद, अजादिराक्टा इंडिका की पत्तियां, स्वर्टिया चिराटा का पूरा पौधा और प्रूनस पर्सिका की पत्तियाँ के जलीय अर्क की कृमिनाशक प्रभावकारिता जानने के लिए किया गया। बकरियों के कुल 1117 मल नमूने पश्चिमी उत्तर प्रदेश के चार जिलों से एकत्रित किए गये और उनकी जांच की गई। बकरियों में उदर - आंत्र परजीवी संक्रमण की कुल व्यापकता 70.55 प्रतिशत पाई गई। इन विट्रो और इन विवो प्रयोग करने के लिए चयनित 7 औषधीय पौधों से जलीय अर्क तैयार किया गया। औषधीय पौधों के जलीय अर्क की कृमिनाशक प्रभाव को जानने के लिए इन विट्रो एग हैच असे, लार्वा पैरालिसिस और एडल्ट मोटिलिटी असे स्ट्रॉंगाइल एग और लार्वा के साथ-साथ हेमोनचस कॉन्टोर्टस वयस्क परजीवी पर भी किया गया। इन विट्रो टेस्ट और साइटोटॉक्सिसिटी के परिणाम के आधार पर स्वर्टिया चिराटा और कोरियनड्रम सैटिवम के जलीय अर्क में तुलनात्मक अधिक प्रभावकारी और कम विषाक्तता पाई गई। 1000 से अधिक ईपीजी वाली स्ट्रॉंगाइल से संक्रमित 48 मादा बकरियों में इन विवो अध्ययन किया गया। कृमिनाशक के प्रभाव का मूल्यांकन एफईसीआर टेस्ट के आधार पर किया गया। क्लोसेंटल के 10 मिलीग्राम/किलोग्राम के दर से उपचार के 14 वें दिन मल में अंडों की संख्या में उच्चतम प्रतिशत (100±0.00) की कमी हुई। औषधीय पौधों के जलीय अर्क से उपचारित समूह में स्वर्टिया चिराटा के जलीय अर्क को 500 मिलीग्राम/किलोग्राम के दर से उपचार के 28 वें दिन मल में अंडों की संख्या अधिकतम प्रतिशत (60.65±0.44) की कमी में हुई। स्वर्टिया चिराटा और कोरियनड्रम सैटिवम के जलीय अर्क से स्ट्रॉंगाइल संक्रमित बकरियों के उपचार के बाद उनके हेमेटो-बायोकेमिकल पैरामीटर में सुधार पाया गया। दोनों पौधों के जलीय अर्क से उपचारित बकरियों पर कोई प्रतिकूल प्रभाव नहीं दिखा। इस प्रकार बकरियों में उदर - आंत्र परजीवियों के नियंत्रित करने के लिए एक वैकल्पिक चिकित्सा के रूप में हर्बल पौधे के अर्क का उपयोग किया जा सकता है।

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