STUDIES ON CLINICO-HAEMATOBIOCHEMICAL AND THERAPEUTIC EFFICACY OF POLYHERBAL FORMULATION AGAINST RESISTANT GASTRO INTESTINAL NEMATODES IN GOATS

Thesis

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By

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CERTIFICATE-I

This is to certify that the thesis entitled **"STUDIES ON CLINICO-HAEMATOBIOCHEMICAL AND THERAPEUTIC EFFICACY OF POLYHERBAL FORMULATION AGAINST RESISTANT GASTRO INTESTINAL NEMATODES IN GOATS"** being submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** with major in **Veterinary Medicine** and minor in **Veterinary Parasitology** of the college of Post graduate studies, G.B. Pant University of Agriculture and Technology, Pantnagar is a record of bona fide research carried out by **Mr. Ashok Singh Rathor, Id. No. 56971,** under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
August, 2023

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CERTIFICATE-II

We, the undersigned members of the Advisory committee of **Mr. Ashok Singh Rathor, Id no. 56971,** a candidate for the degree of **Doctor of Philosophy** with major in **Veterinary Medicine** and minor in **Veterinary Parasitology** agree that the thesis entitled **"STUDIES ON CLINICO-HAEMATOBIOCHEMICAL AND THERAPEUTIC EFFICACY OF POLYHERBAL FORMULATION AGAINST RESISTANT GASTRO INTESTINAL NEMATODES IN GOATS",** may be submitted in partial fulfillment of the requirement of the degree.
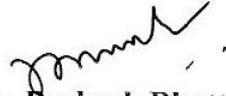
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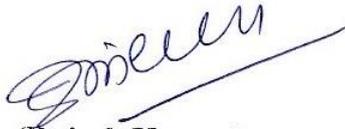
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ABBREVIATIONS

@	At the rate
>	Greater than
<	Less than
=	Equal to
±	Plus Minus
%	Percentage
℃	Degree Celsius
μg	Microgram
μl	Microliter
AR	Anthelmintic Resistance
ALT	Alanine amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate transaminase
AWMA	Adult Worm Mortality Assay
aq.	Aqueous
A/G	Ratio Albumin/ Globulin
b.wt.	Body Weight
BZ	Benzimidazole
^{0}C	Degree Celsius
CAT	Catalase
Cm^2	Centimetre square
EDTA	Ethylene diamine tetra acetic acid
et al.	Et alli / alia (And Others)
EPG	Egg per Gram
etc.	Et cetera
e.g.	Exempli gratia

FECRT	Faecal Egg Count Reduction Test
FAMACHA	FAffa MAlan CHArt
FEC	Faecal Egg Count
Fig.	Figure
ºf	Degree Fahrenheit
F.S	FAMACHA Score
G I, GII, GIII, GIV	Group I, Group II, Group III, Group IV
GSHPx	Glutathione peroxidase
GIN	Gastrointestinal Nematode
gm/dl	Gram per decilitre
gm	Gram
g	Gram
GI	Gastro - Intestinal
GOD-POD:	Glucose oxidase and Peroxidase
HB	Hemoglobin
Hrs.	Hours
IU/L	International Unit per Litre
i.e.	That is
mg/kg	Milligram per kilogram
ml	Millilitre
mg/dl	Milligram per decilitre
M eq/l	Mill equivalent per litre
µg/ml	Microgram per milliliter
µmol/L	Micro mole per liter
mmol/L	Milimoles per liter
mm	Millimetre
mM	Millimolar

mg/ml	Milligram per millilitre
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
n	Number
nm	nanometer
nM	Nanomolar
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NSS	Normal Saline Solution
Na	Sodium
OH+	Hydroxyl
O.D.	Optical density
pg	Picogram
PBS	Phosphate buffer saline
PUFA	Polyunsaturated fatty acids
PCV	Packed Cell Volume
ROS	Reactive oxygen species
RBC	Red Blood Cells
r.p.m	Revolution Per Minute
spp.	Species
S.D	Standard Deviation
S/C	Sub Cutaneous
SGOT	Serum Glutamic - Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
S.E.M	standard Error Mean
SPSS 25	Statistical product and Service Solutions 25

S.E	standard Error
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TST	Targeted Selected Treatment
TLC	Total Leucocyte Cell
TEC	Total Erythrocyte Cell
UV	Ultra - violet
vs	Versus
viz.	Vicelike (Namely)
WAAVP	World Association for the Advancement of Veterinary Parasitology
WBC	White Blood Cell
Wks	Weeks
u/L	Unit per liter
U/ml	Unit per milliliter

Introduction
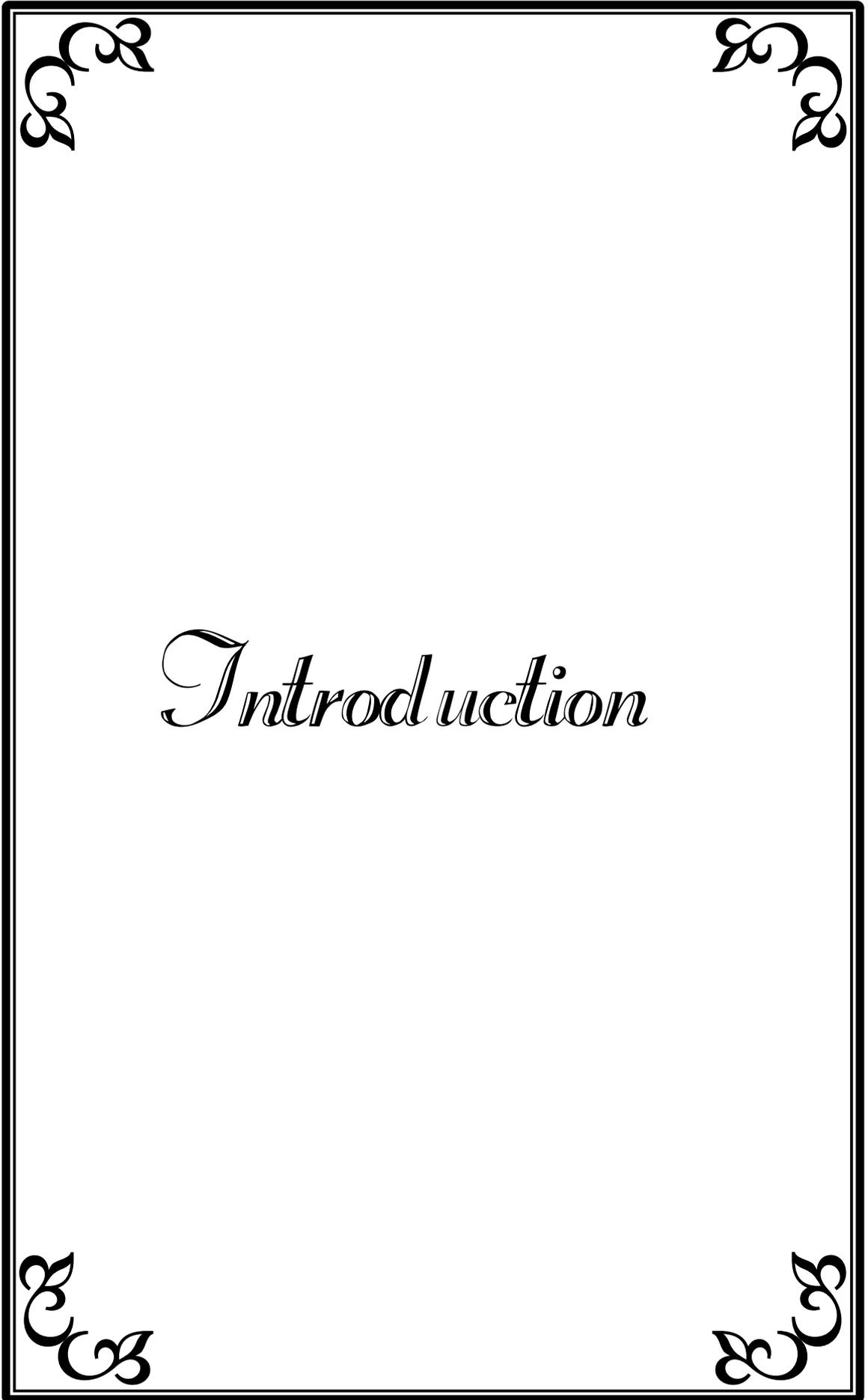
Chapter 1   INTRODUCTION

Livestock sector of India being one of the largest in the world, contributes around 4.11% of total GDP of India. As per 2020 economic survey, the Livestock sector in India has risen at annual growth rate of 7.0 % during last 5 years. As per 2021 economic survey, the contribution of livestock in total agriculture and allied sector has increased from 24.3% in 2014-15 to 28.63% in the year 2018-19. Livestock-generated earnings have emerged as a significant supplementary income stream for families residing in rural areas. Small ruminants mainly sheep and goat play an important role in income generation, employment, improvement of household nutrition, stability and wealth. With around 148.88 million goats **(Livestock Census, 2020)** goat population in India ranks first and constitutes 26.40 % of the total livestock population.

For centuries, goats have been utilized for their milk, meat, hair, skin, manure, and more, making them one of the earliest animals to be domesticated. Since they can survive in various agro climatic conditions therefore they are integral part of rural economy. Milk of goat is easily digestible, wholesome and nutritious. Unlike other ruminants goats prefer browsing rather than grazing. Their diet comprises shrubs, leaves, bushes, kitchen wastes etc. They attain sexual maturity at an early age with short gestation period and give single, triplet but mostly twins during birth. Quadruplets are also seen rarely. Due to comparatively lesser input involved in raising and feeding, it is referred as poor man's cow. Unique ability of goats to survive anywhere and maintain production even in unfavourable climatic conditions has resulted in goat farming as the best choice in developing countries amongst the rural people. It is extremely popular being a quick source of income with minimum risk, low investment, wide adaptability, lower feed and managemental practices and high feed conversion efficiency. However, goat farming can be affected by poor nutrition, hygiene, precarious housing conditions and improper disease management thus resulting in high incidence of diseases caused by various pathogens *viz*. bacteria, virus, fungi and parasites.

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Helminths, especially gastrointestinal (GI) nematodes can be a serious and most common problem affecting small ruminants throughout the world resulting in reduced productivity. Helminths pose a huge veterinary health problem to farm yard animals and are responsible for 3%−8% of their weight loss and 28% of death (**Singh *et al.*, 2022**)

Gastrointestinal nematodosis results from a range of nematodes found within various segments of the animal's GI tract. The common nematodes of small ruminants are *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Oesophagostomum*, *Trichuris*, *Chabertia*, *Strongyloides Bunostomum* and *Cooperia spp*. etc. This condition can lead to diverse pathological consequences such as anaemia, inflammation of the intestines, damage to the intestinal villi, and textured intestinal lining. As a result, it stands as a significant source of illness (**Tyasi and Tyasi, 2015; Tyasi *et al.*, 2015**), contributing to financial setbacks due to decreased weight, stunted development, reduced dietary consumption, diminished milk yield, compromised reproductive capabilities, and heightened susceptibility to additional infections due to compromised immune defences. Within these gastrointestinal nematodes, *H. contortus* is widely acknowledged as the prevalent and extremely harmful parasite due to its aggressive blood-sucking behaviour, which triggers profound anaemia, notable body weight reduction, and decreased overall productivity, potentially culminating in fatality, (**Jabbar *et al.*, 2006; Aumont *et al.*, 1997; Agrawal and Banerjee, 2007; Khalafalla *et al.*, 2011**). The problem of GI nematodosis can be controlled by use of biological agents, chemical drugs, vaccines, and other managemental practices. The Anthelmintics mainly used against GI nematodes belong to avermentin, benzimidazole and imidothiazole groups (**Singh and Yadav, 1997**). Anthelmintics are administered in cases where animals exhibit nonspecific clinical symptoms such as diarrhoea or test positive on faecal examination, without assessing infection intensity. Drug efficacy has been reduced drastically due to rampant and indiscriminate use of anthelmintics finally resulting in increased parasitic resistance.

1.1   FAMACHA AND TARGETED SELECTIVE TREATMENT

The FAMACHA system is an affordable and validated method for evaluating the extent of anaemia, primarily attributed to *H. contortus* infection. **Dr. Francois**

Malan conceptualised it in South Africa and it was named after the first letters of the names of the research farm (FA) and the university (Machaland). By employing a conjunctival-colour scoring mechanism, this system facilitates the identification of animals that are at a higher risk of carrying a substantial parasite load. When addressing parasite control, goats present a more formidable task in contrast to cattle and horses, as they are more susceptible to infestations by worms. Selective treatment is a sustainable approach strongly suggested for effective, long-term herd health management, as it reduces anthelmintic usage by omitting treatment for certain animals, all while ensuring overall herd well-being. It is the most effective approach, especially for slowing down the emergence of anthelmintic resistance.

Bath *et al.* (**1996**) noted that a major challenge in adopting a selective treatment approach in animals was the lack of an efficient and cost-effective method for identifying animals that require treatment. In the year 2002, **Gareth Bath, Francois Malan and Van** introduced an innovative method known as FAMACHA in South Africa that allows for the detection of anaemia in goats/sheep due to *Haemonchus contortus*. In later research, **Van and Bath (2002)** discovered that implementing FAMACHA by farmers and conducting treatments based on FAMACHA scores, rather than PCV assessments, led to a 58% decrease in treatment frequency on 10 farms, compared to the prior year. This system helps farmers and veterinarians identify individual animals that may be severely affected by parasitic infestations, allowing targeted treatment and reducing the risk of parasite resistance to dewormers, **Bandhaiya** *et al.* (**2020**).The practice of selective treatment is an enduring strategy highly advocated for the comprehensive management of herd health over time. It effectively reduces anthelmintic consumption by refraining from treating certain animals, all the while upholding the overall well-being of the herd.

1.2 ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES

The continuous emergence of resistance to anthelmintic drugs has made it difficult for veterinarians to control the disease (**Jackson and Coop, 2000**). Moreover, the affordability, accessibility, and the safety of anthelmintic compounds continue to raise apprehensions as they can potentially enter the food chain through livestock-

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derived food products **(Waller, 1994; Nunomura *et al*., 2006; Dewanjee *et al.*, 2007; Saddiqi *et al.*, 2010).** The desire for animal products devoid of drug residues has been steadily growing. This global problem has led to an interest in finding alternatives to synthetic chemicals for helminth control **(Waller, 1999)**. Traditional ethnobotanical remedies known for their anthelmintic efficacy are gaining recognition as innovative strategies, especially within temperate and tropical regions (**Akhtar *et al*., 2000).** These medicinal plants are cost-effective, readily available, have a wide margin of safety, are biodegradable, and eco-friendly **(Hammond *et al*., 1997).**

Several reports on resistance to various groups of anthelmintics against gastrointestinal nematodes in small ruminants have been reported from different countries **(Prichard, 1980; McKenna, 1997; van Wyk *et al.*, 1987; Pomray *et al.*, 1992). Varshney and Singh (1976)** documented the emergence of anthelmintic resistance to phenothiazine and thiabendazole in *H. contortus* within India as pioneers**.** There after anthelmintic resistance has been reported in various regions of the country, **Laha and colleagues (1999), Yadav (1990), Yadav and Uppal (1993), Jaiswal and co-authors (2013), Garg and team (2007), Singh *et al*., (1995), Buttar and co-authors (2012), Maharshi** and **team (2011, 2012), Rialch** and **co-authors (2013).**

In South America, cases of anthelmintic resistance have been recorded from various countries such as Argentina, Brazil, and Chile. Similarly, in South Africa, anthelmintic resistance has been reported against benzimidazole, levamisole, and macrocyclic lactones. Reports indicate resistance to benzimidazole, levamisole, and macrocyclic lactones within the United Kingdom. In Australia, anthelmintic resistance against benzimidazole, levamisole, and macrocyclic lactones has been observed in various studies conducted in different regions.

Wondimu and **Bayu (2022)** reported that *Trichostrongylus*, *Haemonchus* and *Teladorsagia* were the dominant nematodes which had developed resistance to ivermectin and albendazole. **Rajagopal *et al.* (2018)** reported that in the post treatment coproculture *Haemonchus spp*. was the most predominant species (98 %) indicating it to be the major contributing species which had developed resistance to benzimidazoles. The emergence of resistance to benzimidazoles resulted from the extended and

intensive utilization of these drugs, coupled with frequent treatments. Goats have rapid hepatic metabolism and lower bioavailability of drugs and they require double the dose of benzimidazoles advocated for sheep. This implies that anthelmintic resistance is of bigger concern in goats rather than in sheep **(Wondimu and Bayu, 2022).**

According to **Ekawardhani *et al.* (2021)** Medicinal plants, especially *Diospyros anisandra*, *Citrus aurantiifolia and Momordica charantia*, may be used as an effective anthelmintic against *A. caninum.* **Sanyal *et al.* (2020)** reported that due to the rapid clearance of anthelmintic by goats, it is recommended to enhance the dosage rate in them. Traditionally, goats have been given anthelmintics at equivalent dosage rates as those used for sheep, aiming to manage gastrointestinal nematode infections. However, the pharmacokinetics of anthelmintics, specifically benzimidazoles and closantel, indicate that goats eliminate these substances from their system quicker than sheep resulting in the reduced bioavailability of anthelmintics. As indicated by **Mphahlele *et al.* (2019),** resistance among nematodes has emerged within the three major anthelmintic categories (levamisole, benzimidazoles and macrocyclic lactones) employed for ruminant treatment. As per **Kalkal *et al.* (2020)** the main recommendation to maximize the effectiveness of anthelmintics and reduce resistance is to minimise the selection pressure for anthelmintic resistance in worm population by reduction in drench frequency, appropriate doses, alteration of anthelmintic classes and preferred use of narrow spectrum anthelmintics when possible depending on parasitic species. Another recommended approach is to follow target selective treatment (TST) instead of systematic ones.

1.3   USE OF HERBAL ANTHELMINTICS

Traditional herbal remedies have proven their effectiveness and safety over generations with minimum side effects. Approximately 80% of the global population, particularly in developing nations, continues to depend on natural resources for their primary healthcare needs **(Yadav and Singh, 2011)**. Lot of studies were done to examine anthelmintic efficacy of medicinal plants **(Adama *et al.*, 2009; Kamaraj *et al.*, 2010; Ademola *et al.*, 2010)**. However, the study on anthelmintic efficacy of plants commonly browsed by goat or sheep remains insufficient and requires further research on various

polyherbal combinations to assess their efficacy as potent dewormers. Polyherbal extracts incorporate a multitude of bioactive elements that effectively engage diverse metabolic and physiological processes spanning the unique life stages of *H. contortus*, thereby hindering continued growth and propagation. This underscores their potential as a prospective foundation for a novel anthelmintic treatment. The manifestation of synergistic impacts has been evidenced across various *in-vitro* pharmacological investigations. **Rahal and co-authors (2022)** have highlighted that medicinal plants demonstrate anthelmintic properties attributed to a range of phytochemical constituents including alkaloids, anthraquinones, saponins, tannins, resins, reducing sugars, glycosides, flavonoids, sterols, and volatile oils present in various plant parts such as roots, leaves, bark, and seeds. The likelihood of developing drug resistance to phyto anthelmintics is lesser than that for chemical anthelmintics **(Bauri *et al.*, 2015).** The anthelmintic potential of *Origanum majorana*, *Butea monosperma* and *Piper longum* extract was affirmed in the findings of **Giri, *et al.* (2021).** The utilization of these botanical extracts holds promise as a prospective substitute for anthelmintic medications, aiming to mitigate excessive usage and potential repercussions on human well-being and the ecosystem. *H. contortus* stands out as the predominant, extensively distributed, and significant parasite affecting sheep and goats, leading to substantial illness and loss of life **Besier *et al.*, (2016).** Up to this point, synthetic anthelmintic medications have been frequently and extensively employed for the management of gastrointestinal nematodes (GIN). Nevertheless, the effectiveness of synthetic anthelmintic agents has diminished due to the emergence of anthelmintic resistance**, Singh *et al.* (2017).** Current trends lean towards alternative strategies, such as herbal preparations, to economically and sustainably manage gastrointestinal nematodes (GINs) without relying solely on synthetic anthelmintics. Numerous researchers have conducted *in vitro* and *in vivo* assessments to confirm the anthelmintic attributes of diverse plant species **(Iqbal *et al.*, 2007; Sunandhadevi *et al.*, 2017)**. Minimization of rampant use of conventional anthelmintics, monitoring the anthelmintic ability of commonly used drugs and exploitation of indigenous plants having anthelmintic properties are prerequisites for effective control strategy against GI nematodosis. According to **Ahuir-Baraja *et al.* (2021),** alternative treatments, and The realm of phytotherapy continues to captivate

researchers, and its incorporation into a comprehensive parasitological control strategy could play a role in the gradual decline of reliance on traditional anthelmintic drugs. Secondary compounds like condensed flavonoids, terpenes, tannins possess established anthelmintic attributes, and their utilization is on the rise important for helminth control in ruminants **(Burke and Miller 2020)**.

According to **Karole *et al*. (2019)**, Sarangdhar Samhita, an Ayurvedic literature, introduced the concept of polyherbalism to enhance therapeutic efficacy. Polyherbal formulations are extensively employed worldwide due to their medicinal and therapeutic uses, recognized as polyherbal therapy or combinations of herbal remedies. Utilizing individual plants active phytochemical constituents alone may not always achieve the desired therapeutic effects. However, when multiple herbs are combined in specific ratios to create polyherbal and herbo-mineral formulations, they can offer enhanced therapeutic benefits while reducing toxicity.

Keeping the above background information in mind and understanding the Potential threat posed by widespread development of resistance to sustainable small ruminant economy in India and Uttarakhand in particular, **the current study has been organized with the following aims in mind:**

1. Evaluation of anthelmintic efficacy and resistance to commonly used drugs (Fenbendazole & Ivermectin) in Goats suffering from GI Nematodosis.

2. To evaluate *in vivo* therapeutic potential of polyherbal anthelmintic formulation in goats naturally infected with GI nematodes.

3. To study pre and post-treatment clinico-haematobiochemical and oxidative stress related parameters due to GI Nematodosis over an extended period.
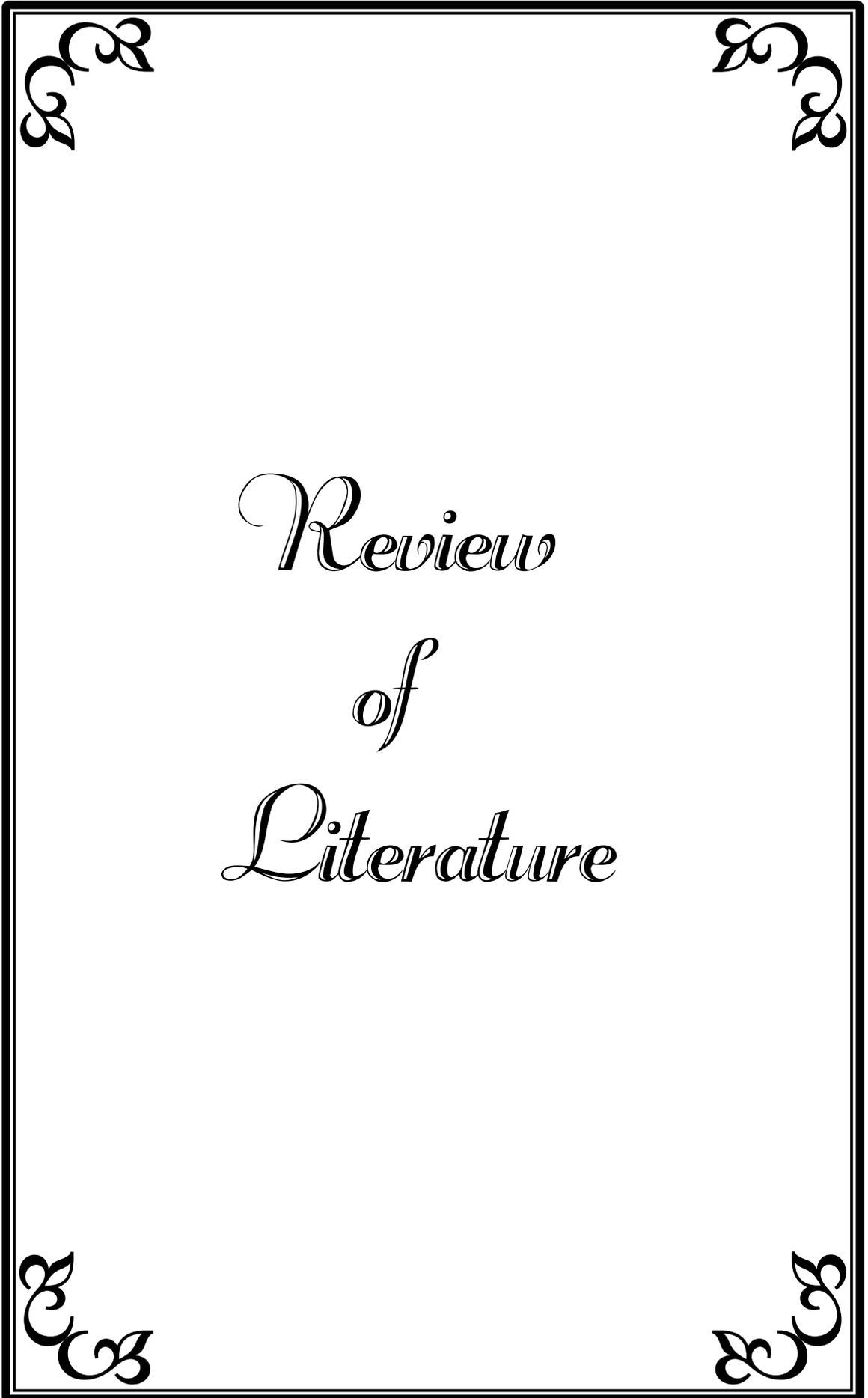
Review
of
Literature

Chapter 2   REVIEW OF LITERATURE

The pertinent literature encompassing investigations into the clinical, hematobiochemical and therapeutic effectiveness of polyherbal formulations against drug-resistant gastrointestinal nematodes in goats has been organized as follows:

2.1   Prevalence study- Indian & Global scenario

2.2   FAMACHA Technique

2.3   Status of anthelmintic resistance in small ruminants

2.4   Anthelmintic properties of herbal plants against gastrointestinal nematodes

2.5   Diagnosis of anthelmintic resistance

2.6   Faecal Egg Count Reduction Test

2.7   Polyherbal ingredients being used –

- Palash seeds (Butea Monosperma)

- Vidanga (Embelia ribes)

- Neem leaves (Azadirachta indica),

- Kali jeeri (Centratheram anthelminticum),

- Pumpkin seeds (Cucurbita maxima)

2.8   Haematobiochemical parameters

2.9   Oxidative stress related parameters

2.1   PREVALENCE STUDY

2.1.1   INDIAN SCENARIO

Sinha and Sahai (1973), recorded in goats the incidence of *O. columbianum* (50%), *T. ovis* (48%), *T. globulosa* (27%), *H. bispinosus* (67.2%), and *H. contortus* (74.2%) in Bihar

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Bali and Singh (1977) conducted epidemiological studies on gastrointestinal nematodes of sheep and goats in Haryana and found the highest prevalence in August and the lowest in February.

Saha *et al.* (1996) reported that In Nadia, West Bengal, parasitic incidence was 75.83%. H. contortus occurred in 32.08%. Incidence peaked in winter (79.41%), moderate in monsoon (76.40%), lowest in summer (72.28%). Double (32.97%) and triple (28.02%) infections were common.

Anthelmintic resistance in sheep's *Haemonchus contortus* was examined in a study carried out by **Swarnkar *et al.* (1999).** They found that fenbendazole was ineffective, tetramisole was only 25% effective, and closantel showed 100% efficacy. The effectiveness of rafoxanide against *Haemonchus contortus* reached 96%.

Chaudhri *et al.* (2000) studied the prevalent helminth parasites that commonly afflict sheep and goats in the region of Haryana, which included *Haemonchus spp.*, *Trichostrongylus spp.*, and *Paramphistomes spp.*

Farhat *et al.* (2000) reported a prevalence rate of 54.77% for *Haemonchus contortus* among sheep in Punjab. The highest infection rates occurred during July (89.55%) and August (87.13%), conversely, the least prevalence was observed in February (20.02%) and March (20%).

Rajkhowa and Hazarika (2001) reported an overall goat infection rate of 5.28%. Age-wise, 1-4 months old goats had the lowest infection rate (34%), while 5-8 months old exhibited the highest (69.35%), and those over 2 years old had 57.76%. Females had slightly higher overage infection rates than males, yet this difference lacked statistical significance.

Arunachalam *et al.* (2002) surveyed sheep farming structures and income in Tamil Nadu and found that many people prefer sheep farming in combination with poultry, buffalo, bullock, cattle, or goats due to its profitability.

In Nagpur, India, **Bhojane *et al.* (2002)** reported a 70% prevalence of helminthic infection among goats. The occurrence of sole infections with *strongylus* (16.57%) and *Haemonchus spp.* (12.23%) exhibited notably elevated levels in comparison to mixed infection of *Fasciola hepatica*, *Oesophagostomum spp. and*

amphistomes were additionally detected. Parasitemia was notably impacted by factors like sex, season, and year.

In Mathura, Uttar Pradesh (India), **Arora *et al.* (2003)** reported a 49.46% prevalence of gastrointestinal parasitism among goats**.** The most prevalent parasite was the bursate worm *Haemonchus contortus.*

Garg *et al.* (2003) conducted an epidemiological investigation into *H. contortus* infection among goats in the Indian semi-arid region. The overall incidence was 56.38%, which was lower in the summer season than in the winter and rainy seasons. A notable positive correlation existed between abomasal worm count and EPG, with statistical significance. The prevalence of female worms was higher than that of males, maintaining an average female-to-male ratio of 1.447.

Thangathurai *et al.* (2003) documented a 47.2% occurrence of enteric parasitism in sheep and goats within the Bidar region of Karnataka. Among the local population, prevalent parasites included *Oesophagostomum*, *Trichuris*, *O. columbianum*, *hookworm and Stilesia hepatica*.

Singh *et al.* (2005), in Ludhiana, Punjab reported that the prevalent infections during both pre- and post-rainy seasons were *strongylus*, *trichuris*, *monezia*, and *paramphistomum*. A positive correlation was observed between the fortnightly mean eggs per gram and climatic factors, including rainfall and temperature. Mixed helminthic infections were found in 11.52% of cases, with *strongyles and moniezia spp* being the most common.

Sreedevi and Murthy (2005) found an overall prevalence of 37.6% of gastrointestinal parasites among Andhra Pradesh sheep. The prevalence was higher in summer and lowest in the monsoon season. Analysis of prevalence based on seasons and age groups revealed that the highest prevalence among sheep occurred during the summer (47.5%), while in lambs, the monsoon season exhibited a higher prevalence (46.1%) of parasitism. The prevailing parasites included *strongyles* and *Moniezia spp.*

Kumar *et al.* (2006), examined the occurrence of parasitic diseases within migratory Nellore sheep flocks, it was observed that *amphistomiasis* exhibited notably greater prevalence in migratory sheep when compared to non-migratory flocks.

Monieziosis was prevalent in both flocks, yet migratory flocks exhibited elevated rates of *fascioliosis* and *strongyle* infections. The primary challenge encountered during migration was attributed to shared water sources, which experienced the highest degree of contamination, infections, and outbreaks. Additionally, the study highlighted that sheep flocks typically exhibited a baseline level of intestinal parasitism under usual circumstances, whether or not accompanied by clinical indications. However, during the migratory phase, the worm burden escalated to a pathogenic level, largely attributed to early morning grazing practices, leading to an increased susceptibility to parasitic infections.

Makawana *et al.* (2009) conducted a study on AR nematode parasite in sheep aged 12-24 months at an organized sheep breeding farm in Patan (Gujarat). The comparative effectiveness of Tetramisole HCl, Albendazole and ivermectin was determined as 54.32%, 66.15%, and 72.73%, correspondingly.

Yadav *et al.* (2009) conducted a comprehensive epidemiological study spanning six years (2001-2006) to investigate the prevalence of gastrointestinal nematodes in sheep across Uttarakhand state. The results indicated that the total occurrence rate of gastrointestinal nematodes was 62.39%, with higher prevalence observed among sheep in the tarai region (67.02%) in contrast to those in the hills (45.01%). The scientists additionally noticed fluctuations in the monthly prevalence of gastrointestinal nematodes. The peak rates were recorded in October (79.43%) for creatures inhabiting the tarai region and in July (65.38%) for those dwelling in the hills. Through coproculture and necropsy investigations, the team pinpointed *H. contortus* as the predominant gastrointestinal nematode, trailed by *T. colubriformis and O. columbianum*.

Bandyopadhyay *et al.* (2010) investigated the prevalence of gastrointestinal parasites in goats intended for human consumption in Shillong, Meghalaya, spanning the period from June 2001 to June 2006.Rainy season exhibited maximum parasitic intensity, contrasting with winter.

Badran *et al.* (2012) examined 810 faecal samples from small ruminants (intensive: 285, extensive: 525). Extensively reared animals showed significantly

higher gastrointestinal parasite prevalence (26.5%) than intensively reared ones (7.9%). *Eimeria spp* exhibited dominance (prevalence of 81.1%, accounting for 34.2% proportion), trailed by *Dictyocaulus spp* (prevalence of 49.1%, proportion of 20.7%), and *Haemonchus spp* (prevalence of 23.1%, proportion of 9.7%). Subsequently, *strongyloides*, *Oesophagostomum*, *Thichuris*, *and Trichostrongylus* followed in sequence.

Arunachalam *et al.* **(2013)** conducted a study investigating the rise of ivermectin resistance in gastrointestinal nematodes among semi-managed goat populations on a farm situated in India's Mathura District. They utilized a variety of *in vivo and in vitro* examinations to identify anthelmintic resistance. Among these, the FECRT method is particularly suitable for assessing anthelmintic effectiveness in commercial flocks. The findings indicated resistance of GI nematodes to fenbendazole, yet susceptibility to levamisole, although there was a suspicion of resistance to ivermectin. This represents the initial record of ivermectin-triggered anthelmintic resistance in gastrointestinal helminths within the Indian subcontinent's goat population.

Akhtar *et al.* **(2014)** explored through *in vivo* and *in vitro* examinations, they assessed the effectiveness of anthelmintic treatments in *H. contortus* in goats. Their investigation demonstrated decreased efficacy of the three employed anthelmintics against this parasite in goats compared to the recognized WAAVP threshold of 95%. Consequently, this current study aimed to explore the effectiveness of diverse anthelmintics within the local context. The evaluation focused on the three frequently utilized anthelmintics: Valbazen, Levamisole, and Dectomax. Results indicated Dectomax's superior performance against *Haemonchus contortus* in comparison to Valbazen and Levamisole. However, all three anthelmintics exhibited effectiveness below the recommended WAAVP standard of 95%, indicating the presence of resistance.

Sharma *et al.* **(2014)** conducted a study at Pantnagar (Uttarakhand) on the seasonal prevalence of GI nematodes in slaughtered goats and sheep. They found a higher mean worm count in sheep compared to goats and the peak and nadir in average

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worm counts were documented in sheep during October and January, respectively. For goats, the peak and nadir occurred in September and January, respectively. The predominant nematodes were *Haemonchus*, *Oesophagostomum*, *Trichuris*, *Trichostrongylus and Strongyloides spp*.

Satyavir *et al.* (2015) undertook a research endeavour to assess the effectiveness of levamisole and ivermectin, both individually and in combination, against gastrointestinal nematodes in sheep. Analyzing infective larvae from faecal cultures before and after treatment, *Haemonchus contortus* was the predominant species identified. The outcomes suggested limited susceptibility of *Haemonchus contortus* to levamisole and ivermectin. Consequently, the investigation proposed that the combined administration of levamisole + ivermectin exhibited heightened efficacy against resistant *Haemonchus contortus* compared to their separate administration.

Amulya *et al.* (2016) undertook an investigation to assess the status of gastrointestinal nematodes in sheep with regards to the emergence of resistance to fenbendazole, a prevalent anthelmintic. Examination of coprocultures unveiled the presence of diverse strongyle larvae infections, encompassing *Haemonchus contortus*, *Trichostrongylus* sp., *Oesophagostomum* spp., *Bunostomum* sp., and *Cooperia* sp. *Haemonchus contortus* held the largest proportion among these species across all four sheep farms scrutinized.

Ramandeep *et al.* (2017) carried out research to assess the effectiveness of fenbendazole., a commonly used anthelmintic, against gastrointestinal nematodes (GIN) in semi-organized sheep and goat farms in Ludhiana and Amritsar Districts using faecal egg count reduction test (FECRT). The objective of the study was to identify the existence of anthelmintic resistance (AR) to fenbendazole in the gastrointestinal nematodes (GIN) affecting sheep and goats within these districts.

Saiful and colleagues (2018) conducted an investigation aimed at ascertaining the occurrence of anthelmintic resistance (AR) among gastrointestinal nematodes (GIN) in controlled farming environments. The research findings indicated the occurrence of albendazole resistance in the gastrointestinal nematodes of structured sheep farms. Additionally, there were indications of potential resistance to levamisole

and ivermectin. Conversely, within structured goat farms, all the anthelmintics utilized in the study exhibited efficacy against the gastrointestinal nematodes.

Dixit *et al.* **(2019)** aimed to assess closantel's effectiveness against benzimidazole-resistant *Haemonchus contortus* infection in sheep. The findings indicated the potential utilization of closantel for targeted selective treatment (TST) in cases of *Haemonchus*-infected sheep. The use of closantel in combination with benzimidazoles may be helpful in reducing pasture contamination and Comprehensive management of gastrointestinal nematode infections.

Priyanka *et al.* **(2019)** conducted a study to detect the presence of AR of commonly used anthelmintic drugs against GIN in goats in village Satnali, District Mehandergargh, Haryana. The study used three drug groups: fenbendazole, morantel, and ivermectin. The investigation unveiled the existence of various instances of anthelmintic resistance (AR) involving fenbendazole, morantel, and ivermectin against *Haemonchus contortus* among goats within Haryana's unorganized sector.

2.1.2 GLOBAL SCENARIO

Le Reiche *et al.* **(1973),** conducted a survey in Cyprus and found 19 species of helminths in sheep and goats, the prevailing nematode species included *Parabronema skrjabini* (1.6%), *Ostertagia circumcincta* (77%), *Haemonchus contortus* (23%), *Bunostomum trigonocephalum* (6%), *O. trifurcata* (77%), *Trichostrongulus spp.* (62%), *T. axei* (53%), *T. ovis* (55%), *Chabertia ovina* (38%), and *Skrajabinema ovis* (9%).

Mollah *et al.* **(1996),** reported that in Bangladesh, *H. contortus* incidence was 70.4% in Black Bengal goats. Highest (97.73%) in autumn, lowest (33.33%) in spring.

Silva *et al.* **(1998),** reported in a study on goat susceptibility, ages 1-2 months most affected. Worm burdens high at 11-12 months in Brazil.

Morales *et al.* **(2001)** researched the influence of rainfall patterns on the occurrence of gastrointestinal nematodes in naturally parasitized ewes in Falcon, Venezuela. Analyses carried out via necropsy during both wet and dry periods revealed *Haemonchus contortus*, *Trichuris ovis*, *Trichostrongylus axei*, *Bunostomum trionocephalum* as the primary prevailing parasites.

Yildiz (2001), documented in Turkey, a substantial occurrence (66.31%) of helminthic infestations within sheep flocks was noted. Prevalent among these were parasites from the *Trichostrongylidae* family, particularly *Ostertagia spp.*, *Haemonchus spp.*, and *Nematodirus spp.*, while *Trichuris spp.* followed in terms of prevalence.

Ismail *et al.* (2004) discovered a prevalence of *Haemonchus contortus* among indigenous goats in Sudan's south Darfur state. In January, the male-to-female ratio was 0:9, while the peak ratio (2:72) was observed in March.

Bitew *et al.* (2011), investigated the prevalence of nematodes in sheep and goats in Hawassa, Ethiopia. They examined 180 gastrointestinal tracts and identified five nematode species were discerned, namely *H. contortus*, *T. axei*, *O. circumcincta*, *T. colubriformis*, *and B. trigonocephalum*, exhibiting prevalence rates of 55.9%, 2.9%, 26.4%, 29.4%, and 23.5%, correspondingly, among goats., respectively.

Khalafalla and colleagues (2011) reported an analysis of the cyclic occurrence of nematode parasites in 173 sheep that underwent slaughter at Al-Mahala abattoir. The outcomes unveiled the utmost frequency of infections in autumn (15.2%), succeeded by summer (11.1%) and winter (9.4%), whereas the least occurrence was witnessed in spring (5.6%).

Adediran *et al.* (2015) assessed the efficacy of Levamisole, albendazole, ivermectin, and Vernonia amygdalina for treating helminth infections in West African Dwarf goats. Outcomes indicated modest resistance in ivermectin and Levamisole, with albendazole displaying effectiveness. The study also hinted at Vernonia amygdalina's potential in addressing helminth infections in goats.

Alcala-Canto *et al.* (2016) examined the efficacy of diverse treatments in decreasing EPG shed by gastrointestinal nematodes (GIN) in sheep across three distinct breeding farms in Mexico. Over a consecutive two-year span, the study assessed the efficacy of Ivermectin and benzimidazole derivatives. The research indicated that the utilization of anthelmintic closantal combined with albendazole may impede the development of anthelmintic resistance (AR) in gastrointestinal nematodes.

Christain *et al.* **(2019)** explored anthelmintic resistance (AR) within gastrointestinal nematodes affecting sheep reared in the mountainous farming conditions of northern Italy. The study implemented oral administration of macrocyclic lactone, benzimidazole (BZ), sometimes combined with salicylanilide (SA), or a blend of imidazothiazole and SA within controlled parameters. The research identified a substantial prevalence of AR in sheep subjected to mountain farming conditions, considering potential concerns about the impact of inadequate dosing on routine treatment outcomes.

Edwin *et al.* **(2020)** explored prevalent practices for worm control and anthelmintic resistance (AR) in sheep farms located in Flanders, Belgium. The findings revealed extensive resistance towards Benzimidazoles (Albendazole, Fenbendazole, and Mebendazole), evident through treatment inefficacy. Subsequent to treatment failure, *Haemonchus contortus* and *Teladorsagia circumcita* were identified as the prevailing species.

2.2   FAMACHA TECHNIQUIES

Bath *et al.* **(1996)** indicated that a primary challenge in implementing a selective treatment strategy has been the absence of a cost-effective and efficient method for identifying animals needing treatment. This predicament has been recently resolved through the introduction of an innovative system originating in South Africa, designed to identify anaemic sheep.

Vatta *et al.* **(2001)** confirmed the applicability of FAMACHA on goat farms in South Africa through validation.

Van and Bath (2002) in subsequent investigations, where FAMACHA was employed by farmers and treatments were guided exclusively by FAMACHA scores, without relying on PCV determinations, a noteworthy 58% reduction in the necessary treatments was observed across 10 farms.

Kaplan *et al.* **(2004)** Originating in South Africa, FAMACHA facilitates the clinical detection of anemia in sheep and goats. Particularly when *Haemonchus contortus* serves as the leading parasitic agent, this approach can be implemented at the

farm level to curtail the frequency of treatments, consequently boosting the proportion of the worm population within refugia. Given that the majority of validation studies for the FAMACHA method have been conducted in South Africa, it is imperative that this technique undergoes testing in diverse regions before its widespread endorsement.

2.3   STATUS OF ANTHELMINTIC RESISTANCE IN SMALL RUMINANTS

Deepa (2005) reported that the most prevalent species identified was *Haemonchus spp.* in the post treatment coproculture (98 per cent) indicating that it was the major contributing species for resistance to benzimidazoles. Suboptimal dosing could be an additional factor influencing resistance development. Goats, characterized by swift hepatic metabolism and decreased drug bioavailability, often necessitate a dosage of benzimidazoles twice as high as recommended for sheep, potentially contributing to this issue.

Lespine *et al.* (2005), found that ivermectin is equally effective against T. colubriformis via subcutaneous or per os administration in goats. Nonetheless, due to lower tissue levels with per os use, efficacy duration may reduce, particularly in poorly conditioned animals in re-infection-prone pastures after treatment.

Van *et al.* (2006) revealed that the reduction in faecal egg counts for *strongyle* worms following treatment with FBZ, closantel, and IVM was documented at 45.0% and 63.0%; 69.0% and 86.3%; and 84.0% and 91.0% on the 7th and 14th day after treatment, respectively. These results imply the emergence of resistance in gastrointestinal nematodes (GINs) against all three drugs—FBZ, closantel, and IVM—as outlined by **Coles *et al.* (1992).**

Akther *et al.* (2015) assessed the efficacy of a polyherbal blend consisting of garlic, turmeric, ginger and black pepper as an anthelmintic agent against gastrointestinal nematodes (GINs) in goats. The findings revealed noteworthy anthelmintic potency of the polyherbal mixture, leading to a considerable decrease in faecal egg counts (FEC) of up to 84%.

Rajagopal *et al.* (2017) documented that the elevated reproductive capacity of gastrointestinal nematodes, particularly *H. contortus*, accelerates the development of

resistance due to the rapid generation of a substantial worm population within a brief timeframe.

Om Prakash *et al.* **(2018)** reported **r**esistance to the majority of the anthelmintics including the Benzimidazole, Salicylanilides, Organophosphates and Imidazothiazoles, Macrocyclic lactones and Amino-acetonitrile derivatives amongst sheep & goat in chennai. The widespread application of drug treatments, whether utilized correctly or incorrectly, coupled with the adaptive capabilities of *H. contortus* to counteract the adverse impacts of these drugs, has fostered the emergence of drug resistance. Consequently, this has contributed to the parasite's persistence and proliferation.

Shakya *et al.* **(2018)** reported the emergence of resistance to levamisole and albendazole in goats, whereas gastrointestinal nematodes exhibited vulnerability to ivermectin. This susceptibility to ivermectin might stem from the fact that oral ivermectin was introduced considerably later than albendazole and levamisole and has not achieved comparable widespread usage, thus avoiding indiscriminate use by farmers.

Váradyová *et al.***, (2018) i**ndicated that employing chemoprophylaxis to combat the gastrointestinal parasitic nematode *Haemonchus contortus* through repeated anthelmintic application heightens the potential for residues in food items and the progression of anthelmintic resistance.

Bihaqi *et al.***, (2020)** highlighted that the frequent and unselective application of FBZ, closantel, and IVM has resulted in the escalation of resistant gastrointestinal nematode (GIN) populations. If not promptly addressed, this trend could eventually lead to the emergence of entirely resistant nematode populations over time. To mitigate further resistance development, the implementation of Targeted Selective Treatment (TST) and FAMACHA is recommended.

2.4 ANTHELMINTIC PROPERTIES OF HERBAL PLANTS AGAINST GASTROINTESTINAL NEMATODES

Iqbal *et al.* **(2001)** illustrated that the methanolic extracts from *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana*, *and Ficus religiosa* exhibited effectiveness

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rates of 100% and 83.4% against *Haemonchus contortus* at 2, 4, 6, and 6 hours following exposure, respectively.

Jangde *et al.* (2001) found that the aqueous extracts of *Artemisia martima* (root) and *Butea frondosa* (seed) were able to cause cessation of mortality and death of *H. contortus* at different concentrations. Pessoa *et al.* (2002) reported that the oil of *Ocimum gratissium* and *eugenol* at 0.05% inhibited egg hatch in *H. contortus*.

Reichling and Saller (2001) emphasized two challenges for veterinarians entering phytotherapy: distinguishing effective from ineffective plants and assessing the physiological effects, risks, and side-effects of herbal treatments. While plant-based preparations are often perceived as safer, they can be as toxic as conventional medicines, causing harm if misused

Alawa *et al.* (2003) conducted an in-vitro trial of *Annona senegalensis* against larvae and eggs of *H. contortus* and observed significant inhibition in egg hatch and larval development at 7.1 mg/ml and 100 mg/ml, respectively.

Assis *et al.* (2003) found that the ethyl acetate and methanolic extracts of *Spigelia anthelmintica* at 50mg/ml concentration recorded 100% and 97.4% inhibition of egg hatching and 81.2% and 84.4% inhibition in larval development, respectively, against *H. contortus*.

Molan *et al.* (2003) observed that flavan-3-ols (monomer unit of condensed tannin) caused 100% inhibition of egg hatch and development of larvae of *Trichostrongylus colubriformis* at 1000 µgm/ml.

Raje *et al.* (2003) found that a mixture of powdered *Azadirachta indica* (bark), *Butea frondosa* (seed), *Nigele sativa* (seed), and *Piper longm* (fruits) resulted in a significant reduction in the faecal egg count in calves.

Raje and Jangde (2003) reported 1%, 4%, 7%, and 10% mortality of *H. contotus* against decoction of *Nicotiana tabacum* after 16, 12, 8.05, and 6.20 hrs post-exposure.

Viegi *et al.*, (2003) indicated that herbs rich in tannins possess a direct antiparasitic effect against internal nematodes in ruminants and can also indirectly enhance host resistance.

Iqbal *et al.* **(2004)** reported a 67.2% reduction in faecal egg count in naturally infected sheep following oral administration of 3gm/kg b.wt. of aqueous extract of *Artemisia brevifolia* on day 14 post-treatment.

Athanasiado *et al.* (2007) noted a shift from anecdotal observations to controlled experiments, validating the ant parasitic activity of plants.

Al-Shaibani *et al.* (2009) examined the *in vivo* anthelmintic potential of *Fumaria parviflora* on sheep's gastrointestinal nematodes. Notably, a substantial decrease in eggs per gram (EPG) by 77.6% and 70.05% was observed with ethanolic and aqueous extracts, respectively, at a dosage of 200 mg/kg body weight on the 14th day post-treatment

Bachaya *et al.* (2009) examined the anthelmintic effectiveness of the crude methanolic extract from *Acacia nilotica fruit and* Ziziphus *nummularia* bark in naturally infected sheep with gastrointestinal nematodes. Remarkably, a reduction of 84.7% and 78.5% in faecal egg count was recorded at a dosage of 3.0 g/kg body weight on the 13th day post-treatment for *Ziziphus nummularia and Acacia nilotica*, respectively. In a study by **Tariq *et al.* (2009)**, orally administered crude ethanolic and crude aqueous extracts of *Artemisia absinthium* resulted in a 90.46% and 80.49% reduction in faecal egg count, respectively, against gastrointestinal nematodes in sheep.

Eugale and Giday (2009) discovered that aqueous extracts derived from *Chenopodium ambrosioides* exhibited a dose-dependent decline in the motility and survival rates of *H. contortus*.

Maphosa *et al.* (2009) observed that unrefined aqueous extracts sourced from *Aloe ferox*, *Leonotis leonurus*, *and Elephantorrhiza elephantina* hindered the hatching of *H. contortus* eggs and the progression of larvae.

Oliveira (2009) observed that ethyl acetate extract of *Cocos nucifera* fruit inhibited the hatching of *Haemonchus* eggs and the development of larvae.

Waghmare *et al.* (2009) reported complete reduction in faecal egg count in sheep infected with *H. contortus* following administration of a seed mixture powder of *Vernonia anthelmintica*, *Butea frondosa*, *Artemisia maritime*, and stem bark of

Holorrhena antidysentrica in equal quantities at 5gm/animal for 10 days. The *in vitro* investigation demonstrated that the hot methanolic extract of *A. indica* and the aqueous extract of *Embelia ribes* displayed larvicidal and adulticidal activity of 56% and 100%, respectively, against *H. contortus* at 20mg/ml after 72 hours. Furthermore, the hot ether extract of *Pongamia glabra* exhibited larvicidal and adulticidal activity ranging from 60% to 74% and 100%, respectively.

Kamaraj *et al.* (2010) discovered that both hydro-alcoholic and aqueous extracts derived from *Melia azedarach L.* leaves effectively hindered the hatching of *H. contortus* eggs and the progression of larvae.

Macedo *et al.* (2010) observed that *Eucalyptus staigeriana* essential oil inhibited the hatching of *Haemonchus* eggs and the development of larvae.

Eguale *et al.* (2011) discovered that hydro-alcoholic and aqueous extracts derived from *Leucas martinicensis*, *Senna occidentalis*, *Leonotis ocymifolia* and *Albizia schimperiana* impeded the hatching of *H. contortus* eggs and the maturation of larvae.

Maithani and colleagues (2011) explored the phytochemical composition of *Azadirachta indica*, revealing the existence of phenolics, alkaloids, glycosides, saponins, tannins, and carbohydrates within the aqueous leaf extract.

Monteiro *et al.* (2011) observed that ethanolic extracts of *Jatropha curcas* seeds inhibited the hatching of *H. contortus* eggs.

Singh *et al.* (2011) evaluated the ovicidal and larvicidal property of *Andrographis paniculata* leaves against *Haemonchus contortus* and found that the extract could reduce embryonation and hatching of eggs at concentration of 1.25 mg/ml.

Adama *et al.* (2012) found that aqueous extracts of *Leuceana leucocephala* and *Gliricidia sepium* inhibited the hatching of *Haemonchus spp.* eggs and the development of larvae.

Ahmed *et al.* (2012) observed that ethanol extracts of *Lespedeza cuneata* induced mortality in *H. contortus*.

Bairwa *et al.* **(2012)** reported that *Trachyspermum ammi* contains carbohydrates, glycosides, saponins, phenolic compounds, volatile oils, protein, fat, fiber, and minerals such as calcium, phosphorous, iron, and nicotinic acid.

Cala *et al.* **(2012)** observed that hexane extracts of *Melia azedarach* and *Trichilia claussenii* inhibited the hatching of *Haemonchus spp.* eggs and the development of larvae.

Getachew *et al.* **(2012)** observed that aqueous and alcoholic extracts from *Myrsine africana*, *Rhus glabrous*, *Jasminum abysinicum*, *Rhus vulgaris*, *Acokanthera schimperi, and Foenicum vulgare* effectively hindered the hatching of *H. contortus* eggs and the subsequent larval development.

Vinoth *et al.* **(2012)** investigated the phytochemical components of *Azadirachta indica* extracts obtained through ethanol, methanol, and acetone solvents. The analysis indicated the occurrence of various compounds, including reducing sugar, flavonoids, saponin, and tannin in the ethanol extract; reducing sugar, glycoside, and terpenoids in the methanol extract; and reducing sugar and glycoside in the acetone extract

Ferreira *et al.* **(2013)** discovered that aqueous extracts obtained from *Annona muricata* leaves successfully impeded the hatching of *H. contortus* eggs and the subsequent larval development.

Mini *et al.* **(2013)** evaluated the anthelmintic ability of *Aristolochia indica* extracts against *H. contortus.*

Mbadianya *et al.* **(2013)** conducted a study on the phytochemical analysis of aqueous extract of *Azadirachta indica* and recorded the presence of tannin, soluble carbohydrate, hydrogen steroid, and flavonoid.

Zhu *et al.* **(2013)** found that the essential oil derived from *Artemisia lancea* at a concentration of 10mg/ml inhibited 77% of larval motility, 93% of larval development, and 99% of egg hatching.

Hamad *et al.* **(2014)** tested the anthelmintic property of a combined formulation of *Nicotiana tabacum* and *Azadirachta indica* against levamisole and

ivermectin resistant *Haemonchus contortus* and recorded a 94.59% reduction in FEC at the dose rate of 4 (2+2) gm/kg b. Wt.

Kumar *et al.* (2014) explored the *in-vitro* impacts of unrefined powder, aqueous, diethyl ether, and methanolic extracts of *Prunus persica* on *H. contortus*. The research revealed complete mortality rates at concentrations of 0.25%, 0.5%, 1%, and 2% within 24 hours of exposure.

Lem *et al.* (2014) conducted an in-vitro anthelmintic study of methanolic and aqueous extracts of the stem bark of *Terminalia glaucescens* (*Combretaceae*) against *H. contortus*. They reported 86.5% and 96% inhibition in egg hatch and 89.3% and 91.1% mortality of L3 at 5000μg/ml.

Saidu *et al.* (2014) found that the methanolic extract of *Azadirachta indica* leaves contains alkaloids, flavonoids, saponins, steroids, tannins, phenolic compounds, and resins as major phytochemical constituents.

Suleiman and co-researchers (2014) examined the effectiveness of unrefined methanolic extracts from *Cassia occidentalis* and *Guiera senegalensis* in combating eggs and larvae of *H. contortus*. The study found 65% and 60% inhibition in egg hatching and 86% and 85% inhibition in larval development at 100mg/ml, respectively.

Alam *et al.* (2015) investigated the effects of ethanolic and aqueous extracts of *Artemisia absinthium* (aerial parts), *Picrorhiza kurroa*, *Nepeta cataria*, *and A. maritime* (roots) against adult *H. contortus* in an in-vitro study. The study found that ethanolic extracts showed 100%, 100%, 65%, and 70% efficacy, respectively, while aqueous extracts showed 80%, 70%, 67%, and 70%, respectively, at 100mg/ml.

Akther *et al.* (2015) in an in-vitro trial of methanolic and aqueous extracts of eight medicinal plants against adult and L3 larvae of *H. contorts*, found that methanolic extract of neem showed 100% mortality against adult worms at 5% and 10%. The study also found that neem and korolla fruits showed 100% mortality, followed by leaves of korolla (80%), and lazzabati (60%) at 10% concentration against L3 larvae within 3 hours.

Bhardwaj *et al.* (2015) conducted an *in-vitro* trial of methanolic extract of the flower of *Tagetes patula* against adult *H. contortus* and recorded maximum efficacy of 83% at 8mg/ml.

Kuamr *et al.* (2015) evaluated the in-vitro anthelmintic efficacy of methanolic extract of *Annona squamosa*, *Eucalyptus globulus*, *Syzygium cumini*, and *Catharanthus roseus* on eggs of *H. contortus*. The investigation demonstrated that all extracts exhibited complete inhibition of egg hatching at a concentration of 50mg/ml.

Sachan *et al.* (2015) performed an anthelmintic experiment involving methanolic, ethyl acetate, and chloroform extracts derived from *Chenopodium album and Annona squamosa* against GI nematodes in goats, utilizing an egg hatch assay. The findings demonstrated that all plant extracts demonstrated full suppression (100%) of egg hatching at a concentration of 100 mg/ml.

Ambalathaduvar and colleagues (2016) investigated both *in vitro and in vivo* the anthelmintic capabilities of leaf extracts from *Indigofera tinctoria* against gastrointestinal nematodes (GIN) in sheep. The findings indicated that the ethanolic extract exhibited a significant 73% suppression of egg hatching at a concentration of 80mg/ml. Furthermore, reductions in faecal egg count were evident, with levels of 47.78%, 41.41%, and 30.82% at doses of 500, 250, and 125mg/kg body weight, respectively.

Kumar *et al.* (2016) assessed the anthelmintic attributes of diverse *Chenopodium album* leaf extracts against adult *H. contortus* in vitro, observing complete efficacy at concentrations of 0.5%, 1%, and 2% within 30, 24, and 24 hours of exposure respectively.

Kusum Lata *et al.* (2017) also recorded anthelmintic activity of herbal formulation and found that faecal egg count in goat showed 20.4% reduction after treatment with seed powder of *A. indica* @ 4g/kg b.wt.

Sunandhadevi *et al.* (2017) suggested the potential for synergistic effects among the phytochemicals of *Butea frondosa*, *Vernonia anthelmintica*, *Artemisia maritima*, and *Holarrhena antidysentrica* in a polyherbal anthelmintic formulation.

Khanolkar *et al.* (2018) noted that following the administration of a polyherbal tablet, a notable and efficient reduction of H. contortus was observed. This outcome

was attributed to the synergistic activity of phytochemicals from four extracts – *Artemisia maritima*, *Butea frondosa*, *Holarrhena antidysentrica*, *and Vernonia anthelmintica* – within the polyherbal tablet. This collaborative effect contributed to gradual weight gain in goats.

Singh *et al.* (**2019**) stated that processed Poly Herbal Mixtures (PHM) exhibited anthelmintic activity comparable to the conventional anthelmintic drug fenbendazole. Notably, a higher dose of crude ethanolic (CEE) extracts from PHM, composed of *Allium sativum* (cloves), *Chenopodium album* (leaves) and *Azadirachta indica* (leaves), demonstrated similar *in vivo* anthelmintic effectiveness as Fenbendazole against prevalent gastrointestinal nematodes in goats. The anthelmintic activity of CEE of PHM may be associated with the various phytochemicals present in the hebal drug such as phenolics compounds, azadirachtin, lupeol, allicin, b-sitosterol, and alkaloids.

These investigations propose that polyherbal formulations might serve as viable substitutes for conventional anthelmintics in addressing GIN infections in small ruminants and cattle. Nonetheless, additional research is essential to assess the extended effectiveness and safety of these formulations across diverse livestock species. Moreover, determining the ideal dosage, treatment duration, and mechanisms of action for these formulations is imperative to promote their broader adoption in livestock farming.

2.5   DIAGNOSIS OF ANTHELMINTIC RESISTANCE

The existence of resistance stemming from anthelmintic use is acknowledged when the reduction percentage in faecal egg count (FECR%) falls below 95% and is associated with a 95% confidence level that extends below 90%. The extent of resistance can be categorized as severe when FECR% is below 60% and moderate when it falls within the range of 60-90%. If either of these two conditions is met, there arises a presumption of anthelmintic resistance (**Coles** *et al.*, **1992).**

Precise and timely detection of anthelmintic resistance holds paramount significance, given the global and national prevalence of anthelmintic resistance. A range of both *in vivo* and *in vitro* methodologies, including molecular approaches for diagnosing resistant parasites, have been employed to ascertain anthelmintic resistance.

In-Vivo **tests** are bioassays/parasitological assays that could be used for all types of anthelmintics. Nevertheless, these assessments are demanding in terms of labour, time, finances, and exhibit limited accuracy and reproducibility.

In-Vitro **Tests** Anthelmintic resistance screening can be performed using in-vitro tests, which are rapid, sensitive, and more cost-effective, compared to in-vivo tests. These tests involve parasitological, biochemical, and genetic assays that do not require the host as the source of primary data. However, their usefulness is limited by their dependence on the pharmacodynamics of specific anthelmintic groups.

2.5.1 Critical Anthelmintic Test –

The critical anthelmintic test was first defined by **Hall and Foster in 1918**. It is based on collecting faeces from animals for at least four days after anthelmintic treatment. The expelled worms are recovered from the collected faeces and their numbers are estimated. The treated animals are then slaughtered, and the residual worm burden is estimated. The calculation of percentage efficacy involves dividing the count of expelled worms by the remaining number and then multiplying the result by 100.However, this test cannot be used for worms that undergo digestion during passage through the intestinal tract **(Reinecke *et al.*, 1962)**.

2.5.2 Controlled Anthelmintic Efficacy Test-

In this test, efficacy of an anthelmintic is evaluated by comparing the parasite population in treated and untreated animals **(Prichard, 1980; Power *et al.* 1982).** The animals are artificially infected with susceptible and/or suspected resistant isolates of the parasite and later treated. All animals are killed, usually 5-7 days after treatment, and the parasites are counted, identified, and efficacy calculated. This approach stands as the most dependable means for assessing anthelmintic effectiveness in ruminants and is advised for dosage adjustment and verification trials. Additionally, this technique can be utilized to ascertain the efficacy of an anthelmintic across various developmental stages of a parasite's life cycle **(Reinecke and Louw, 1994)**. Nonetheless, this examination is resource-intensive, demanding substantial time and expenses for both animals and labour.

2.6   Faecal Egg Count Reduction Test –

The test stands as a prevalent and highly recommended examination by WAAVP for identifying anthelmintic resistance within real-world settings (**Presidente, 1985; Dash *et al.*, 1988; Coles *et al.*, 1992**). The interval between treatment and sampling is critical, as temporary suppression of egg production by gravid female may occur after treatment, giving a false high efficacy if the interval is short. If the interval is long, a new patent infection may be acquired, giving a false low efficacy. To overcome this, **Coles *et al.*, 1992,** suggested that faecal egg counts should be done at 10-14 days post-treatment. However, if the egg counts prior to treatment are low, the FECRT may fail to detect resistance unless the response is consistent among most animals in the group **(McKenna, 1997).** Additionally, the FECRT does not detect the presence of immature worms, which can play a significant role in post-treatment egg counts.

It can be used with ruminants, horses, and pigs, with all types of anthelmintics and with all species of nematodes in which eggs are shed in the faeces. This method does not necessitate extensively trained personnel, remains cost-effective, and eliminates the need for advanced equipment. The effectiveness of an anthelmintic treatment can be gauged through the FECRT, utilizing either arithmetic or geometric means of EPG. The interval between treatment and sampling is critical, as temporary suppression of egg production by gravid female may occur after treatment, giving a false high efficacy if the interval is short. If the interval is long, a new patent infection may be acquired, giving a false low efficacy. To overcome this, **Coles *et al.* (1992)** suggested that faecal egg counts should be done at 10-14 days post-treatment. Anthelmintic resistance is present if FECR% is less than 95% associated with a 95% confidence level extending below 90%. The severity of resistance may be classified as severe. However, if the egg counts prior to treatment are low, the FECRT may fail to detect resistance unless the response is consistent among most animals in the group **(McKenna, 1997).** Additionally, the FECRT does not detect the presence of immature worms, which can play a important role in post-treatment egg counts.

2.7   POLYHERBAL INGREDIENTS BEING USED-

2.7.1   BUTEA FRONDOSA

Butea frondosa, commonly known as Palas, is a herb from the leguminosae family.

Garg and Metha (1958) conducted an *in vitro* trial that revealed the anthelmintic efficacy of *B. frondosa* seeds against *Bunostomum trigonocephalum* and *Haemonchus similis* in animals.

Kirthikar and Basu (1975) stated that *Butea frondosa*, commonly known as 'palash', is prevalent across India and possesses anthelmintic properties. The researcher documented its utilization in combating roundworm infections.

Lal *et al.* (1978) reported that Palasonin, a *Butea monosperma* compound, showcased substantial anthelmintic potential against *A. lumbricoides in vitro.*

Kumar *et al.* (1995) explored the biochemical mechanism underlying the action of Palasonin, the active component found in *B. frondosa* seeds, *on A. galli*. Palasonin was observed to suppress both the frequency and strength of spontaneous contractions in *A. galli* while also enhancing the spasmogenic impact of Ach on the toad's rectus abdominis muscle. *Pippali Rasayana* (PR), an Ayurvedic herbal formulation derived from Palash (*Butea monosperma*) and Pippali (*Piper longum*), exhibited complete efficacy in managing *Giardia lamblia* when orally administered thrice daily over a 15-day period.

Agarwal *et al.* (1997) found that Seeds of Butea monosperma (Lam.) Kuntze, commonly known as Palash in Hindi, contain *cantharidin palasonin*, which has anthelmintic properties, particularly against roundworms and tapeworms. The unrefined seed powder displayed a dose and duration-dependent anthelmintic impact on a variety of gastrointestinal nematodes present in naturally infected sheep. Anthelmintic properties have also been documented in Butea species against a range of parasites including *Ascaridia galli*, *earthworms*, *oxyurids*, *Dipylidium caninum*, *Toxocara canis*, *Ascaris lumbricoides and Taenia spp.*

Mackeen *et al*. (1997) found that Butea monosperma, was ubiquitous in India, holds folk medicinal value. Its methanol extract displayed anthelmintic, anticonvulsive, hepatoprotective, antifertility, anti-diarrheal, and antifungal potential.

Hussain *et al*. (2006) treated live parasites (*Haemonchis controtus* and *pheretime posthuma*) with *Butea frondosa* extract and found that concentrations of 5.00%, 2.25%, and 1.25% caused death of parasites in 1-1.5, 2-4, and 4-5 hours post-exposure, respectively, comparable to piprazine's anthelmintic activity.

Iqbal *et al*. (2007) indicated that *Butea frondosa*, referred to as Palash seeds, showcases anthelmintic characteristics attributed to the presence of bioactive compounds including palasonin, phenolics, and flavonoids. These constituents disrupt the energy dynamics and metabolic processes of parasites by impeding glucose absorption and depleting glycogen reserves.

Vihan *et al*. (2007) evaluated the anthelmintic potential of *B. frondosa* against *H. contortus* using concentrations of 2, 4, and 8 mg/ml. The chloroform extracts demonstrated notably substantial larvicidal activity across different time intervals (24 hrs, 48 hrs, and 72 hrs). Additionally, a range of *B. frondosa* plant extracts displayed notable larvicidal activity, reaching levels as high as 50-60%.

Swarnkar *et al*. (2008) observed that the aqueous extract derived from *Butea frondosa* caused 90.73% larval mortality of *H. contortus* at 72 hours post-incubation, while other extracts showed lower activity. He reported that chloroform and ether extracts of *B. frondosa* seeds exhibited embryonicidal activity, while the alcoholic extract displayed ovicidal activity and aqueous extract had larvicidal activity against *H. contortus*.

Dhiraj Arora, 2009 documented that the LD50 values for the Aqueous and Ethanolic extracts of *Butea Monosperma* (L) Toub leaves, following OECD guidelines, surpassed 2000mg/kg (LD50>2000mg/kg). Furthermore, no instances of toxicity or fatality were noted at the administered dose levels in the tested animals. Consequently, the biological dosages for both extracts were established at 100 and 200mg/kg.

Pandhare *et al*. (2009) assessed the anthelmintic efficacy of *Butea frondosa* leaves against adult Indian earthworms *Pheretima posthuma*. The alcoholic extract

demonstrated considerable activity at concentrations of 25, 50, and 100 mg/ml, comparable to the reference standard piperazine citrate.

Borkar *et al.* **(2010)** demonstrated that phenolic compounds found in *B. frondosa* seed have anthelmintic activity, similar to synthetic phenolic anthelmintics that interfere with energy generation in helminth parasites.

Rai *et al.* **(2011)** documented the antihelmintic activity of Palasonin, a compound derived from *Butea monosperma* seeds. The administration of seeds in crude powder form at varying doses (1, 2, and 3 g/kg) to sheep naturally infected with mixed species of gastrointestinal nematodes revealed a dose-dependent and time-dependent anthelmintic impact. Notably, the most significant reduction of 78.4% in eggs per gram of faeces was observed on the 10th day post-treatment with a dose of 3 g/kg. Furthermore, the anthelmintic efficacy of various Butea species has been reported against parasites such as *Toxocara Canis*, *Ascaridia Galli*, *Earthworms*, *Oxyurids*, *Ascaris Lumbricoides and Dipylidium Caninum* among others.

Rajput *et al.* **(2011)** found that *Butea monosperma* leaves contained active constituents such as sterols, triterpenes, glycosides, flavonoids, and proteins. The petroleum ether extract contained sterols and triterpenes, while the chloroform extract contained sterols, triterpenes, and glycosides. The methanol extract revealed the presence of flavonoids and proteins.

Mamta (2012) found that among different extracts of *Butea frondosa* methanolic extract revealed the presence of flavonoids, tannins, anthraquinones, resins and phenolic compounds however alkaloids, glycosides, sterols, flavonoids, tannins, reducing sugars and saponins were present in the hydroalcoholic extract. The aqueous extract showed presence of alkaloids, sterols, amines, flavonoids, reducing sugars, anthraquinones and saponins.

Mansoor *et al.* **(2013)** indicated that the crude methanolic extract derived from *B. frondosa* seeds displayed noteworthy anthelmintic activity against earthworms (*Lumbricus terrestris*). Notably, the extract achieved 100% mortality of the worms when used at a concentration of 50 mg/ml.

Singh *et al.* (2015) stated that *Butea frondosa*, commonly known as Palash seeds, possesses anthelmintic properties attributed to its active constituents, including palasonin, phenolic compounds, and flavonoids present in the seeds. These compounds contribute to the disruption of energy and metabolic processes within the worm by impeding glucose uptake and depleting glycogen reserves.

Singh *et al.* (2015) disclosed that a phytochemical analysis of *Butea monosperma* (Lam.) Kuntze highlighted a substantial accumulation of total phenolic and flavonoid content within the plant, indicating its potential suitability for anthelmintic purposes. Moreover, the plant exhibited elevated nutritional value as deduced from proximate analysis. Additionally, the presence of a balanced concentration of copper and zinc, acknowledged for their direct wormicidal effects and indirect enhancement of the immune system, alongside their gastro-protective properties, positions it as a fitting candidate for a comprehensive approach to managing *haemonchosis* in sheep and goats.

Yadav *et al.* (2015) mentioned that the active principle of *B. frondosa* is an alkaloid that possesses physostigmine-like pharmacological action, which can be demonstrated by specific atropine blockade, acetylcholine response, and the antagonism of curare action. Treatment of *F. gigantica* infected cattle with powdered seeds of *B. frondosa* at 1gm/day showed a significant decline in faecal egg count (EPG) by the 15th day post-treatment, and the therapy demonstrated enhanced efficacy compared to the same dose of *B. frondosa* given with powdered *Withania somnifera* (Ashwagandha) at 0.15 gm/kg wt, perhaps due to the immunomodulating activity of Ashwagandha.

Sunandhadevi *et al.* (2017) suggested the potential for synergistic interactions among the phytochemicals of *Artemisia maritima*, *Butea frondosa*, *Vernonia anthelmintica* and *Holarrhena antidysentrica* within a polyherbal anthelmintic formulation.

In conclusion, *Butea frondosa* has been widely investigated for its anthelmintic activity against various parasites. Its active principle, Palasonin has been demonstrated to reduce the occurrence and strength of autonomous contractions in *A. galli* and amplify the spasmogenic influence of Ach on the rectus abdominis muscle in toads.

Saiyam *et al.* (2021) reported in the *in vivo* assessment, the aqueous extract of *B. frondosa* seeds demonstrated the highest reduction (73.33%) in egg count per gram of faeces, significantly so (p<0.01), on the 14th day following treatment. Conversely, the methanolic extract exhibited the highest reduction (57.14%), which was also statistically significant (p<0.05), on the 21st day after treatment. Notably, no indications of toxicity were observed at the experimental doses administered at 100 mg/kg body weight orally on days 0, 3rd, 7th, and 21st. In the adult mortality test, both the aqueous and methanolic extracts of *B. frondosa* seeds achieved 100% mortality at concentrations of 100 mg/ml and 50 mg/ml.

Parul *et al.* (2022) conducted a study focused on the aqueous extraction of *Butea frondosa (Lam.)* seeds to assess its *in vitro* anthelmintic potential against *H. contortus*. The chemical profiling of the extract unveiled elevated levels of tannins, phenolics and flavonoids. The recorded data indicated that at 4 hours post-exposure, the parasites exhibited reduced activity and movement when exposed to concentrations of 25 and 50 mg/ml. Furthermore, after 6 hours of exposure, the extract demonstrated complete mortality of adult *H. contortus* worms at a concentration of 100 mg/ml.

2.7.2 EMBELIA RIBES,

Embelia ribes, also known as Barbarang, is a herb from the Myrsinaceae family.

Gajjar UH (2009), stated that the ethanolic extract derived from *E. ribes* seeds exhibited moderate antibacterial effects against *Klebsiella pneumonia*, *Enterobacter aerogenes and. Staphylococcus aureus*. However, no significant antimicrobial activity was observed against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *and Escherichia coli*.

Kekuda *et al.* (2009) indicated that the aqueous extract of *Embelia ribes* at concentrations of 3% and 5% displayed greater potency compared to equivalent concentrations of the standard drug.

Swarnkar (2009) reported that both aqueous and alcoholic extracts of *Embelia ribes* seeds demonstrated potent *in-vitro* ovicidal activity and mild larvicidal effects against the external stages of *Haemonchus contortus*. Moreover, the oral administration

of these extracts, given at a dose of 6.0 ml per sheep, resulted in a moderate decline in faecal egg count in sheep deliberately infected with *H. contortus.*

Tambekar *(2009)* stated that a notable reduction in faecal eggs per gram (EPG) count was observed in goats afflicted with mixed gastrointestinal nematode infections when administered a combination of *Embelia ribes* fruit extract and *Veronica anthelmintica* seed extract at a dosage of 1g/kg. Additionally, the ethanolic extract isolated from the fruit component of *Embelia ribes* exhibited a potent anthelmintic activity, achieving up to 93% efficacy against larvae of the gastrointestinal nematode *Haemonchus contortus.*

Kumar *et al.* **(2010)** reported an 80% and 84.46% decline in faecal egg count in sheep and goats, respectively, following administration of crude powder of Embelia ribes leaves for 3 consecutive days at 10gm orally, 10 days post-treatment.

Mahendran *et al.* **(2010)** reported LD50 of *embelin* in rats and mice is 2000 mg/kg, demonstrating no mortality or adverse effects and affirming its safety.

Rohilla and Patel (2010) noted a complete elimination of faecal egg count in both sheep and goats upon administering anthelmintic 28, comprising *B. frondosa*, *C. papaya*, *C. maxima*, *E. ribes*, *Meilotus ohillipinensis*, *Paedaria foetida*, *and Punica gr*anatum, at a dose of 50 gm/day/animal as a single administration. This significant reduction was observed 7 days post-treatment.

Bharat (2013) Stated that *Embelia ribes* harbors *embelin* as a prominent active component, contributing to a diverse range of activities including antibacterial, antifertility, antiprotozoal, alleviation of constipation, antifungal effects, management of mouth ulcers and sore throats, potential against pneumonia, anti-obesity properties, analgesic effects, anti-inflammatory benefits, antioxidant action, anthelminthic activity, antidiabetic potential, anticonvulsant properties, anticancer effects, and facilitation of wound healing.

Sharanbasappa Durg *et al.* **(2017)** Indicated that the LD50 of *Embelia Ribes* (embelin) is 2000 mg/kg in rats and mice, with no recorded mortality or adverse effects, thus highlighting its safety profile.

Sharanbasappa *et al.* (2017) reported that ER extract and its major active constituent embelin as well its derivatives showed beneficial effects in blood glucose, HbAlc, insulin, and lipid profile. In addition, ER extract and embelin normalized HR, SBP, LDH, CK and oxidative stress markers in diabetic rats.

Sinha *et al.* (2020) documented that the combined aqueous and alcoholic extracts from *Embelia ribes* fruits and *Vernonia anthelmintica* seeds resulted in a 72% decrease in faecal egg count. Positive changes in hematological and biochemical parameters indicated a reduction in worm infestations. Examination of faeces revealed *Haemonchus contortus* as the prevailing *strongyle* species. As a result, the formulation exhibited notable effectiveness against *Haemonchus contortus*, the most harmful worm in small ruminants, which has shown resistance to various anthelmintic treatments.

Choudhary *et al.* (2021), conducted research that highlighted *E. ribes* as possessing superior anthelmintic properties compared to other plants, such as *Celastrus paniculata*, *Impatiens balsamina*, *Gynandropsis gynandra* and *Mucuna pruriens*. The ethanolic extract from the fruit of *E. ribes* exhibited remarkable anthelmintic activity of up to 93% against the larvae of the gastrointestinal nematode *Haemonchus contortus*. Furthermore, an investigation into the antinematodal activity of a preparation combining *Vernonia anthelmintica seed and E. ribes* fruit extract was conducted on goats.

2.7.3 NEEM (*Azadirachta indica*)

Azadirachta indica, commonly referred to as neem, is a sizable evergreen tree extensively grown in India. It holds a traditional history of application in both human and veterinary medicine to address diverse health issues, including helminth infections.

Ahmad *et al.* (1994) reported 35% and 40 % efficacy of *Azadirachta indica* (Neeem) seed at 75 mg and 100 mg/kg b. wt. against GIN in sheep.

Arora *et al.* (2007) assessed the effectiveness of methanol extracts (hot and cold), ether extracts, and aqueous extracts from *A. indica* leaves against both larvae and adult *H. contortus*. In vitro experimentation indicated that the hot methanolic extract at a concentration of 20 mg/ml exhibited the highest larvicidal activity (56%) after 72

hours, achieving a 100% adulticidal outcome. In vivo studies demonstrated that the hot methanol extract showcased efficacy rates of 80.95% and 85.71% at doses of 150 and 200 mg/kg body weight, respectively, on the 7th day post-treatment. Application of leaf powder exhibited effectiveness rates of 80.0% and 88.89% on the 2nd and 3rd days post-treatment.Altogether, *A. indica* has displayed anthelmintic attributes and has been traditionally employed for this purpose in both human and veterinary medical practices. Various extracts and compounds derived from the plant have been shown to impede helminth activity, suggesting its potential as a natural alternative to synthetic anthelmintics.

Swarnkar *et al.* (2008) recorded 44.1% and 56.9% decline in faecal egg counts on 7 and 10 days post-treatment following oral administration of alcoholic extract of *Azaridachta indica* @ 50mg kg b.wt. against *haemonchus spp.* in sheep.

Rabiu and Subhasish (2011) stated that the aqueous extract of Neem (*Azadirachta indica*) demonstrated a potent anthelmintic effectiveness of up to 93%, when compared to the performance of pyrantel tartrate**.**

Mbadianya *et al.* (2013) performed a study involving the phytochemical analysis of the aqueous extract derived from *A. indica*, revealing the presence of various compounds including tannins, soluble carbohydrates, hydrogenated steroids, and flavonoids.

Hamad *et al.* (2014) tested the anthelmintic property of a combined formulation of *N. tabacum* and *A. indica* against levamisole and ivermectin-resistant H. contortus and found a 94.59% decline in faecal egg count at 4(2+2) gm/kg b.wt orally.

Nawaz *et al.* (2014) conducted both *in vitro* and *in vivo* anthelmintic trials using the aqueous extract of *Azadirachta indica*, *Dalbergia sisso*, *and Morus alba*. The study focused on their effects on eggs and adult worms of *H. contortus*. Results indicated reductions in faecal egg count on the 12th day after treatment.

Saidu *et al.* (2014) reported alkaloids, flavonoids, saponins, steroids, tannins, phenolic compound and resins were the major phytochemical constituents present in methanolic extract of *A. indica* leaves.

Braga *et al.* **(2021)** indicated that the LD50 value of *Azadirachta indica* was determined to be greater than 5000 mg/kg. An ethanolic extract of *A. indica* leaves, administered orally to mice at a dose of 5 g/kg b.w. over a period of 7 days, did not result in any acute toxicity symptoms or fatalities. Consequently, the calculated LD50 value was established as being higher than 5 g/kg.

Dehuri, *et al.* **(2021)** investigated the anthelmintic potential of aqueous extracts from various parts of *Azadirachta indica*, including leaves, stem, and root barks. The aqueous extracts from leaves and bark displayed inhibitory effects on nematode egg hatch. *In vivo*, a faecal egg count reduction test (FECRT) was performed using crude methanolic extract against *Strongyle* nematodes, revealing a significant reduction (p < 0.05) in egg per gram counts by day 7 post-treatment.

2.7.4 KALI ZEERI (*Centratheram anthelminticum)*

Centratherum anthelminticum, also known as "Kali Jeeri" or "Black cumin", is a plant species that has been traditionally used for medicinal purposes, including as an anthelmintic in both humans and animals. Scientific evidence exists to substantiate the anthelmintic attributes of *Centratherum anthelminticum* in animals.

Singh *et al.* **(1985)** exhibited in vitro anthelmintic efficacy of the alcoholic extract derived from *C. anthelminticum* seeds against *Hymenolepis nana*, *Fasciolopsis buski and Ascaris lumbricoides* worms.

Lambertini *et al.* **(2004)**. noted that certain bitter-tasting components present in Centratheram anthelminticum seeds are accountable for the observed anti-parasitic effect.

Iqbal and co-authors (2006) demonstrated the anthelmintic effectiveness of *C. anthelminticum* seeds through both in vitro and in vivo studies conducted on naturally infected sheep with gastrointestinal nematodes.

Waghmare *et al.* **(2009) n**oted a complete reduction of faecal egg count in sheep infected with *H. contortus* after administering a mixture of *Artemisia maritima* (Kirmaniova), *B. frondosa* (Palas), and *Holorrhena antidysentrica* (Kura) stem bark in equal proportions at a dosage of 5 gm per animal for duration of 10 days.

Paydar et al. **(2013)** stated that the employment of *Centratherum anthelminticum* as a remedy can induce the expulsion of parasitic intestinal worms, yielding effective outcomes in deworming both young children and adults. The pharmacological impact of *C. anthelminticum* spans a broad range, encompassing antioxidant, anti-diabetic, anti-microbial attributes, and, more recently, revealed anti-cancer potential. The plant's diverse components, encompassing fatty acids, sterols, flavonoids, and carbohydrates, exceeding 120 in number, have been identified from various plant parts. Numerous of these active elements were sourced from the seeds and have undergone assessment for diverse biological functions.

Ghai (2017) reported that Centratheram anthelminticum may be used as convenient, effective and economical anthelmintic in place of conventional anthelmintics

2.7.5 PUMPKIN SEEDS (*Cucurbita maxima*)

The anthelmintic effect of pumpkin seeds is attributed to cucurbitine, which effectively paralyzes turbellaria, as indicated by various studies (**Guarrera, 1999; Baytop, 1999; Asımgil, 2004; Gonzales *et al.*, 1974; Pieroni *et al.*, 2005**).

Mahmoud *et al.* (2002) reported that oral administration of pumpkin seed extract prepared with boiled water to puppies infected with Heterophyes heterophyes for two weeks resulted in the destruction of parasite eggs.

Cruz, *et al.* (2006) noted that the mean lethal dose (LD50) of *Cucurbita maxima* Duch. (Cucurbitaceae) pumpkin seeds surpasses 5000 mg/kg, while sub acute treatment resulted in weight gain.

Ayaz *et al.* (2015) documented that both water and ethanol extracts of pumpkin seeds exhibited notable anthelmintic efficacy (81% and 85%, respectively) against *A. tetraptera* in rats.

2.8 HAEMATO – BIOCHEMICAL STUDIES

Sahai (1966) studied *Haemonchus contortus* infection in lambs and kids, noting decreased Hb and PCV levels, along with altered leukocyte populations.

Barowicz and Patryszak (1970) observed reduced Hb in lambs infected with nematodes, including eosinophilia. Profound changes occurred after 135 days due to *H. contortus*.

Georgieva and Vladimirova (1975) delved into lambs infected experimentally with 2500-5000 *H. contortus* larvae, revealing electrophoretic analysis modifications. Notable were albumin decrease and globulin rise.

Symons and Jones (1978) linked protein loss to larval dose, impacting phosphorus and calcium absorption.

Uppal and Rai (1978) found a drop in serum proteins post 10, 000 *H. contortus* larvae infection, impacting albumin and globulin fractions, though A and B globulins increased.

Dakkak *et al.* (1981) noted pepsinogen increase post-infection in lambs, correlated with larval doses, while Radio Immuno Assay displayed gastrin concentration rise in *H. contortus*-infected sheep.

Bennet (1983) identified *H. contortus* as a major cause of anaemia and hypoproteinemia in both sheep and goats.

Costa and Pant (1983) studied goats naturally infected with *Haemonchus*, observing varying R.B.C. counts and eosinophilia based on breed and seasons.

Ahmad and Ansari (1989) found negative correlations between worm burdens and blood values in sheep and goats.

Ahmad *et al.* (1990) reported albumin decrease and varying globulin levels in *H. contortus*-infected lambs, which later normalized with treatment.

Bhat and Sharma (1990) associated eosinophillia with antigenic stimulation or parasite burden, showing increased lymphocyte count.

Siddiqui *et al.* (1990) reported hematological changes in Black Bengal goats due to intestinal parasites, including decreased Hb and increased eosinophils.

Brar *et al.* (1991) noted Pol-Dorset sheep infection with *H. contortus*, resulting in lowered serum protein, increased albumin, serum and blood glucose levels. Acute *haemonchosis* led to elevated blood urea and uric acid.

Rahman and Collins (1991) highlighted the greater impact of goat-derived *H. contortus* strain on weight gain and blood components compared to sheep-derived strain.

Abdel (1992) highlighted lowered hemoglobin and RBCs in h*aemonchosis*, possibly due to abomasal bleeding

Fox *et al.* (1992) observed raised blood gastrin and pepsinogen in Malaysian goats infected with 10500 L3 *H. contortus*.

Chakraborty and Lodh (1994) reported biochemical changes in goats afflicted with various species.

Partani *et al.* (1995) found reduced serum proteins, albumin, globulin, and serum urea nitrogen in camels with gastrointestinal nematode infection.

Urquhart *et al.* (1996) reported that *Haemonchosis* leads to anemia via bloodsucking worm activities.

Brar *et al.* (1998) revealed altered sodium, calcium, and potassium concentrations, in acute *haemonchosis* in Pol Dorset Sheep.

Arora (2000) highlighted decreased Hb, PCV, and TEC values in infected groups, attributed to active feeding by developing *bursate* worms.

Sharma *et al.* (2000) A notable decrease in packed cell volume (PCV), hemoglobin (Hb), and total erythrocyte count (TEC) was observed in goats affected by *haemonchosis* in the region of Mathura, Uttar Pradesh.

Swarnkar *et al.* (2000) reported that in lambs experimentally infected with *Haemonchus contortus*, a significant decrease was observed in mean PCV, Hb concentration, and erythrocyte count. Additionally, there was a negative correlation between worm burden, faecal egg count, body weight, Hb%, PCV and total erythrocyte count (TEC).

Arora *et al.* (2001) significant reductions in total protein, albumin, and blood glucose levels were observed during *bursate* worm infections in both sheep and goats.

Sharma *et al.* (2001) evaluated biochemical parameter during experimental *Haemonchus contortus* infection in goats and found a notable increase in levels of SGPT and SGOT, accompanied by a significant decrease in total serum protein levels when compared to the control group of animals.

Arora *et al.* (2003) explored relationships between EPG levels and hematological/biochemical parameters in both sheep and goats, revealing correlations between EPG, Hb, TLC, and protein levels.

Lakra *et al.* (2007) assessed diverse biochemical constituents in goats naturally afflicted with prevalent gastrointestinal nematodes such as *Ostertagia*, *Haemonchus*, *Bunostomum*, *Trichuris*, *Oesophagostomum* and others. A substantial reduction in key parameters such as hemoglobin (Hb), total erythrocyte count (TEC), and packed cell volume (PCV). serum calcium, inorganic phosphorus, copper (Cu), and zinc (Zn) levels was observed during infection. This decline in biochemical values was attributed to the blood loss caused by blood-sucking nematodes. Furthermore, hypoproteinemia, hypoglycaemia, hypoalbuminemia, and lowered serum phosphorus levels were attributed to reduced feed intake, compromised absorption, and alterations in carbohydrate metabolism due to the infection.

Ibrahim (2009) studied gastrointestinal nematodes in Hyderabad's small farmer sheep during 2004-05. Monitoring various indicators, seven nematode species were identified. Infection prevalence ranged from 42.1% (coprological) to 44.75% (necropsy). Seasonal variations significantly affected measures like EPG, larval counts, TLC, and PCV.

Jain and Sahni (2010) treated goats with herbs, showing improved glucose, total protein, and albumin levels.

2.9 OXIDATIVE STRESS RELATED PARAMETERS

Slater (1984) reported that in lipid per oxidation process, initially reactive free radical interacts with polyunsaturated fatty acids to commence a complicated series of reactions that may leads to genesis of various degradation products, among which majority are cytotoxic aldehydes such as malondialdehyde and 4-hydroxynonenal.

Level of Lipid peroxidation is directly indicated by malondialdehyde which may give idea about oxidative stress.

Padh (1991) inferred that the antioxidant status is the representative of health status of the body as they are important part of body defense mechanism by scavenging free radicals (ROS: Reactive oxygen species) which damages the biological system.

Halliwell and Chirico (1993) indicated that within molecules such as proteins, DNA, and lipids, proteins and DNA frequently emerge as primary targets susceptible to oxidative damage. In contrast, lipid peroxidation generally occurs subsequently in the injury progression. The process of lipid peroxidation primarily revolves around the oxidation of polyunsaturated fatty acids, constituting a nonenzymatic chain reaction intertwined with the presence of reactive oxygen species (ROS).

Kargin *et al.* (2001) reported that Increased levels of lipid peroxidation products such as MDA have been re- ported in a range of diseases like Dicro- coelium dendriticum infection in sheep (**Simsek *et al.*, 2006**) and kidney diseases in dogs.

Dede *et al.* (2002) revealed that the antioxidant mechanism offers cellular defence against oxidative stress induced by parasitic intrusion, thereby safeguarding cells, organs, and tissues from potential harm.

Marshall *et al.* (2002) documented that the oxidative condition is subject to variability and can be altered by various factors. Furthermore, even during physical activity, oxidative stress is induced, leading to a reduction in antioxidant capacity.

Agarwal and Prabhakaran (2005) indicated that the combined action of SOD along with catalase and GSH-Px serves to eliminate intracellular and extracellular superoxide radicals, thus thwarting lipid peroxidation. Among the primary cellular enzymes, SOD holds significance in shielding cells from the detrimental impact of superoxide radicals generated amidst stress factors such as elevated temperature and humidity. Consequently, it stands as a predominant antioxidant enzyme accountable for upholding the oxidative/antioxidative equilibrium within various cellular compartments and the extracellular milieu.

Urban *et al.* (2009) reported that **i**mbalance between the generation of radicals and their elimination results into Oxidative stress, leading to an excessive build-up of ROS.

Kumar *et al.* (2011) stated that ROS in excess can overwhelm the action of antioxidants, causing oxidative stress, resulting in suppression of the immune system and reduced productivity**.**

Esmaeilnejad *et al.* (2012) documented that within Babesia Ovis-infected goats, there was a notable increase in MDA concentration within erythrocytes. MDA levels displayed an inversely proportional relationship with PCV and key antioxidant enzymes including SOD, CAT, GSH-Px, and TAC, as observed by Abd E.M.R.

Abd (2013) noted that the presence of free radicals contributes to the development of many parasitic infections, which have been linked to lipid peroxidation. Antioxidant mechanisms function along various lines of defence, spanning from curtailing the initial generation of reactive species to quenching propagating radicals.

Machado *et al.* (2014), suggested that infections caused by nematodes could trigger the generation of reactive oxygen species. These species have the potential to harm the parasites while concurrently inducing oxidative stress within the host organisms

Duhig *et al.* (2016) stated that functionally active ROS (reactive oxygen species) assume a critical function in diverse cellular processes, encompassing cell signalling, gene and protein expression, intrinsic apoptosis, and overall cellular homeostasis maintenance.

Omidi *et al.* (2017) noted that the presence of stress triggers the generation of reactive oxygen species (ROS). In typical physiological states, the animal's antioxidant defense mechanism acts as a counterforce to counteract ROS. Nevertheless, an imbalance between ROS production and the effectiveness of the antioxidant defense system leads to oxidative stress.

Váradyová *et al.* (2018), noted the possibility that herbal neutraceuticals might offer innate antioxidants, which could have the potential to reduce oxidative stress in lambs afflicted with nematode infections.

Jaheed *et al.* **(2020),** documented a notable decline in total antioxidant capacity within the serum biochemistry of the infected animals. Among goats afflicted by gastrointestinal nematodosis, there was a marked reduction in glutathione peroxidase levels, while malondialdehyde exhibited an opposing pattern of change.

Rasha Alam *et al.* **(2020),** reported that serum SOD, catalase and GSH-Px level significantly decreased in heavy infection, compared to control whereas Serum lipid peroxidation (MDA) level revealed a notable rise amongst infected sheep and goats.

Alborzi1 *et al.* **(2023)**, reported that enzymes like SOD, CAT, and GPx are actively involved in the primary defence mechanism. Lipid peroxidation emerges as a robust gauge of the extent of reactive oxygen species (ROS) responsible for inducing widespread biological harm. Among goats and sheep, lymph nodes exhibited a noteworthy reduction in SOD and CAT activity in serum, particularly in those with elevated parasite loads and greater injuries. Additionally, infected animals displayed notably higher GPX and MDA levels compared to their non-infected counterparts. The diminished SOD and CAT activity in infected animals might be associated with oxidative stress, consequently leading to a pronounced upsurge in lipid peroxidation and MDA levels.

Charinya So-In and Sunthamala (2023), documented elevation in blood MDA levels in goats subjected to stress. Stress has the potential to elevate oxidative harm, leading to MDA production and heightened generation of free radicals, which may serve as an indicator of oxidative stress.
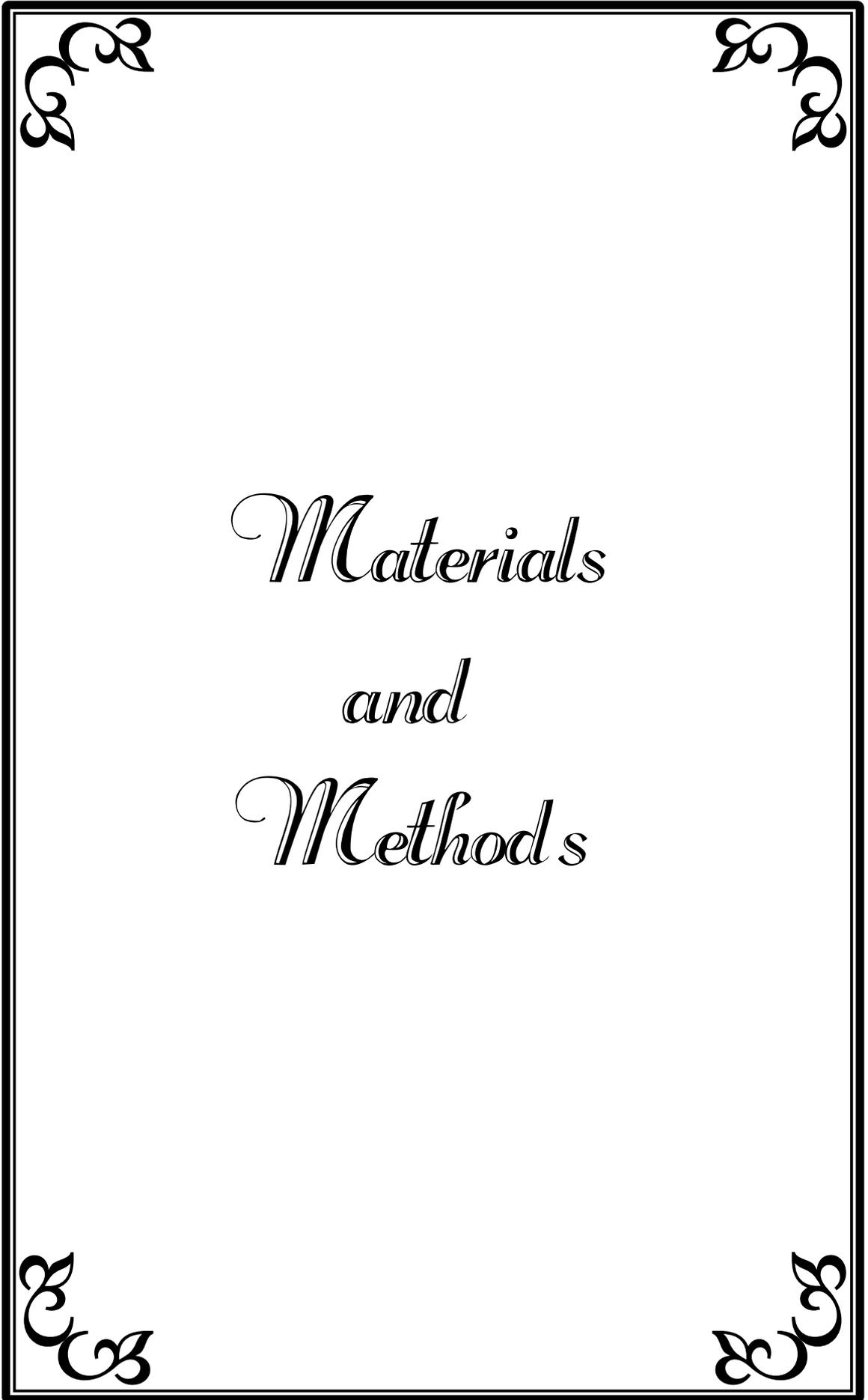
Materials

and

Methods

Chapter 3   MATERIALS AND METHODS

3.1   SCREENING AND SELECTION OF ANIMALS

The present study was conducted between Nov 2021 to Dec 2022. The research was conducted at goat farms/herds of Tarai region around Pantnagar. Screening of goats infected with gastro intestinal (GI) nematodes was done using "Faffa Malan Chart" (FAMACHA) technique (table 3.1). The FAMACHA scoring system uses a colour chart with five different shades, ranging from pale pink (score 1) to bright red (score 5), **Kaplan *et al*., (2004)**. The user compares the colour of the inner eyelid (conjunctiva) of the animal against the colour chart to determine its FAMACHA score. Anaemia is indicated by paleness of the conjunctiva, and the more anaemic the animal is, the higher the FAMACHA score will be. It allows farmers, livestock keepers, and veterinarians to quickly and easily detect anaemia caused by the blood-sucking parasites especially *Haemonchus contortus*, also known as the barber pole worm in goats and other small ruminants.

Table 3.1: FAMACHA scoring system

Score 1 (F1)	Red or normal color	Non-anaemic
Score 2 (F2)	Pink color	Non-anaemic
Score 3 (F3)	Pale pink color	Mildly-anaemic
Score 4 (F4)	Pale white color	Anaemic
Score 5 (F5)	White color	Severely anaemic

Target selective treatment (TST) which involves treatment of nematodosis in goats using specific strategies to manage and control parasitic nematode infections while minimizing the development of resistance was followed during the course of investigation. Use of anthelmintic drugs was done selectively based on FAMACHA score, faecal egg count (FEC) results and clinical signs of illness, avoiding mass treatments of the entire herd. A total of 146 goats having FAMACHA score from 3 to 5

were examined to determine the prevalence of helminthosis in the Tarai region around Pantnagar. Out of these 146 goats, 103 goats having FAMACHA score between 3-5 were suspected to be suffering from GI nematodosis. From these 103 goats, 24 goats of either sex (n=24; 9 months to 5 years of age with 10 to 30 kg body wt.) having egg counts (>200), were selected for the research work.

3.2   PILOT STUDY AND SAFETY TRIALS

A pilot study was conducted on three goats to rule out any adverse effects of the polyherbal formulation used in the experimental study. Initially *in-vitro* evaluation of polyherbal drug using Adult Worm Motility Assay (AWMA) was carried out.

After *in vitro* trial by AWMA, *in- vivo* trial of the polyherbal drug at three different concentrations of 5 gm, 6 gm and 6.5 gm per 30 kg body wt. as a single dose was carried out and various physiological and haematobiochemical parameters were monitored for 7 days to rule out any adverse effect of the polyherbal formulation.

Table 3.2: Formulation of Polyherbal anthelmintic preparation used for the research.

Poly Herbal Ingredients	Vidanga dried fruits (*Embelia ribes*)	Palash seeds (*Butea frondosa*)	Neem leaves (*Azadirachta indica*)	Pumpkin seeds (*Cucurbita maxima*)	Kalijiri seeds (*Centratherum anthelminticum*)
Dose of polyherbal extracts	**1000 mg**	**1000 mg**	**2000 mg**	**1500 mg**	**1000 mg**
Kingdom	Plantae	Plantae	Plantae	Plantae	Plantae
Order	Ericales	Fabales,	Sapindales	Cucurbitales	Asterales
Family	Myrsinaceae	Fabaceae, Peas, legumes	Meliaceae	Cucurbitaceae	Asteraceae
Species	*Embelia ribes*	*Butea monosperma*	*Azadirachta indica*	*Cucurbita pepo*	*Centratherum anthelminticum*
English name	False Black Pepper	Bastard Teak, Bengal Kino	*Azadirachta Indica*	Gourds, Squashes, Citrouilles	Purple Flebane
Hindi name	Vayavidanga, Bhabhiranga,	Dhak, Palas, Tesu, Palaash	Neem or Margosa	Kaddu, Petha, Kumrha, Kohala	Kali Jeeri

Plate 3.1: Depiction of Tarai region goats grazing in the pastures

Plate 3.2: Depiction of Tarai region goats returning from the pastures

Plate 3.3: Selection of goats using FAMACHA score card

3.3 EXPERIMENTAL DESIGN

The selected 24 goats were divided randomly into four equal groups and different coloured tags were placed around the neck for identification. Based on history of regular deworming by the farmers and high EPG seen during faecal examination, anthelmintic resistance (AR) to commonly used anthelmintics was suspected in the goats selected for the research work.

For a change ivermectin which is mostly given subcutaneously as an anthelmintic in this belt has been given orally in order to see its efficacy on oral administration at the same dose rate (**Lespine *et al.*, 2005, Shakya *et al.*, 2018**).

In previous studies (**Waruiru *et al.,* 1998, McKenna and Watson, 1987; Swann and Gross, 1985**) have evaluated the effectiveness of oral ivermectin administration in goats. They reported over 99% efficacy for ivermectin at 0.2 mg/kg given orally. In field studies, lower efficacy of subcutaneous administration of ivermectin in goats has been observed using FECRT or worm load reduction (**Pearson and Rutherford, 1988, McKenna, 1991, Rahman, 1997**).

- **Group I:** (n=6) was treated with fenbendazole @ 5mg/kg body weight orally, (**Plumb, 2008, Sharma *et al.*, 2015, Papich, 2016**).

- **Group II**: (n=6) was treated with ivermectin @ 0.2mg/kg body weight orally (**Lespine *et al.*, 2005, Sharma *et al.*, 2015, Shakya *et al.*, 2018, Myers *et al.*, 2020**).

- **Group III**: (n=6) was treated with single dose of polyherbal formulation @ 6.5 gm/30 kg body wt. orally (**Based on safety trial/pilot study, Parsani *et al.*, 2020, Kalkal and Vohra 2021, Khanolkar *et al.*, 2018**). Composition of polyherbal formulation used in the study is shown in table no 3.2.

- **Group IV**: (n=6) was kept as untreated control.

The experimental design is depicted in table no 3.3.

Plate 3.4: **Depiction of: (A) Weighing of goat selected for trial, (B) & (C) Goats with coloured ribbons for identification**

Table 3.3: Experimental design

Treatment Groups	GROUP I Fenbendazole (G I)	GROUP II Ivermectin (G II)	GROUP III Polyherbal anthelmintic formulation (G III)	GROUP IV Control (No Drugs given) (G IV)
'O' day	• Note down age, sex, weight and put identification tag • Collection of Faecal sample directly from rectum for examination by modified Mc Master technique (**EPG Calculation**) • Taking 5 ml blood from jugular vein into a sterile vial • Testing of **haematological, biochemical and oxidative stress related parameters** of all blood samples with standard procedures.			
Day 7, 14 & 21	• Collection of 5ml blood from jugular vein into sterile vials under asceptic conditions. • **Testing of haematological, biochemical and oxidative stress related parameters of all blood samples with standard procedures.** • Collection of Faecal sample directly from rectum for examination by modified Mc Master technique & **Faecal egg count reduction test (FECRT)**			

3.4 PLANT MATERIALS FOR POLYHERBAL DRUG

The ingredients of polyherbal drug (plant materials and seeds) were procured from authentic sources / local market at Rudrapur, Uttarakhand. The identification of plant samples was done at Botanical Survey of India, Dehradun and seeds were identified at Uttarakhand Seeds & Tarai Development Corporation Ltd., Haldi, Udham Singh Nagar (**Annexure 1).**

3.4.1 Processing of plant material & preparation of extract

The plant materials were chopped, air dried and powdered. The extracts of all the plants were prepared in ethanol by the method of cold percolation, following the

standard protocol being practiced for the preparation of extracts (**Kumar *et al.*, 2017**). The different amounts (Table 3.4) of the powdered material of each plant were soaked in 99.9% ethanol in a stoppered container and allowed to stand at room temperature for a period of minimum seven days with frequent agitation at different time intervals. The process helped in breaking and softening the plants cell walls to release the soluble phytochemicals. After seven days, the mixture was strained by filtration using Whatman filter paper No. 1. The solvent was removed by evaporating it through steam distillation. The yield of the extracts were recorded and stored at 4ºC for further analysis.

Table 3.4: Yield of extracts obtained from different polyherbal ingredients

S. No.	Plant	Botanical name	Dry weight for extraction (gm)	Weight of extract obtained (gm)	% Yield
1.	Neem leaves	*Azadirachta indica*	380	21.12	5.56
2.	Pumpkin seeds	*Cucurbita maxima*	530	20.3	3.83
3.	Kalijiri seeds	*Centratherum anthelminticum*	850	49.1	5.78
4.	Palash seeds	*Butea frondosa*	550	16.03	2.91
5.	Vidanga dried fruits	*Embelia ribes*	680	18.7	2.75

Plate 3.5: **Depiction of: (A) Dried Neem leaves** *(Azadirachta indica)***, (B) Crushed Neem leaves, (C) Ethanolic extracts of Neem leaves**
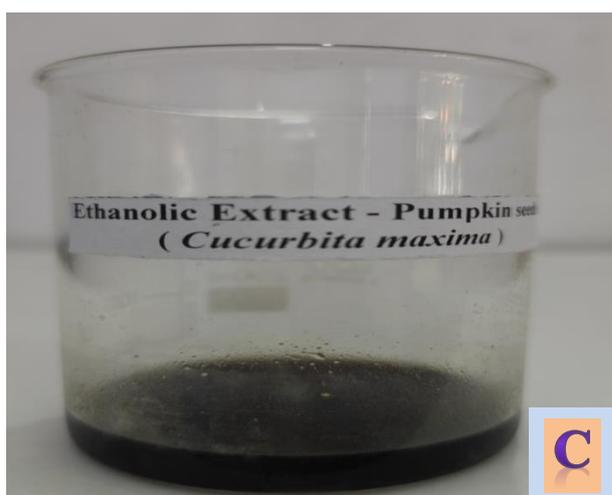
Plate 3.6: Depiction of: (A) Pumpkin Seeds *(Cucurbita maxima)*, (B) Crushed Pumpkin Seeds, (C) Ethanolic extract of Pumpkin Seeds

Plate 3.7: Depiction of: (A) Kalijiri seeds (*Centratherum anthelminticum*), (B) Crushed Kalijiri seeds, (C) Ethanolic extract of Kalijiri seeds
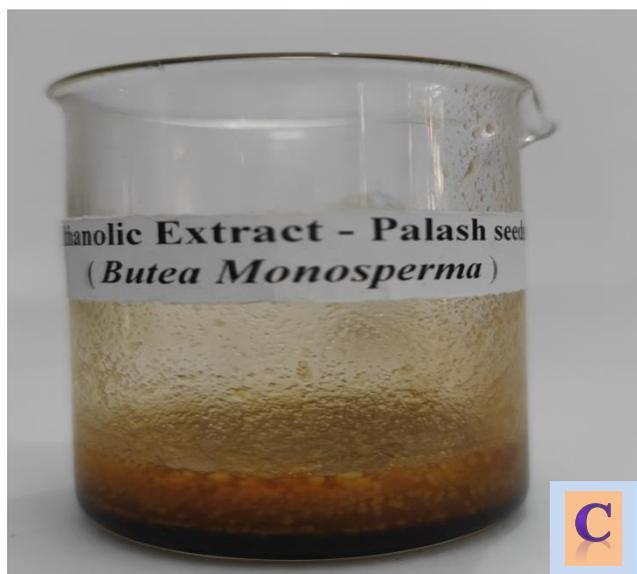
Plate 3.8: Depiction of: **(A) Palash seeds** *(Butea frondosa)*, **(B) Crushed Palash seeds, (C) Ethanolic extract of Palash seeds**

Plate 3.9: Depiction of: **(A) Vidanga dried fruits** *(Embelia ribes)***, (B) Crushed Vidanga, (C) Ethanolic extract of Vidanga dried fruits**
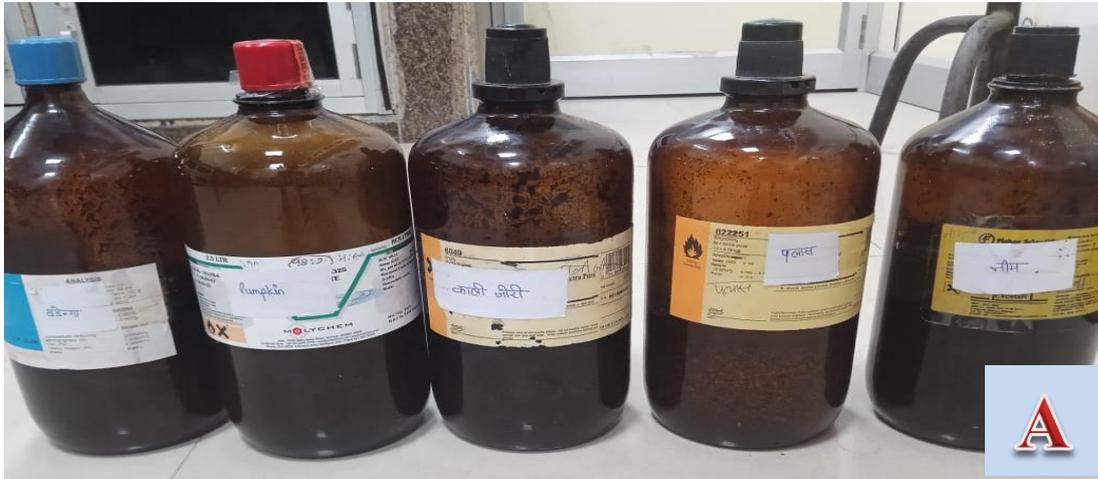
Plate 3.10: Depiction of: (A) Polyherbal ingredients soaked in ethanol, (B) The ingredients after filteration (C) Ethanolic extracts of polyherbal ingredients

Plate 3.11: **Depiction of:** **Polyherbal formulation in bolus form for use as an anthelmintic**

3.5   ADULT WORM MORTALITY ASSAY

The *in-vitro* technique to check efficacy of polyherbal formulation included adult worm motility assay (AWMA) against adult GI round worms. They were collected from the freshly slaughtered goat abomasum at slaughter house/butcher shop at Lalkuan, Nainital. The motile adult worms were collected and put in beaker filled with lukewarm normal saline solution. Further the worms were transferred in beaker containing Phosphate-buffered saline (PBS) at 37°C **(Bhatnagar *et al.*, 1961).**

From the ethanolic extracts of polyherbal formulation, four concentrations *viz.* 10 mg/ml, 25mg/ml, 50 mg/ml and 100 mg/ml were prepared in PBS for evaluation of their anthelmintic activity. Ten adult round worms were taken in each small petridish having different dilutions of polyherbal extracts. The petri dishes were kept in an incubator at 37° C. The inhibition of motility, activeness and mortality of the worms was observed at an interval of 30 min, 1 hr, 2 hr, and 4 hr. The number of motile (alive) and non-motile (dead) worms were counted and recorded for each concentration. For all the ten worms, minimum lethal time was recorded in each extract. Pinch technique was used to check viability of the worms (Non - motility for 5-6 seconds) as proposed by **Neogi *et al.* (1964); Eguale and Giday (2009).**

3.6   COLLECTION OF BLOOD AND SERUM SAMPLES

The blood samples for haematological and biochemical studies were drawn directly from jugular vein as recommended by **Benjamin (1986)** from the selected twenty four animals. Approximately 06 ml of blood was collected from jugular vein of each goat at 0, 7, 14 and 21 days of trial. Of this 2.0 ml of blood was transferred into a clean, dry, test tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant, for use in haematology and remaining 4.0 ml blood sample was transferred into a clean and dry test tube without any anticoagulant and allowed to clot in slanting position for about one to two hours. After centrifugation for 10 minutes at 2, 000 to 3, 000 rpm in a labelled dry Eppendorf, separated serum was harvested gently using micropipette and preserved at -20^{0}C in a deep freezer for further use.

3.6.1   HAEMATOLOGICAL ANALYSIS

It was done on day 0, 7, 14 and 21st day of trial. Haematological studies were carried out to study the effect of GI nematodosis and effect of various therapeutic regimes on haematological profiles of goats selected for the trial.
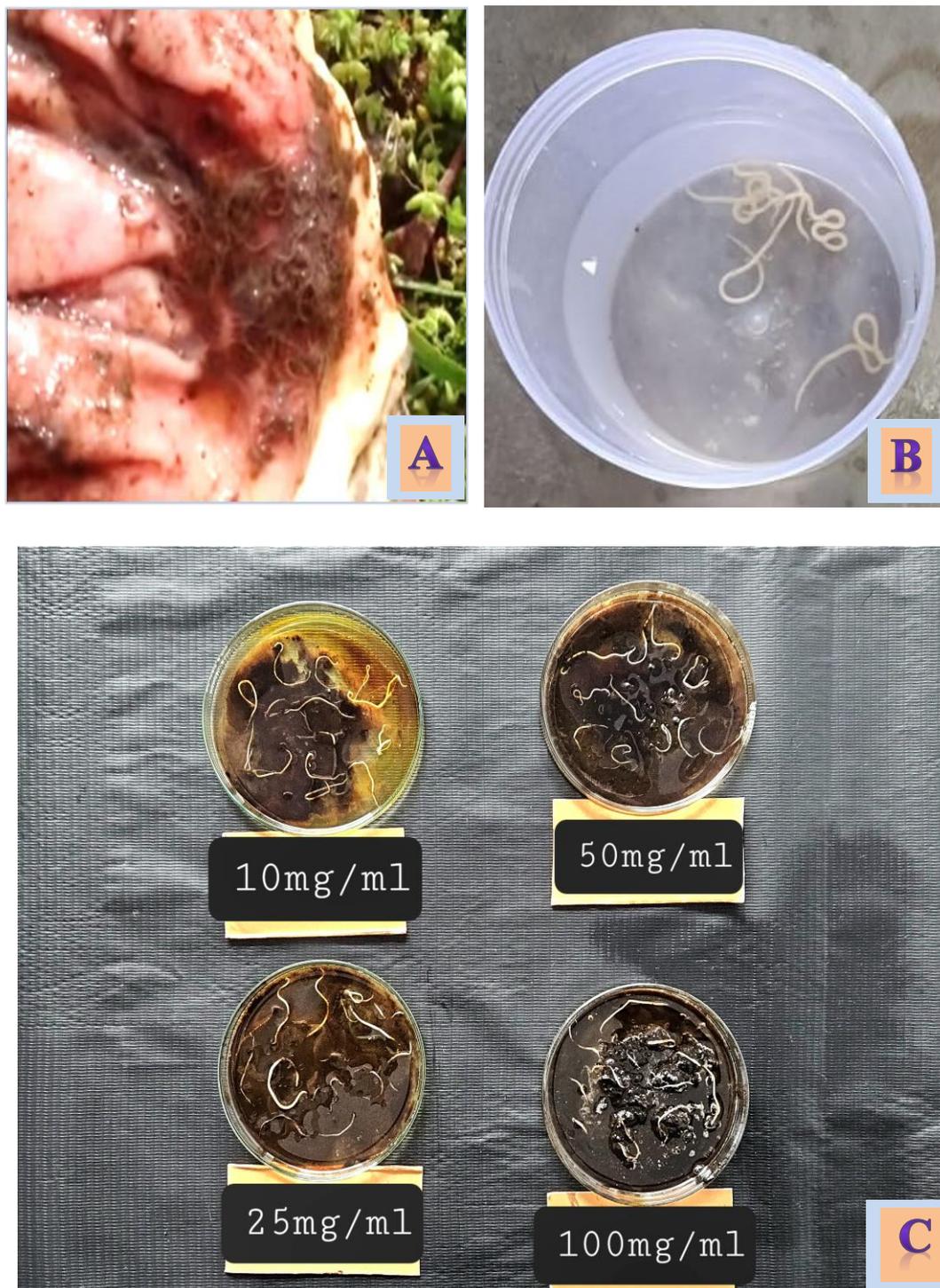
Plate 3.12: **Depiction of: (A) and (B) Adult round worms collected from goat abomasum (C) Different concentrations of polyherbal formulation for adult worm mortality assay**

Plate 3.13: Depiction of blood collection from the jugular vein of goats selected for Haematobiochemical examination

Following haematological parameters were estimated; Red Blood Cell Count (RBC), Hemoglobin (HB), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count, White Blood Cell Count (WBC) and Differential Leukocyte Count (DLC; Neutrophil, Lymphocyte, Monocytes, Eosinophils, Basophils) using standard procedures and protocols with help of commercially available kits following the standard methods.

3.6.1.1 Haemoglobin (Hb) Estimation:

Hemoglobin concentration was estimated by using Sahli's Haemoglobinometer by acid hematin method as described by **Oser (1979)** and findings were expressed in g/dL of blood.

3.6.1.2 Total erythrocyte counts (TEC):

Using a Neubauer's haemocytometer and RBC (red blood cell) diluting fluid, the TEC (total erythrocyte count) was calculated as described by **Jain (1986)**. The calculated values were then expressed as millions per microliter of blood ($\times 10^6/\mu l$).

3.6.1.3 Total leukocytes count (TLC):

Total leukocytes count was performed using Neubauer's haemocytometer as described by **Jain (1986)** and values were indicated in thousands per microliter ($\times 10^3/\mu l$) of blood.

3.6.1.4 PCV (Packed cell Volume)

PCV was estimated using the micro haematocrit method, as described by **Grindem (2011)** and the results were expressed as a percentage.

3.6.1.5 Differential leukocyte count (DLC)

The calculation of the differential leukocyte count was conducted following **Cole's (1986)** method, employing Leishman's staining technique. A blood smear was prepared using a spreader slide, and the air-dried smear was subsequently stained with Leishman's stain. The stained smear was then examined under oil immersion to count the number of neutrophils, lymphocytes, eosinophils, and basophils. The relative proportions of each leukocyte type were expressed as percentages.

3.6.1.6 Platelet count

Estimation of platelet count was done with help of Neubauer chamber and values indicated in x 10^3/ µl.

3.6.1.7 Erythrocytic Indices

MCV, MCH & MCHC were calculated according to **Chauhan and Agrawal (2006).** MCV (fL) femto lit, MCH (pg) pico gram, MCHC (gm /dl).

3.6.2 BIOCHEMICAL ANALYSIS

It was done on day 0, 7, 14 and 21st day of trial to study the effect of GI nematodosis and effect of various therapeutic regimes on biochemical profiles of goats selected for the trial. Analysis of serum samples was carried out for Total protein, albumin, globulin, A:G ratio, blood glucose, Serum Glutamic Oxaloacetic Transaminase/Aspartate Aminotransferase (SGOT/AST), Serum Glutamic Pyruvic Transaminase/ Alanine Aminotransferase (SGPT/ALT), Alkaline phosphatase (ALP), Urea, Creatinine, Uric Acid, Sodium (NA$^+$), Potassium (K$^+$) and Chloride (CL$^-$) by using commercially available diagnostic kits.

3.6.2.1 Glucose Estimation:

The glucose level was measured by using diagnostic kit (GOD-POD method, End Point) using a kit from Erba diagnostics, Mannheim, Germany at 505 nm wavelength against blank reagent and the results of the glucose measurement were reported in mg/dl.

3.6.2.2 Total Protein Estimation:

Calculation of Total serum protein was done by the modified Biuret and Dumas method as described by **(Young, 1997)**, using a kit from Erba diagnostics, Mannheim, Germany at 546 nm wavelength against blank reagent. Total protein values were expressed as gm per deciliter (g/dL).

3.6.2.3 Serum Albumin

Albumin level in serum sample was estimated by BCG-Dye method as described by **Doumas *et al.* (1997)**, using a kit from Erba diagnostics, Mannheim, Germany at 630 nm wavelength. The results were expressed in gm/dL.

3.6.2.4 Serum Globulin

To get value of serum globulin, subtraction of the value of the total serum albumin was done from total serum protein and expressed in gm/dL.

Serum globulin = total serum protein − serum albumin.

3.6.2.5 Albumin Globulin ratio (A/G ratio)

For this purpose total serum albumin was divided by total serum globulin.

3.6.2.6 Alkaline phosphatase (ALP)

The Serum alkaline phosphatase level was determined using Erba diagnostic kits, following the method described by **Wilkinson and Winston (1980)**. Results of the alkaline phosphatase measurement were expressed in international units per liter (U/L).

3.6.2.7 Alanine amino transferase (ALT)

Level of Alanine Aminotransferase (ALT) was estimated using the 2, 4-DNPH method, following the protocol established by **Reitman and Frankel (1957)**. The measurement was carried out using an Erba diagnostics kit, and the values of ALT were reported in international units per liter (U/L).

3.6.2.8 Aspartate amino transferase (AST)

The Aspartate Aminotransferase (AST) level was determined utilizing the 2, 4-DNPH method, following the procedure described by **Reitman and Frankel (1957)**. The measurement was conducted using an Erba diagnostics kit, and the results of the AST measurement were reported in international units per liter (U/L).

3.6.2.9 Urea Estimation:

Estimation of urea in serum samples was performed by using a kit from Erba diagnostics, Mannheim, Germany (GLDH-Urease method, Initial rate) and values were expressed as mg/dL.

3.6.2.10 Uric Acid-

Uric Acid was estimated by colorimetric method. After reaction with reagent mixture and acid, uric acid produced coloured derivative which was detected at 411

nm. Estimation was done using **Colorimetric Test kit** by Uric acid enzymatic assay kit (commercially available) from Erba diagnostics, Mannheim, Germany, and values were expressed as mg/dL.

3.6.2.11 Serum creatinine

The serum creatinine level was measured using Erba diagnostic kits by modified Jaffe's Alkaline picrate method. The results were expressed in mg/dL.

3.6.2.12 Estimation of Serum electrolytes Potassium (K+), Sodium (Na+) and chloride (Cl⁻)

Determination of serum electrolytes was done using Electrolyte Colorimetric Test kit (Colorimetric method) by Beacon diagnostics PVT. LTD, Kabilpore, Navsari, India, and values were expressed in Unit mmol/L.

3.6.3 OXIDATIVE STRESS RELATED PARAMETERS

Oxidative stress related parameters were measured on day 0, 7, 14 and 21 to study the effect of GI nematodosis and effect of various therapeutic regimes on biochemical profiles of goats selected for the trial. Estimation of Glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) Catalase (CAT) and lipid peroxidation marker, malondialdehyde (MDA) were carried out by spectrophotometric assays using respective activity assay kit from Real Gene Labs, Ghaziabad, India, following the instructions provided in the user manual. This method assesses the functional capacity of the enzyme to act on its substrate. CAT activity was measured using the peroxidative function of CAT. Total SOD activity was measured using a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. GPX activity was indirectly determined by a coupled reaction with glutathione reductase. Estimation of Malondialdehyde (MDA) was done by Thiobarbituric Acid (TBA) Assay.

3.6.3.1 Glutathione peroxidase (GSH-Px)

Estimating glutathione peroxidase (GSH-Px) activity in goat serum is important for assessing the antioxidant status and overall health of the animals.

Detection principle

Glutathione Peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione (GSH) to produce H_2O and oxidized glutathione (GSSG). The activity of glutathione peroxidase can be expressed by the rate of enzymatic reaction. The activity of glutathione can be calculated by measuring the consumption of reduced glutathione. Hydrogen peroxide (H_2O_2) and reduced glutathione can react without catalysis of GSH-Px, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH reacts with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which showed a stable yellow colour. The absorbance was measured at 412 nm, and amount of GSH calculated. **(Lubos *et al.,* 2011).**

Assay protocol

I. **Enzymatic reaction**

a) 0.2 ml of 1 mmol/L GSH standard solution was taken into 5 ml EP tube (Non-enzyme tube). Another 0.2 ml of 1 mmol/L GSH standard solution was taken in which 0.1 ml of serum sample was mixed thoroughly (Enzyme tube).

b) The tubes were pre-heated at 37°C water bath for 5 min. Reagent 1 application solution was preheated at 37°C for 5 min at the same time.

c) 0.1 ml of reagent 1 application solution was added to the tubes and mixed thoroughly. It was left to react at 37 °C for 5 min accurately.

d) In non-enzyme tube 2 ml of reagent 2 and 0.1 ml of sample was added. In the enzyme tube 2 ml of reagent 2 was added.

e) Tubes were thoroughly mixed and centrifuged at 3000 rpm for 10 min. After that 1 ml of the supernatant was taken for chromogenic reaction.

II. Chromogenic reaction

a) 1 ml of supernatant from non-enzyme tube was taken in 5 ml EP tube and 1 ml of supernatant was taken from enzyme tube into 5 ml EP tube.

b) Took 1 ml of GSH standard application solution to 5 ml EP tube (Blank tube) and another 1 ml of 20 µmol/l GSH standard solution was taken in 5 ml EP tube (Standard tube).
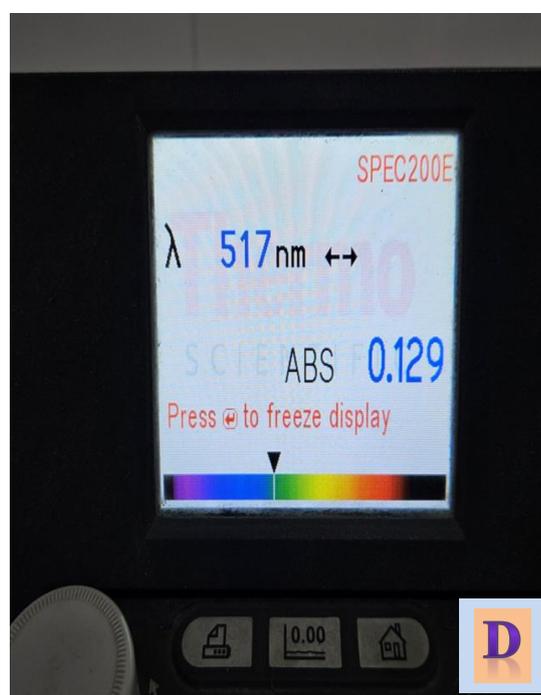
Plate 3.14: Depiction of: (A) and (B) lab work, (C) and (D) UV visible spectrophotometer

c) 1 ml of Reagent 3 application solution, 0.25 ml of Reagent 4, 0.05 ml of Reagent 5 application solution was added to each tube.

d) Tubes were thoroughly mixed and kept at room temperature for 15 min. The spectrophotometer was adjusted to zero using double distilled water and the optical density (OD) measurements for each tube were recorded at 412 nm with 1 cm optical path cuvette.

Calculation

Definition: "The activity of GSH-Px in 0.1 ml of the sample is determined by measuring its ability to consume 1 µmol/L of reduced glutathione (GSH) at 37°C for 5 minutes, after accounting for the contribution of non-enzyme reactions. This defined activity is considered as 1 unit of GSH-Px."

$$\text{GSH-Px activity (U)} = A_1 \div A_2 \times c \times f_1 \times f$$

Note:

$$A_1: (OD_{\text{Non-enzyme tube}} - OD_{\text{Enzyme tube}}) \text{ and } A_2: (OD_{\text{Standard}} - OD_{\text{Blank}})$$

c: the concentration of standard, 20 µmol/l.

f: dilution factor of sample before test.

f_1: dilution factor of serum/plasma in enzymatic reaction, 6 times.

f_2: dilution factor of tissue, cells or cell culture supernatant in enzymatic reaction, 5 times.

3.6.3.2 Superoxide dismutase (SOD)

Superoxide dismutase exists in all oxygen- metabolizing cells to protect cells from excessive superoxide.

Detection principle

The superoxide anion free radical (O_2^-) can be generated through the xanthine and xanthine oxidase reaction system. This O_2^- radical can further oxidize hydroxylamine to form nitrite, which turns purple when reacted with the developer. However, in the presence of samples containing Superoxide Dismutase (SOD), the SOD specifically inhibits the superoxide anion free radical (O_2^-). This inhibitory effect

of SOD reduces the formation of nitrite, resulting in a lower absorbance value in the sample tube compared to the control tube **(Cristiana *et al.*, 2014).**

Assay Protocol

a) 1 ml of reagent 1 working solution was added to 0.1 ml sample inside the sample tube and 1 ml of reagent 1 working solution was added to 0.1 ml of double distilled water in the control tube.

b) 0.1 ml of reagent 2, 0.1 ml of reagent 3, 0.1 ml of reagent 4 working solutions were successively added into the tubes of Step 1.

c) They were thoroughly mixed and incubated for 40 min at 37 °C.

d) 2 ml of chromogenic agent was added into the tubes of Step 3.

e) Left for 10 min at room temperature after thorough mixing.

f) The spectrophotometer was adjusted to zero using double distilled water and the optical density (OD) measurements for each tube were recorded at 560 nm with 1 cm optical path cuvette.

Calculation

Definition: The SOD activity unit (U) is defined as the amount of SOD required to achieve a 50% inhibition ratio in a 1 ml reaction solution. In other words, when half of the superoxide radicals in the reaction are effectively inhibited by SOD, the SOD activity is considered as 1 unit (U).

$$\textbf{T-SOD activity (U/ml)} = i \div 50\% \times \frac{V_1}{V_2} \times f$$

Note:

$$i : \text{inhibition ratio}, i = \frac{OD_{control} - OD_{sample} \times 100\%}{OD_{control}}$$

V_1: the total volume of reaction solution, ml. V_2: the volume of sample added, ml.

f: dilution factor of sample before test.

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3.6.3.3 Catalase (CAT)

Catalase is a vital enzyme found in organisms, responsible for efficiently decomposing hydrogen peroxide into harmless substances.

Detection principle

Catalase (CAT) efficiently decomposes H_2O_2, and this reaction can be rapidly halted using ammonium molybdate. The remaining H_2O_2 reacts with ammonium molybdate, forming a yellowish complex. CAT activity can be measured by quantifying the production of this yellowish complex at 240nm (**Aebi, 1984 and Nicholls *et al.*, 2000**)

Assay Protocol

a) The control tube was prepared by adding 1 ml of reagent 1 into the 5 ml EP tubes. The sample tube was prepared by adding 0.1 ml of the sample and 1 ml of Reagent 1 into the 5 ml EP tubes.

b) The tubes were incubated at 37°C for 5 minutes.

c) Next, 0.1 ml of reagent 2 was added into each tube, and the contents were mixed fully. The tubes were then allowed to react at 37°C for 1 minute accurately.

d) For the sample tube, 1 ml of reagent 3 application solution and 0.1 ml of reagent 4 were added and mixed fully. In the control tube, 1 ml of reagent 3 application solution, 0.1 ml of reagent 4, and 0.1 ml of sample were added and mixed fully.

e) The tubes were left undisturbed for duration of 10 minutes at ambient temperature. Subsequently, the optical density (OD) of each tube was gauged at 240nm with 0.5 cm optical path cuvette after setting the instrument to zero with double distilled water.

Calculation

Definition- Catalase activity is defined as the amount of CAT in 1 ml of serum or plasma that decomposes 1 μmol of H_2O_2 per minute at 37°C, expressed as 1 unit.

$$\text{CAT activity (U/ml)} = \frac{A \times 32.5 * \times f}{1 * \times V}$$

- A : Absolute OD (OD_control – OD_sample)

- OD_control : Optical density of the control tube

- OD_sample : Optical density of the sample tube

- V : Volume of the sample in milliliters (ml)

- f : Dilution factor of the sample before the test

- 32.5* : The reciprocal of the slope obtained from the calibration curve.

- 1* : Reaction time in minutes.

3.6.3.4 Malondialdehyde (MDA)

The body produces oxygen free radicals through the enzymatic systems and non-enzymatic systems, which can attack unsaturated fatty acid on biofilm and lead to lipid peroxidation and form lipid peroxide, such as aldehyde group Malondialdehyde (MDA), keto-, hydroxyl, carbonyl, etc.

Detection principle

MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce red compound, which has a maximum absorption peak at 532 nm. (**Nielsen, 1997)**

Assay Protocol

a) Blank tube: 0.1 ml of absolute ethanol was taken into the 10 ml glass test tubes.

Standard tube: 0.1 ml of 10 nmol/ml Standard was taken into the 10 ml glass test tubes.

Sample tube: 0.1 ml of the test Sample was taken into numbered 10 ml glass test tubes.

Control tube: 0.1 ml of the test Sample was taken into numbered 10 ml glass test tubes.

b) 0.1 ml of reagent 1 was added into each tube of Step 1.

c) 3 ml of reagent 2 application solution was added into each tube of Step 2.

d) In the Blank tube, Standard tube, and Sample tube, 1 ml of reagent 3 application solution was added. In the Control tube, 1 ml of 50% glacial acetic acid was added.

e) The tubes were mixed fully and the mouth of each tube was fastened with plastic film. A small hole was pricked with a needle. Then, the tubes were incubated at 95-100°C for 40 minutes.

f) The tubes were cooled to room temperature using running water and then centrifuged at 3100 rpm for 10 minutes.

g) 3 ml of the supernatant from each tube was taken. The spectrophotometer was calibrated to zero using double-distilled water, and the optical density (OD) was assessed at a wavelength of 532 nm employing a cuvette with a 1 cm optical path length. (the precipitation was not added to the cuvette).

Calculation

For serum and plasma

$$\text{MDA content (nmol/ml)} = \frac{A_1}{A_2} \text{ x C x f}$$

Note:

A_1: OD sample – OD control, A_2 = OD $_{standard}$ - OD $_{Blank}$

C: The concentration of standard, 10 nmol/ml.

f: Dilution factor of sample before tested.

3.7 COLLECTION AND EXAMINATION OF FAECAL SAMPLES

Faecal samples were collected directly from rectum on 0 day (Pre-Treatment), 7th day, 14th day and 21st Day (post-treatment) in sterilized polybags from goats of Pantnagar and nearby areas and transported to the laboratory for further processing

3.7.1 MICROSCOPIC EXAMINATION OF FAECAL SAMPLES AND CALCULATION OF EPG

The intensity of EPG of each faecal sample was determined by modified Mc Master Technique **(MAFF, 1971).** Mc Master counting chamber is made up of two glasses, separated by 3 or 4 narrow transversely placed stripes of glass 1.5 mm thick, so that 2 or 3 spaces of 1.5 mm depth are obtained between the two slides. On the under surface of the upper slide an area of 1 cm^2 is ruled over each space. The volume under this ruled area will therefore be 0.15 ml.

One gram of faecal sample was taken and triturated in mortar and pestle after adding of 14 ml of saturated salt solution. Sample was sieved through tea strainer and transferred into plastic / glass test tube. The chamber of McMaster egg counting slide was charged with the prepared filtrate of faecal suspension and was allowed to settle for 30 second to1 minute. All the eggs under the two ruled grids were counted at 10X magnification. As total volume of each chamber was 0.3 ml, the number of eggs counted was multiplied by 50 (dilution factor) to determine the EPG of the faecal sample. Faecal egg count of each animal was ascertained by modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Thus the eggs per gram of faeces (EPG) were calculated by multiplying the nematode eggs counted in two squares of the McMaster slide by a dilution factor of 50.

Egg per gram = Total of eggs in both the chambers x 50

The faecal egg count data was analyzed by faecal egg count reduction test (FECRT %) for the anthelmintic efficacy of the polyherbal extracts and conventional anthelmintics on a particular day using the formula given by **Dash *et al.* (1988).**

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

3.8 THERAPEUTIC AND ANTHELMINTIC RESISTANCE STUDY

The Faecal Egg Count Reduction Test (FECRT) stands as the most prevalent technique for assessing anthelmintic effectiveness **(Presidente *et al.*, 1985; Kalkal *et al.*, 2020)**, and it is applicable to a wide range of anthelmintic agents. It is a common method used to assess the efficacy of anthelmintic drugs (dewormers) in controlling

gastrointestinal parasites in goats and other livestock. Gastrointestinal parasites, such as nematodes, can negatively impact the health and productivity of goats, making deworming an essential management practice. FECRT and its associated calculations help in evaluating the efficacy of anthelmintic treatments, identifying possible drug resistance in parasites, and guiding appropriate parasite control strategies in goat populations.

FECRT Principle:

1. Pre-treatment Faecal Egg Count (FEC): Before administering the anthelmintic treatment, a faecal sample is collected from a representative group of goats. The faecal sample is then analysed to determine the number of parasite eggs per gram of faeces (EPG). This serves as the baseline measurement of the parasite burden in the goats.

2. Anthelmintic Treatment: The goats are treated with the chosen anthelmintic drug, following the manufacturer's recommended dosage and administration guidelines.

3. Post-treatment Faecal Egg Count (FEC): Approximately 10 to 14 days after treatment, another faecal sample is collected from the same group of goats. This sample is also analysed to determine the post-treatment FEC, which indicates the number of parasite eggs after treatment.

4. Calculating FECR: The calculation of Faecal Egg Count Reduction (FECR) involves the application of the subsequent formula: FECR (%) = [(Initial FEC - Final FEC) / Initial FEC] x 100

5. The FECR percentage represents the reduction in the number of parasite eggs in the faeces after treatment. Higher FECR percentages indicate a more effective anthelmintic treatment.

6. Interpretation: If the FECR is below a certain threshold (usually 90-95%), it suggests that the parasites are showing resistance to the anthelmintic drug used, and alternative treatments or management strategies may be needed. On the

other hand, if the FECR is above the threshold, it indicates that the anthelmintic has been effective in reducing the parasite burden.

In the present trial two commonly used anthelmintics (Ivermectin & Fenbendazole) were administered to measure anthelmintic efficacy by the FECRT based on the WAAVP (World Association for Advancement of Veterinary Parasitology) guidelines on interpretation and conduct of studies to determine the efficacy of anthelmintic drugs in animals reared for meat & milk **(Thomas Geurden,** *et al.***, 2022; Kaplan** *et al.,* **2022).**

The drug was considered fully effective when it reduced the egg counts by more than 95% and lower confidence limits were higher than 90%. The drug was considered moderately resistant when it reduced the egg counts between 60% to 95% and considered severely resistant when the reduction in egg counts was below 60% along with lower confidence limits below 90%. (**Kalkal and Vohra, 2021**)

 Step 1: Calculation of the percent reduction for each observation:

$$\text{Percent Reduction} = \frac{[(\text{Pre-treatment EPG - Post-treatment EPG})}{\text{Pre-treatment EPG}]} \text{ x } 100$$

Step 2: Estimation of the mean and standard deviation of the percent reduction:

Step 3: Calculation of the lower and upper confidence intervals using the t-distribution:

With 6 observations, the degrees of freedom (df) = 6 - 1 = 5. Using a 95% confidence level, the t-value is approximately 2.571.

Lower Limit = Mean Percent Reduction - {t * (Standard Deviation / sqrt (n)}

Upper Limit = Mean Percent Reduction + {t * (Standard Deviation / sqrt (n)}

t-value is a critical value from the Student's t-distribution table. For a 95% confidence level, the t-value is typically selected, which corresponds to a 95% confidence level (two-tailed) with n-1 degrees of freedom.

n: The sample size of the treated group

3.9 COPROCULTURE FOR LARVAE IDENTIFICATION

For identification of gastro intestinal nematodes (GIN), pooled faecal sample was crushed with spatula and mixed with adequate quantity of activated charcoal to avoid fermentation. The faeces were then packed in Petri dishes, loosely covered with gauge and were incubated at 27°C in BOD incubator for a week. To maintain 80% humidity, water was sprinkled every alternate day to maintain moisture level during incubation. The separation of infective larvae (L_3) was done by means of (Baermann's apparatus) for identification and to assess the composition of larvae. The apparatus was left undisturbed over night at room temperature so that larvae migrated out of the faeces through wire mesh and got settled in the stem of funnel. After 24 hours, the collecting tubes were detached gently from rubber tube by pressing the spring clip.

The sediment containing larvae were transferred into a centrifuge tube and washed by filling the tube up to two–third with distilled water. After centrifugation at 1500 rpm for 5 minutes the supernatant fluid was discarded and again two more similar washings were given.

The larvae thus collected were examined under microscope and were identified according to morphological characteristics as per the key given by **Soulsby (1982)** and **MAFF (1986).** For this, a drop of sediment fluid was taken on clean glass slide and the larvae were fixed by putting a small drop of luke warm formalin and stained with Lugol's iodine and then identified under 10X magnification of compound microscope

Plate 3.15: Depiction of: (A) Pooled sample for Coproculture (B) Baermann's apparatus

3.10 PHYTOCHEMICAL STUDY OF EXTRACTS

The extracts of polyherbal ingredients were analysed for detection of major phytochemical groups by standard methods and protocols. (**Das *et al.*, 1964; Harborne, 1973; Sofowara, 1982**) at CBSH Pantnagar.

The solvents used in the extraction method, and the chemicals used in the research, were of analytical and laboratory quality and were acquired from Hi- Media, Merck, and SD Fine. Prior to their use, the solvents were distilled according to protocol. Thermo Scientific 10S **UV-VIS Spectrophotometer** was used to perform the Spectrophotometric protocols. Qualitative chemical analysis of extracts of *A. indica* (leaves), *B. frondosa* (seeds), *E. ribes* (berries), pumpkin seeds (*Cucurbita maxima*) and Kali Jeeri (*Centratheram anthelminticum) was* done to detect total flavonoid, phenols, antioxidants, tannins and reducing sugars by standard methods (**Das *et al.*, 1964; Peach and Tracey, 1956 and Harborne, 1973).**

3.10.1 Total phenols estimation: -

Estimation of total phenol content was done using the Folin–Ciocalteu method (**Chaubey *et al.*, 2017**). A volume of 1 ml of sample extract was mixed with 1 ml of 95% (V/V) ethanol before being diluted with 5 ml of distilled water. An aliquot of 0.5 ml of FCR (Folin – ciocalteu reagent) 50% (V/V) was added to each sample extract. Subsequently the mixture was kept at room temperature for 3 min before adding 2 ml of Na_2CO_3 (20%) and incubating for 60 mins again at room temperature. Absorbance was measured at 650 nm. Total phenols were measured using gallic acid as a reference. The results were calculated in terms of mg/gm gallic acid equivalent (GAE).

3.10.2 Total flavonoid estimation

Using an aluminium chloride colorimetric assay, the total flavonoid content was determined as per **Woisky and Salatino (1998)**. Approximately, 10 mg of extract was dissolved in10 ml volume of 80% methanol to make a stock solution of 1mg/ml of the sample extracted. In a test tube, 1 ml of stock solution was combined with 1.25 ml water and 0.75 ml of 5% $NaNO_2$. For 5 min mixture was allowed to incubate at room temperature, and 0.15 ml of 10% $AlCl_3$ was added to it. Before adding 0.5 ml of 1 N

NaOH and 275 µL of distilled water, a 6-minute interval was allowed. At 510 nm, intensity of pink colour was measured after properly mixing the solution. Various concentrations of catechin were used to validate the flavonoid content standard curve and the concentrations of samples were calculated using calibration curve. Total flavonoid content of sample extracts was measured in mg catechin equivalents per 100g dry weight. Testing of samples was done in triplicates.

3.10.3 Total Antioxidant Content

The extract's total antioxidant activity was calculated using the phosphomolybdenum method (**Prieto *et al.*, 1999; Costa and Fidelis, 2010**). Around 3 ml of reagent solution was combined with 1ml of sample extract (0.5 mg/ml) (0.6 M H_2SO_4, 4 mM ammonium molybdate and 28 mM sodium phosphate). As a blank, a 4 ml aliquot of reagent solution was collected. After that, the mixture was incubated for 90 min at 95°C. After cooling to room temperature, absorbance was measured at 695 nm. Using catechin as standard, the catechin equivalent of total antioxidant content (TAC) was used.

3.10.4 Estimation of Total Tannin Content

Estimation of tannins was done by Folin-Ciocalteu method (**Vijay and Rajendra, 2014**). The procedure for preparing the sample extract involved the addition of 0.1 ml of the extract to a 10 ml volumetric flask. To this flask, 7.5 ml of distilled water and 0.5 ml of Folin Ciocalteu phenol reagent were added, followed by 1 ml of 35% sodium carbonate solution. With distilled water the solution was diluted to a final volume of 10 ml. The flask was left at room temperature after thorough mixing, for 30 minutes. Concurrently, a series of reference standard solutions of tannic acid having concentrations of 20, 40, 60, 80, and 100 µg/ml were prepared using the same method as mentioned earlier. Measurement was done against the blank at 700 nm with UV/ Visible spectrophotometer of both the test and the standard solution. The estimation of tannin content was done in triplicate. The tannin content was expressed in terms of tannic acid equivalents in mg / g of dried sample.

3.10.5 Total Reducing sugar

Three ml of the unknown solution was pipetted out and 3 ml of DNS reagent was added to it followed by heating the contents in boiling water bath for 5 min. Once the tube's contents had reached a warm temperature, a carefully measured 1 ml of a unique 40% Rochelle salt solution was introduced. Subsequently, the solutions were allowed to cool, leading to the emergence of a captivating and rich dark red hue. To quantitatively assess this intense colour, a spectrophotometer measured the light absorbance at 510 nm, utilizing both a blank and a set of carefully prepared glucose standards for calibration (0 to`250 µg) and the standard graph was plotted (**Sadasivam and Manickam, 1992**).

3.11   STATISTICAL ANALYSIS

Data were analyzed using two-way analysis of variance (ANOVA) in a completely randomized design (CRD) and means were compared using DMRT (Duncan's Multiple Range Test) with SPSS 25 software. Statistical significance level for all comparisons was established at P<0.05. P values less than 0.05 were considered significant.

Results

and

Discussion
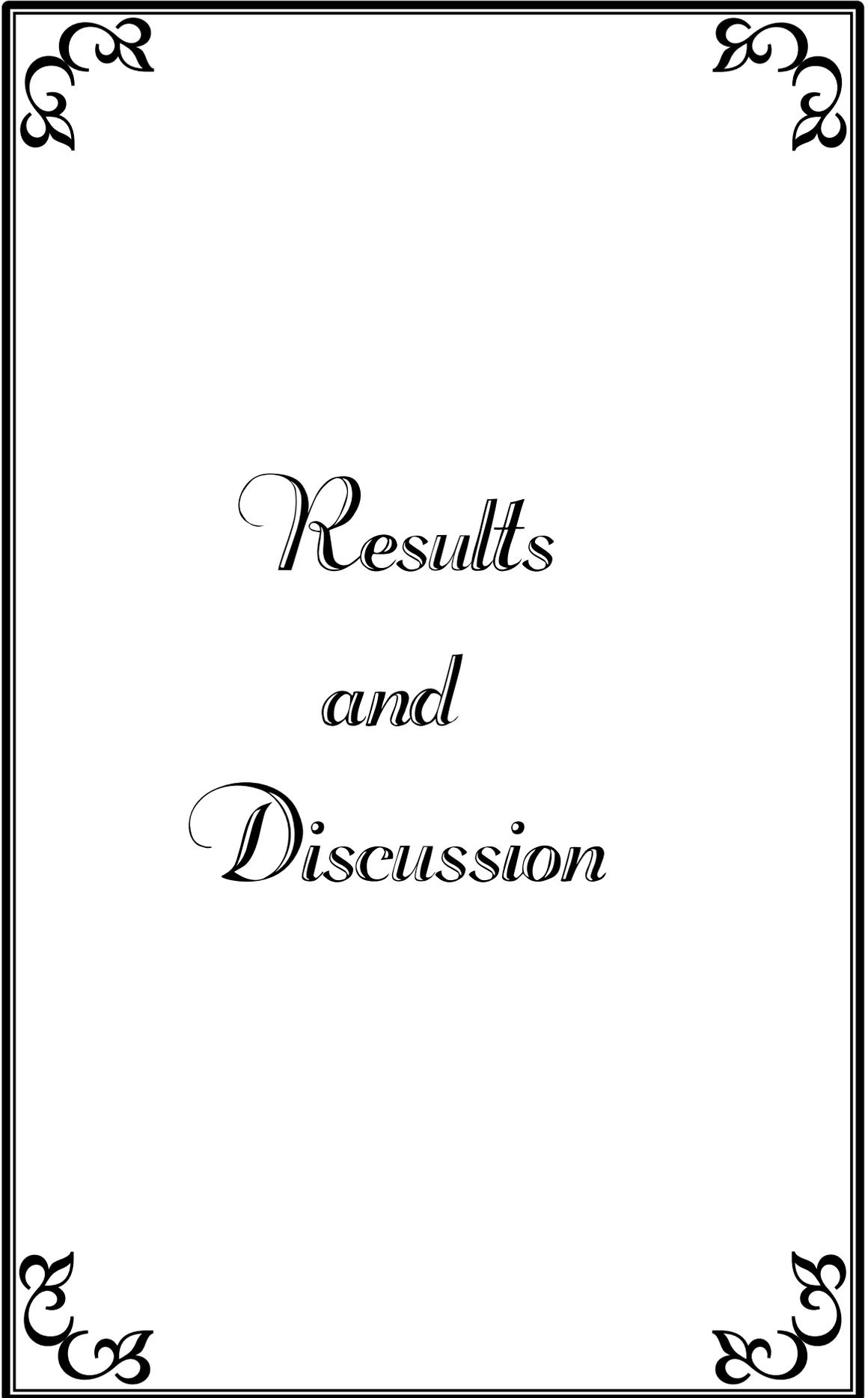
Chapter 4   RESULTS AND DISCUSSION

The present study in goats suffering from gastrointestinal (GI) nematodosis and its therapeutic management by a polyherbal formulation was conducted between Nov 2021 to Dec 2022 at goat farms / herds of Tarai region around Pantnagar. The effect of commonly used anthelmintics *viz.* fenbendazole and ivermectin on haematobiochemical parameters and biomarkers of oxidative stress in goats suffering from gastrointestinal nematodosis was carried out and their efficacy was compared with the polyherbal formulation used in the trial. A gastrointestinal parasitic infection in goats is clinically characterized by enteritis, anemia, emaciation, dehydration, and mortality. These alterations impact animal growth, reproduction, weight, and yield, leading to financial loss for farmers. **(Sharma *et al.,* 2014).**

The local goat farms/herds were screened for GI nematodes using FAMACHA technique. The FAMACHA technique is a cost-effective and validated approach for assessing anaemia levels, primarily caused by *H. contortus* infestation. **Van Wyk** and **Bath (2002)** revealed a 58% reduction in the quantity of treatments required at 10 farms in comparison to previous year, when farmers employed and executed interventions guided by the FAMACHA score, as opposed to relying on PCV assessments.

The practice of polyherbalism introduces synergism, which provides unique advantages not accessible in single herbal formulations. Polyherbal formulations have demonstrated high effectiveness in treating various diseases, even at high doses safely. Synergism in polyherbalism operates through two main mechanisms: pharmacodynamics and pharmacokinetics. Pharmacokinetic synergism focuses on how one herb facilitates the metabolism, absorption, elimination and distribution of other herbs. While, pharmacodynamics synergism explores the combined effect of bioactive components possessing comparable healing properties that target diverse mechanisms of action (**Karole, 2019**).

Traditional phytomedicines demonstrate a multi-target therapeutic approach, leveraging their diverse bioactive compounds to synergistically combat various

ailments **(Spiegler *et al.,* 2017; Brusotti *et al.,* 2014**). Phytosaponins are known to impact cell membrane permeability, leading to vacuolization and tegumental disintegration in parasites **(Melzig *et al.,* 2001).** Alkaloids, on the other hand, induce worm paralysis affecting their central nervous system. Moreover, alkaloids help in reducing nitrate generation, thereby disrupting local homeostasis crucial for helminth development **(Roy *et al.,* 2010).** Phenolic compounds, including triterpenoids and tannins, act by inhibiting energy production via oxidative phosphorylation and interfere with the parasite's cell surface glycoprotein **(Roy *et al.*, 2010).**

Tannins, such as palasonin, can bind to free proteins in the host's alimentary tract or the cuticle glycoproteins of the parasite. Furthermore, tannin-rich herbs have demonstrated direct antiparasitic activity against internal nematodes in ruminants by enhancing host resistance, as evidenced by significant reductions in parasitic load in herb-treated groups during necropsy **(Hoste *et al.*, 2006).**

Additionally, plant extracts rich in flavonoids possess antioxidant properties that help mitigate oxidative stress-related diseases **(Spiegler *et al.,* 2017; David *et al.*, 2016; Barrau *et al.*, 2005).** These flavonoids synergistically augment the activity of condensed tannins, as supported by research **(Klongsiriwet *et al.,* 2015).**

Rahal *et al.* (2022) emphasized that the integration of drug therapies offers promising potential to enhance overall treatment outcomes for various diseases. This approach not only reduces side effects through dose optimization but also targets distinct bio molecular elements, including metabolic interdependence, mediators, and risk factors. Although herbal medicine synergy research is still nascent, the potential of this holds significant promise for future progress. The activities of herbal anthelmintic formulations have been well-documented in various studies conducted *in vivo* as well as *in vitro* (**Iqbal *et al.,* 2006; Hordegen *et al.*, 2006 and Singh *et al.,* 1985**). Phytochemical investigations have revealed that *Butea frondosa* possesses anthelmintic properties because of palasonin, phenolic compounds, and flavonoids present in its seeds. These active principles disrupt energy and metabolism in the worms by interfering with glucose uptake and depleting glycogen stores (**Kumar *et al.* 1995;**

Prashant *et al.* **2001; Singh** *et al.* **2015; Iqbal** *et al.* **2006**). Aqueous extracts of *Embelia ribes* were found to be more potent than standard drugs (**Kekuda** *et al.* **2009**).

The ethanolic extracts of *Azadirachta indica, and Embelia ribes* have demonstrated significant anthelmintic efficacy, up to 93% compared to pyrantel tartrate (**Hordegen** *et al.*, **2006**). The presence of phenolics such as flavonoids, tannins, and anthraquinones in these extracts may be responsible for the observed anthelmintic effects.

Components of *Centratheram anthelminticum* seeds, like *vernolic acid vernodalol and vernodlin,* were identified which are known for their bitter taste, and might contribute to their anthelmintic activity (**Lambertini** *et al.,* **2004, Asaka** *et al.,* **1977**). Triterpenol derivatives like *azadirachtin* from *Azadirachia indica* (Neem) have also demonstrated anthelmintic properties (**Verma** *et al.,* **1995**). The heated methanolic extract of neem leaves (*Azadirachta indica*) demonstrated a substantial 89.95% decrease in faecal egg count. (**Arora** *et al.*, **2007**).

4.1   PREVALENCE STUDIES

The hot humid tropical climate of this part of country is suitable for development and survival of pre parasitic stages of gastrointestinal nematodes throughout the year. Several phenomena in host parasite relationship favour success for the parasite to develop. Numerous investigations have been carried out regarding the occurrence of gastrointestinal parasitic infections both within this nation and internationally. (**Ghosh** *et al.,* **1976; Saha** *et al.,* **1996; Arora** *et al.,* **2010; Bandyopadhyay** *et al.*, **2011** and **Pandey** *et al.,* **1994**). Fluctuations in the occurrence of parasitic infestations are influenced by variations in agro-climatic conditions and the presence of susceptible hosts. (**Radostits** *et al.*, **1994**).

A group of 146 goats, with FAMACHA scores spanning from 3 to 5, was analyzed to establish the occurrence of gastrointestinal nematode infections using targeted selective treatment (TST). Among these 146 goats, 103 were identified as having gastrointestinal nematode infections. From these 103 goats, 24 goats of either sex (n=24; 9 months to 5 years of age with 10 to 30 kg body wt) having egg counts (>200), were selected for the research work.

4.2 SYMPTOLOGY AND CLINICAL PARAMETERS

The key clinical indicators observed in goats afflicted with GI nematodosis encompassed blanched mucous membranes, weakness, growth retardation, and diarrhoea. The diarrheic faeces exhibited a fluid to semi-solid consistency, appearing light black to tarry in colour. Furthermore, most cases displayed whitish to light pinkish conjunctival mucous membranes, accompanied by facial swelling and oedema. Additionally, affected goats showed a lack of appetite, high mortality rates, weight loss, stunted growth, overall weakness, emaciation, dehydration and moderate hypothermia. It was noted that all affected goats had a history of grazing in both field and forest environments.

4.2.1 FAMACHA Score Card

The average FAMCHA score of goats was close to 3 at goat farms / herds of Tarai region around Pantnagar. Majority of the goats kept under semi – intensive system of farming had FAMACHA score between 3 to 5 in the Tarai region around pantnagar and the mucous membrane colour of goats varied from whitish to light pinkish in colour as depicted in table 4.1 and fig. 4.1.

Table 4.1: FAMACHA Score in goats in correlation to **egg per gram (EPG)**

Attributes	FAMACHA scores				
	1 (F1)	2 (F2)	3 (F3)	4 (F4)	5 (F5)
Egg per gram (EPG)	100-300	400-600	700-1000	1100-1200	1300 and above
Number of Goats	16	27	41	30	32
FAMACHA chart	Red or normal colour (non-anaemic)	Pink (non-anaemic)	Pale Pink (mildly-anaemic)	Pale white colour (anaemic)	White colour (severely-anaemic)
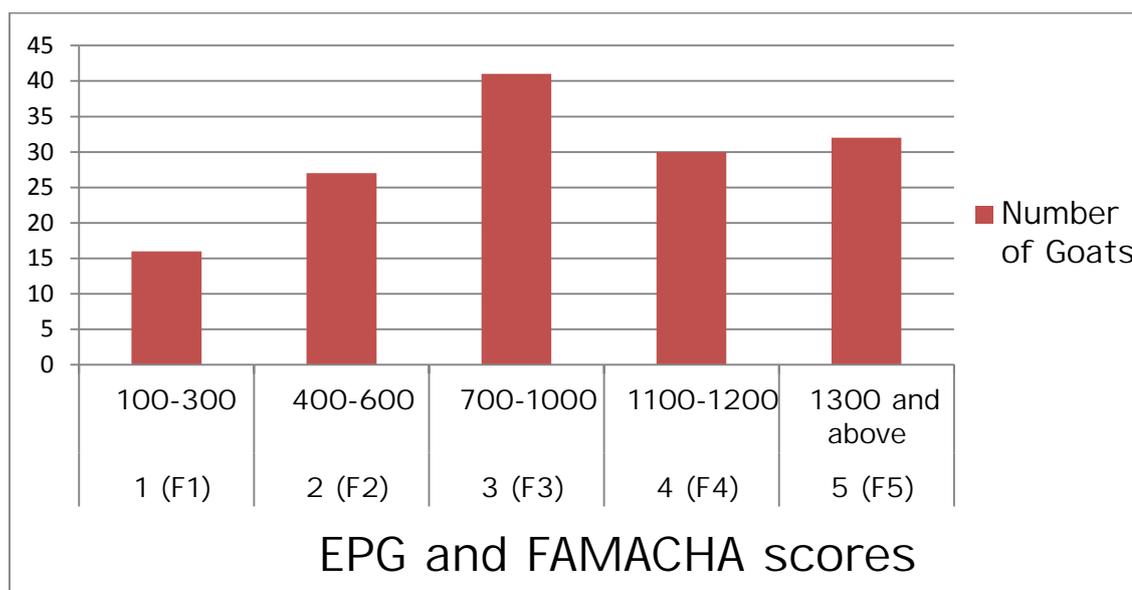
Fig. 4.1: FAMACHA Score in goats in correlation to egg per gram (EPG)

4.2.2 Temperature (°F)

The temperature of goats suffering from GI nematodosis was normal to slightly low. Only in few cases rise in body temperatures was seen. The mean temperature was found to be 38.97 ± 0.033°C.

4.2.3 Pulse (per minute)

The mean pulse rate found in the goats suffering from GI nematodosis was 71.33 ± 0.667.

4.2.4 Respiration (per minute)

The mean respiration rate found in the goats suffering from GI nematodosis was 22.33 ± 0.667.

4.3   *IN VITRO* - TRIAL

In the present study evaluation of *in- vitro* anthelmintic activity of polyherbal formulation (ethanolic extract) against round worms isolated from goats affected with GI nematodosis was carried out. Anthelmintic activity was determined in terms of concentration of polyherbal formulation and time taken in mortality of adult worms. Ethanolic extract of polyherbal formulation (PHF) showed total mortality at 100 mg/ml at the exposure time of 2 hrs. The concentrations of 50mg/ml and 25 mg/ml resulted in

total mortality at the exposure time of 4 hrs. The absence of motility by using pinch technique for an observation period of 5-6 seconds was used to determine the worms' viability, as described by **Eguale and Giday (2009).** At 100 mg/ml concentration 50% mortality was recorded at 1 hr post exposure. The LC50 at 1 hr was 100 mg/ml, and at 2 hrs interval both 50 mg/ml and 25 mg/ml were effective as the LC 50 dose. The efficacy of the ethanolic extract of polyherbal formulation (PHF) in different dose dependent concentrations revealed that it has good efficacy at the concentrations of 100, 50 and 25 mg/ml at the exposure time of 2 hrs and 4 hrs against the adult round worms of goat origin in the *in vitro* trials as depicted in Table 4.2. The present finding is in agreement with the previous published research work **(Prashant *et al.* 2001; Iqbal *et al.* 2006 and Singh *et al.* 2013)**.

Table 4.2: *In-vitro* anthelmintic effect of ethanolic extract of polyherbal formulation against adult round worms of goats (AWMA)

Conc. of PHF (mg/ml)	No of parasites exposed	No. of parasites found dead at various times of exposure				% mortality
		30 min	1 hr	2 hr	4 hr	
100	10	2	5	10	10	100
50	10	0	3	5	10	100
25	10	0	1	5	10	100
10	10	0	0	0	2	20

4.4   PILOT STUDY AND SAFETY TRIALS

A pilot study was conducted on three goats to rule out any adverse effects caused by the polyherbal formulation (PHF) clinically. Initially *in-vitro* evaluation of polyherbal formulations using Adult Worm Motility Assay was carried out as described at para 4.3. After that, *in-vivo* trial of the polyherbal formulation at dose rate based on literature and products already available in market **(Parsani *et al.,* 2020, Hardeep Kalkal *et al.,* 2020)** was carried out. The various clinico-haematobiochemical parameters were recorded for seven days to check any adverse effects caused by the polyherbal formulation as depicted in table 4.3. All the parameters fell within the

standard range. EPG count was recorded as depicted in Fig.4.2. Following the safety trial, a dosage of 6.5 grams of the polyherbal formulation (PHF) per 30 kilograms of body weight as a single dose was finalised for investigation.

Table 4.3: Effect of polyherbal formulation on different clinical and haemato-biochemical parameters at 0 Day, 3rd Day and 7th day in the three goats (G1, G2 and G3).

PARAMETERS		TIME IN DAYS								
		0 Day			3rd Day			7th Day		
Goats for Pilot study		G1	G2	G3	G1	G2	G3	G1	G2	G3
Clinical Parameters	Age (yrs)	2	1.7	1.9	2	1.7	1.9	2	1.7	1.9
	Sex	M	F	F	M	F	F	M	F	F
	Body Wt (Kg)	18	20	20	18	20	20	18	20	20
	FAMACHA Score	3	3	3	3	3	3	3	3	3
	Respiration /min	21	23	23	22	22	23	22	23	23
	Pulse rate/min	70	72	72	70	72	72	71	70	72
	Temperature °C	39	38.9	39	39	39	39.1	38.9	39	39
Haematological Parameters	Haemoglobin (g/dl)	9.5	9.3	8.9	9.5	9.5	9	9.5	9.6	9.1
	TLC (x10^3/mm3)	9.9	11.4	11.8	9.9	11.4	11.8	9.9	11	11.2
Biochemical Parameters	Total plasma protein (g/dl)	8.5	7.2	7.8	8.5	7.4	7.8	8.6	7.4	7.8
	Blood glucose (mg/dl)	42	51	45	42	52	46	43	52	46
	ALT (IU/L)	25.6	26.7	23.2	28	29	28	28	30	29
	AST (IU/L)	166	131	184	186	156	218	188	156	210
	Urea	40	34.4	38	41	35	38	41	35.3	40
Faecal egg count	EPG	2300	2250	2400	2150	2050	1900	2000	1950	1650

G1- was administered 5 gm Polyherbal formulation orally
G2- was administered 6 gm Polyherbal formulation orally   — Single Dose
G3- was administered 6.5 gm Polyherbal formulation orally
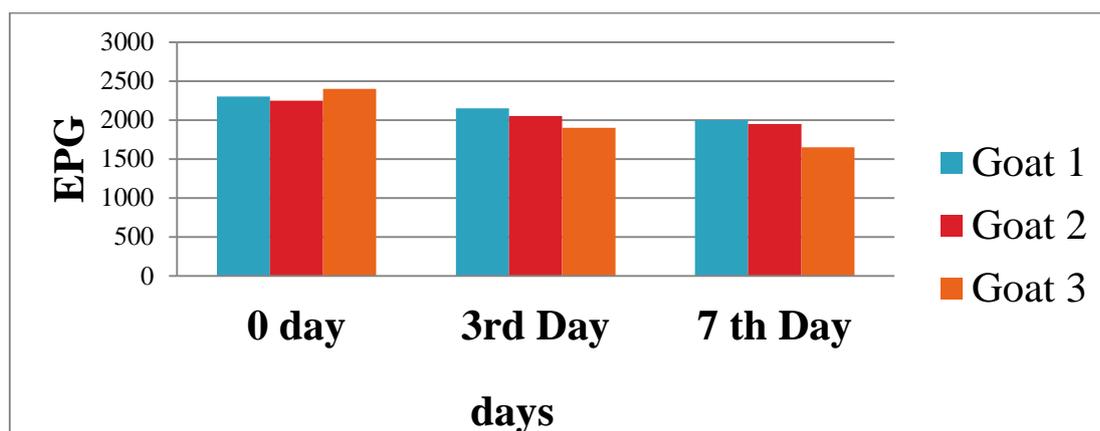
Fig. 4.2: Pilot study- (safety trial) effect of PHF on EPG at 0 day, 3rd day and 7th day.

G1- was administered 5 gm Polyherbal formulation orally
G2- was administered 6 gm Polyherbal formulation orally — Single Dose
G3- was administered 6.5 gm Polyherbal formulation orally

4.5   *IN -VIVO* TRIAL

The selected 24 goats were divided randomly into four equal groups and different coloured tags were placed around the neck for identification.

Group I (G I): (n=6) was treated with fenbendazole @ 5mg/kg body weight orally (**Plumb, 2008, Sharma *et al.,* 2015, Papich, 2016).**

Group II (G II) : (n=6) was treated with ivermectin @ 0.2mg/kg body weight orally (**Lespine *et al.*, 2005, Sharma *et al.,* 2015, Shakya *et al.*, 2018, Myers *et al.*, 2020**).

Group III (G III) : (n=6) was treated with polyherbal formulation @ 6.5 gm/30 kg body wt. orally as a single dose (Based on safety trial/pilot study**, Parsani *et al.*, 2020, Kalkal and Vohra 2021, Khanolkar *et al.,* 2018**).

Group IV (G IV): (n=6) was kept as untreated control.

The efficacy of oral administration of ivermectin in goats has been assessed by previous works (**McKenna and Watson, 1987; Swann and Gross, 1985; Waruiru *et al.,* 1998**). These authors reported efficacy of more than 99% for per os ivermectin at 0.2 mg/kg.

Haematological, biochemical, and oxidative stress-related parameters were examined at intervals of 0, 7, 14, and 21 days.

In vivo experiments involved a comparison of the effectiveness of polyherbal formulation with traditional anthelmintic drugs, specifically fenbendazole and ivermectin, on the basis of reduction in EPG count after oral administration of all the drugs. Collection of faecal samples directly from rectum for examination by modified Mc Master technique & FECRT was done on faeces collected on day 0 (Pre-treatment) and 7, 14 and 21st day (post treatment).

In vivo studies also included before and after treatment study of alterations in the haematological and biochemical profiles including oxidative stress biomarkers of all blood samples collected on 0 day (Pre-treatment) and 7, 14 and 21st day (post treatment).

4.6   EFFECTS ON HEMATOLOGICAL PARAMETERS

In the GI Nematodosis infected goats there was significant increase in the mean values of Hb, PCV and TEC after treatment with Polyherbal formulation & Ivermectin, as compared to control group. A notable rise in Hemoglobin (Hb), Packed Cell Volume (PCV), and Total Erythrocyte Count (TEC) subsequent to treatment indicated its positive effect on health of animals along with cessation of blood loss. The substantial elevation in Hb, PCV, and TEC could also be attributed to the replenishment of iron deficiency and the swift regeneration of erythrocytes. The present results are consistent with the research outcomes of previous studies such as those conducted by **Priyanka *et al.* (2019**), **Amulya *et al.* (2016), Rajguru *et al.* (2003), Akhtar *et al.* (2014), Sharma *et al.* (2014), Islam *et al.* (2003), Yadav *et al.* (2009)** and **Arunachalam *et al.* (2013).**

Haematological investigations have unveiled intriguing findings in goats afflicted by *Haemonchus contortus* parasitic infection. Specifically, these infected goats exhibited decreased levels of Hb (hemoglobin), PCV (packed cell volume), and TEC (total erythrocyte count). The notable reduction in these essential blood components can be attributed to a dual impact. Firstly, the parasitic infestation leads to blood loss as the *Haemonchus* worms pierce the mucosal lining, causing bleeding and anaemia in the host. The adult and L4 (fourth larval stage) worms are known to be hematophagous, meaning they feed on the host's blood, further exacerbating the blood loss. Researchers have quantified the average blood loss caused by *H. contortus* infection to be approximately 0.05 ml per day per worm, as reported in a study by **Urquhart *et al.* (2000)**. This steady loss of blood over time contributes to the decline in Hb, PCV, and

TEC counts observed in infected goats. Secondly, *Haemonchus* parasites can inflict injuries on the abomasums (fourth compartment of the stomach) of the goats. These injuries, described by **Abdel** in **1992**, result from the action of the parasites while feeding and contribute to the reduced values of Hb, PCV, and TEC counts in the infected groups.

In conclusion, the haematological changes observed in goats with *Haemonchus contortus* parasitic infection and other blood sucking GI nematodes can be attributed to the dual effects of blood loss through mucosal piercing and hematophagy by the parasites, along with injuries to the abomasum. Comprehending these mechanisms is vital for devising effective management and treatment approaches aimed at mitigating the adverse effects of this parasitic infection on goat well-being and productivity.

The notable decrease in mean Total Leukocyte Count (TLC) observed on the 14th and 21st days might be attributed to the anthelmintic effects of the polyherbal formulation. It's possible that it also possesses an added antioxidant effect. **(Farida and Iswahyani, 2018).** The current results are in accordance with the studies conducted by **Sena (2000) and Rajkowa (2003**). Similar observation was also made by **Qamar and Makbool (2012).**

4.6.1. Hemoglobin

Haemoglobin is a molecule made up of proteins and is present within red blood corpuscles where it works as a carrier of gases. It comprises of four protein molecules (globulin chains), attached together. No statistically significant distinction was noted in the mean haemoglobin values between groups G I, G II, G III and G IV on day 0 (before treatment).

In the present study it was revealed that in G I goats, the haemoglobin value of day 21 was significantly different (P<0.05) with same day values of G II goats and also significantly different (P<0.01) with same day value of G III goats. Among G II goats values of day 21, differed significantly (P<0.01) in comparison to the values of day 0. In case of G III goats day 21 value was significantly different (P<0.01) with day 0 values of the same group. The day 14 value of G III goats were significantly different (P<0.05) from G IV goats. However the mean haemoglobin concentration was somewhat higher in the G II and III goats in comparison to G I and IV goats. The

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study's findings are consistent with the research conducted by **Mamta (2012), Shashank *et al.* (2019)**, **Biswas *et al.* (2017)** and **Rasha *et al.* (2020).** Similar kinds of improvement of these blood parameters after anthelmintic treatment have been previously reported in sheep too by **Aruwayo *et al.* (2011) and Rahman (2002).**

Mean values along with SE of Hb (g/dL) of different treatment groups and within the treatment group have been given in table 4.4 and fig. 4.3.

Table 4.4: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Hb in goats suffering from GI nematodosis. Normal range 8-12 (Schalms Hematology, 2010)

Duration	0 day	7th day	14th day	21st day
Group I	5.47 ± 0.2^{A}	5.93 ± 0.11^{Bab}	6.53 ± 0.07^{Cc}	8.62 ± 0.09^{Cb}
Group II	5.2 ± 0.09^{A}	6.25 ± 0.04^{Aa}	7.48 ± 0.06^{Bb}	10.12 ± 0.05^{Ba}
Group III	5.72 ± 0.13^{A}	6.28 ± 0.08^{Aa}	8.33 ± 0.06^{Aa}	10.58 ± 0.04^{Aa}
Group IV	6.07 ± 0.09^{A}	5.73 ± 0.09^{Bb}	5.63 ± 0.08^{Dd}	6.53 ± 0.08^{Dc}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
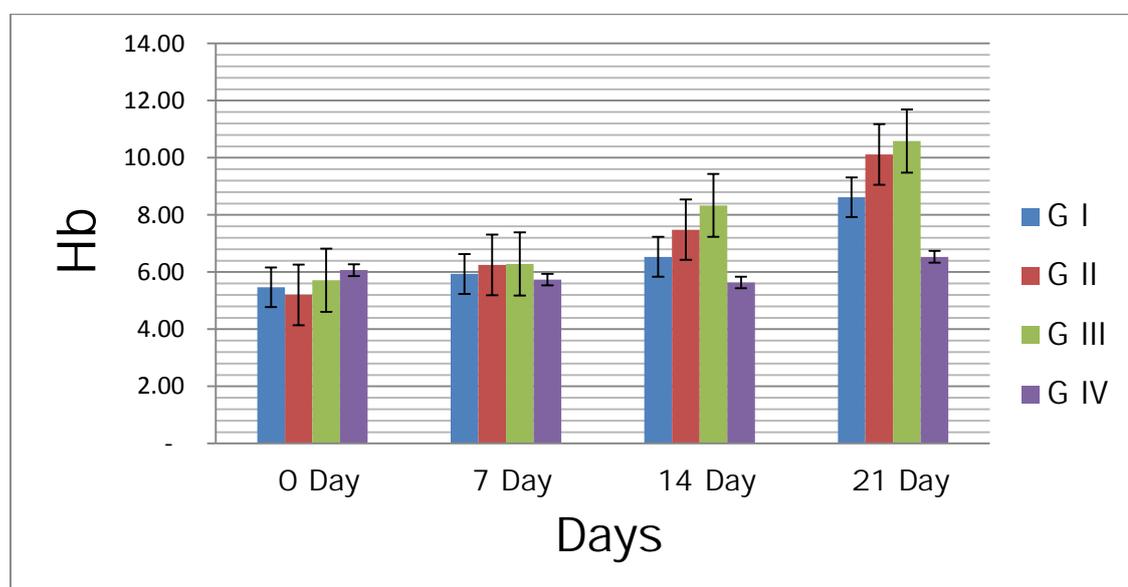
Fig. 4.3: Influence on Hb (g/dL) in Goats afflicted with GI Nematodosis following administration of Fenbendazole, Ivermectin, and Polyherbal formulation

4.6.2. Total Erythrocyte Count (TEC)

On the 21st day following treatment, Group III exhibited a notable rise in TEC values. In contrast, the goats within group IV displayed insignificant impacts on erythrocyte counts when compared to the remaining groups. Comparable results were documented in studies conducted by **Rasha *et al.* (2020), Biswas *et al.* (2017), Contreras *et al.* (1976), Misra *et al.* (1996)** and **Moudgil *et al.* (2017).**

The decrease in Hemoglobin (Hb), Packed Cell Volume (PCV), and Total Erythrocyte Count (TEC) observed in goats infested with nematodes can be attributed to the blood-sucking behaviour of the parasites and the consequent losses through leakage, which results from extensive damage to the gastrointestinal mucosa. This is in line with the findings reported by **Urquhart *et al.* (1996) and Rajpoot *et al.* (2022).** Mean values along with SE of TEC (x 10^6/μl) of different treatment groups and within the treatment group have been given in table 4.5 and fig. 4.4.

Table 4.5: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on TEC (x 10^6/μl) in goats suffering from GI nematodosis. Normal range 8-18 (Schalms Hematology, 2010)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	5.46 ± 0.14^A	5.83 ± 0.12^B	7.03 ± 0.06^{Cb}	9.4 ± 0.14^{Bb}
Group II	4.46 ± 0.11^B	6.22 ± 0.06^A	8.07 ± 0.03^{Ba}	9.48 ± 0.03^{Bb}
Group III	5.23 ± 0.06^A	5.68 ± 0.02^B	8.53 ± 0.05^{Aa}	10.55 ± 0.05^{Aa}
Group IV	$5.6^a4 \pm 0.13^A$	5.59 ± 0.09^B	5.58 ± 0.09^{Dc}	6.18 ± 0.09^{Cc}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.4: Influence on TEC (x 10^6/μl) in goats afflicted with GI Nematodosis following administration of Fenbendazole, Ivermectin, and Polyherbal formulation

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4.6.3. Total leukocytes count (TLC)

For all groups (I, II, III, and IV), TLC values generally decreased over time. Group III had the most significant decrease in TLC values compared to the other groups. Group II also showed a substantial decrease in TLC values over time. Group I and IV demonstrated non-significant decrease in TLC values. Similar findings were recorded by **Qamar *et al.* (2012), Jas *et al.* (2008), Ahmed *et al.* (2015)** and **Shashank *et al.* (2019).**

Mean values along with SE of TLC (x 10^3/μl) of different treatment groups and within the treatment group have been given in table 4.6 and fig. 4.5.

Table 4.6: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on TLC ($\times 10^3$ / μl) in goats suffering from GI nematodosis. Normal range 4-13 (Schalms Hematology, 2010)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	13.95 ± 0.25^A	13.26 ± 0.26^A	12.55 ± 0.23^{Aa}	10.48 ± 0.18^{Ab}
Group II	13.63 ± 0.22^A	11.15 ± 0.12^B	10.65 ± 0.07^{Bb}	9.12 ± 0.05^{Bc}
Group III	14.25 ± 0.38^A	11.52 ± 0.23^B	9.97 ± 0.11^{Bb}	8.93 ± 0.03^{Bc}
Group IV	11.67 ± 0.58^B	12.62 ± 0.45^A	13.98 ± 0.33^{Aa}	11.93 ± 0.28^{Aa}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.5: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on TLC ($\times 10^3$ / μl) in goats suffering from GI nematodosis.

4.6.4. PCV (Packed cell Volume)

PCV values in Group I, II and III showed a significant increase on day 21st after treatment compared to the pre-treatment values of day 0. Group IV showed decrease in PCV values which is obviously due to the persisting worm burden being the untreated control group.

The rise in mean PCV after treatment might be associated with the increase of Hb%, as these parameters are closely interrelated with each other. The improvement of blood PCV level in the treated group could be due to the reduced EPG. The findings are in agreement with **Rahman *et al.* (2009), Mamta (2012), Biswas *et al.* (2017), Shashank *et al.* (2019)** and **Rasha *et al.* (2020).** Similar kinds of improvement of these blood parameters after anthelmintic treatment were reported in sheep too by **Aruwayo *et al.* (2011) and Rahman (2002).**

Mean values along with SE of PCV (%) of different treatment groups and within the treatment group have been given in table 4.7 and fig. 4.6.

Table 4.7: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on PCV (%) in goats suffering from GI nematodosis. Normal range 22-38 (Schalms Hematology, 2010)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	21.17 ± 0.13^{B}	23.23 ± 0.28^{A}	24.33 ± 0.23^{Ab}	25.33 ± 0.14^{Aa}
Group II	24.33 ± 0.49^{A}	22.33 ± 0.14^{B}	25.5 ± 0.25^{Aab}	26 ± 0.24^{Aa}
Group III	22.3 ± 0.33^{B}	24 ± 0.3^{A}	26.33 ± 0.27^{Aa}	25.58 ± 0.17^{Aa}
Group IV	23.67 ± 0.31^{A}	22 ± 0.24^{B}	22 ± 0.24^{Bc}	21.83 ± 0.25^{Bb}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.6: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on PCV (%) in goats suffering from GI nematodosis

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4.6.5. Differential leukocyte count (DLC)

The haematological parameters of DLC (differential leukocyte count) were measured before and after treatment. DLC provides valuable insights into the distribution of various types of WBC, namely Neutrophils (N), Lymphocytes (L), Monocytes (M), and Eosinophils (E), which are crucial for immune function.

The study revealed following changes in DLC values in response to the treatment given to the groups;

- Neutrophils (N): The percentage of Neutrophils increased in G I (from 48.83% to 61.17%). G IV had non-significant change, whereas decrease in the values was seen in group II (56.17% to 41.33%) & III (60.50% to 43.17%) suggesting that the treatment might have had a positive effect on the neutrophil values in bringing them back to normal range.

- Lymphocytes (L): The percentage of Lymphocytes showed significant decrease in the values in G I (44.83% to 34.50%) whereas significant increase in values was seen in G II (37.50% to 55.00%) and G III (33.67% to 53.17%). There was no statistically significant change observed in the values within Group IV (34.50% to 35.00%). This finding indicates that the treatment might have had a positive effect on the lymphocyte values in bringing them back to normal range.

- Monocytes (M): The percentage of Monocytes increased slightly in G I and decreased non significantly in G II & III, suggesting that the values remained within normal range during the course of treatment.

- Eosinophils (E): The percentage of eosinophils remained relatively constant and fell within the standard range in most groups after the treatment (21st Day), compared to the levels before treatment (0 Day), indicating that the treatment may not have had a substantial effect on Eosinophil populations.

Comparable results were documented by **Rasha *et al.* (2020)**. Mean values along with SE of DLC (%) of different treatment groups and within the treatment group have been given in table 4.8 and fig. 4.7.

Table 4.8: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on DLC (%) in goats suffering from GI nematodosis. Normal range as per Schalms Hematology, 2010 are; Neutrophil (30-48), Lymphocyte (50-70), Monocytes (0-4), Eosinophils (1-8) and Basophils (0-1)

Haematological parameters		Before Treatment (0 Day)				After Treatment (21st Day)			
		G I	G II	G III	G IV	G I	G II	G III	G IV
DLC(%)	N	48.83	56.17	60.50	61.17	61.17	41.33	43.17	60.67
	L	44.83	37.50	33.67	34.50	34.50	55.00	53.17	35.00
	M	1.83	4.83	3.67	2.50	2.50	1.83	1.83	2.50
	E	4.50	1.50	2.17	1.83	1.83	1.83	1.83	1.83
	B	0	0	0	0	0	0	0	0

Fig. 4.7: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on DLC (%) in goats suffering from GI nematodosis

4.6.6. Platelet count

For all groups (I, II, III, and IV), platelet count values generally decreased on 21st day post treatment compared to the pre-treatment values on 0 day however the variation was within normal range. Mean values along with SE of Platelet count (x 10^3/ µl) of different treatment groups and within the treatment group have been given in table 4.9 and fig. 4.8.

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Table 4.9: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Platelet count (x 10^3/ μl) in goats suffering from GI nematodosis. Normal range 300-600 (Schalms Hematology, 2010)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	359.5 ± 13.63^A	326 ± 12.72^A	338.83 ± 11.91^{Aab}	339.17 ± 8.53^A
Group II	391.83 ± 8.97^A	338.67 ± 8.13^A	362 ± 5.63^{Aa}	328 ± 3.12^A
Group III	399.67 ± 10.61^A	368.17 ± 6.95^A	360.33 ± 4.8^{Aa}	329.83 ± 1.46^A
Group IV	367 ± 17.42^A	318 ± 10.6^A	285.83 ± 7.84^{Bb}	343.5 ± 10.14^A

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
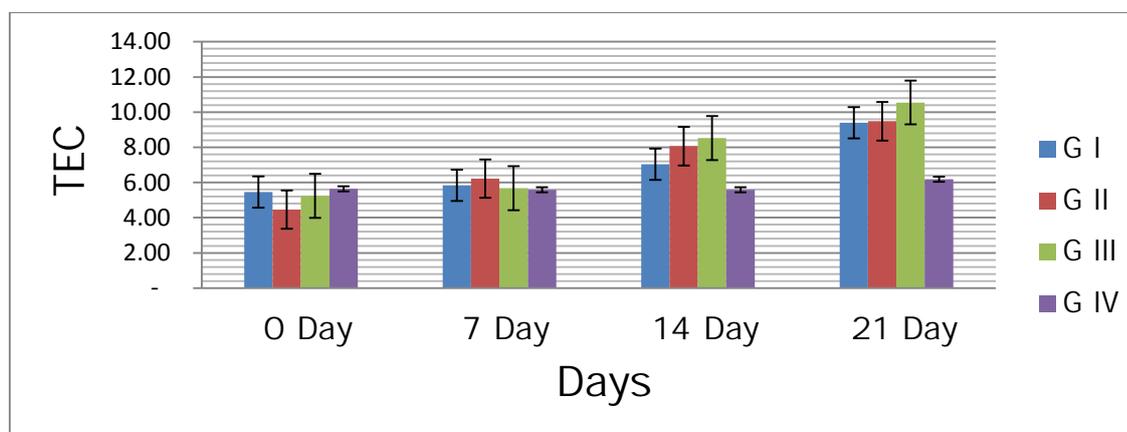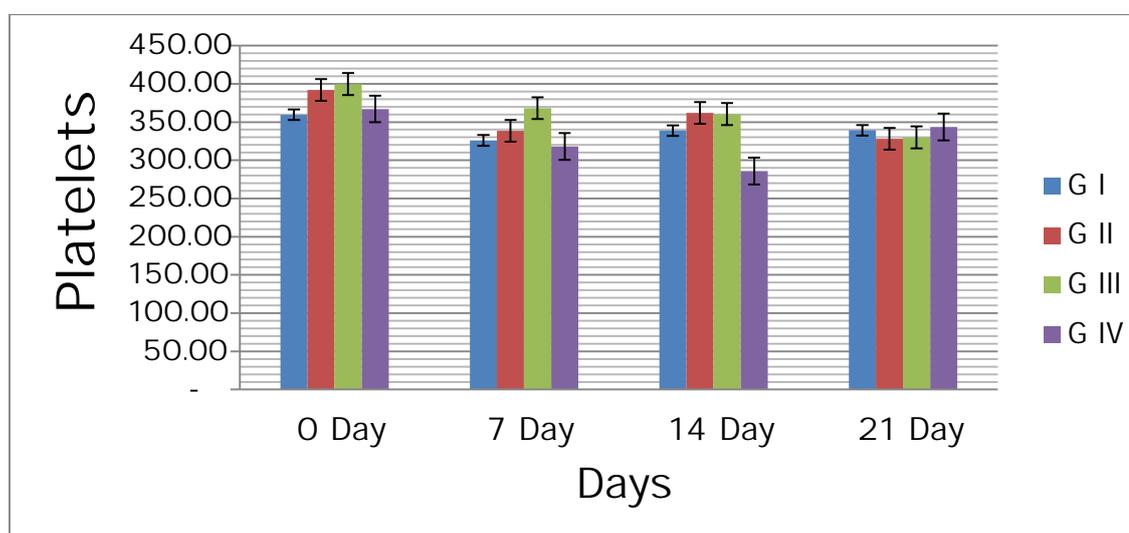

Fig. 4.8: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Platelet count (x 103/ μl) in goats suffering from GI nematodosis

4.6.7. Erythrocytic indices (MCV, MCH & MCHC)

Erythrocytic indices provide valuable information about the size, content, and concentration of red blood cells, which are essential for oxygen transport and overall physiological function.

Based on the data obtained after treatment, we can infer the following:

• Mean Corpuscular Volume (MCV) - After treatment (21st Day), the MCV values in G I, II and III decreased significantly, reaching a level equal to the normal range of healthy goat whereas values in the untreated control group did not have any significant deviation.

- Mean Corpuscular Hemoglobin (MCH) - After treatment (21st Day), the MCH values in G I, II and III decreased significantly and were close to the normal values of a healthy goat whereas values of the untreated control group were devoid of any significant variation.

- Mean Corpuscular Hemoglobin Concentration (MCHC) - The values after treatment (21st Day), were devoid of any significant variation among the groups.

Similar outcomes were documented by both **Rasha *et al.* (2020)** and **Shashank *et al.* (2019).** Table 4.10 and fig. 4.9. provides mean values and standard errors (SE) for MCV, MCH and MCHC across various treatment groups as well as within the treatment group.

Table 4.10: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on MCV (fL), normal range 16-25, MCH (pg), normal range 5.2-8 and MCHC (g/dl), normal range 30-36, in goats suffering from GI nematodosis.

Erythrocytic indices	Before Treatment (0 Day)				After Treatment (21st Day)			
	G I	G II	G III	G IV	G I	G II	G III	G IV
MCV(fL)	63.83 ± 2.21^B	72.17 ± 0.45^A	73.17 ± 0.41^A	70.45 ± 0.89^A	52.17 ± 0.73^{Bb}	36.67 ± 1.18^{Cc}	35.67 ± 0.57^{Cc}	69.67 ± 0.58^{Aa}
MCH (pg)	24.5 ± 0.42^A	26.17 ± 0.59^A	26.5 ± 0.49^A	25.2 ± 0.6^A	16 ± 0.59^{Bb}	13.13 ± 0.44^{Cbc}	11.93 ± 0.22^{Cc}	28.17 ± 0.64^{Aa}
MCHC (g/dl)	32.45 ± 0.27^A	32.17 ± 0.39^A	32.93 ± 0.39^A	32.45 ± 0.35^A	29.67 ± 0.42^A	30.33 ± 0.55^A	31.33 ± 0.27^A	32.33 ± 0.29^A

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
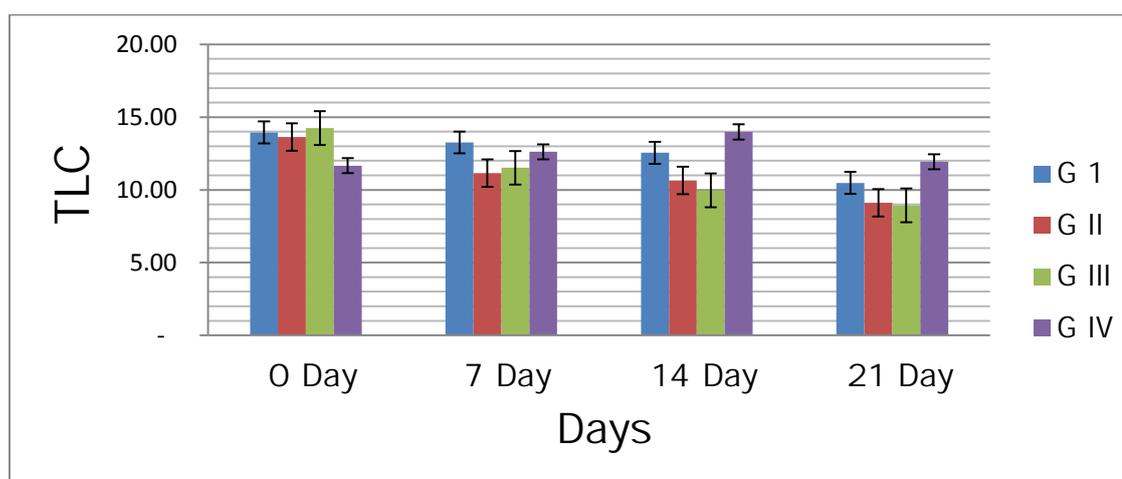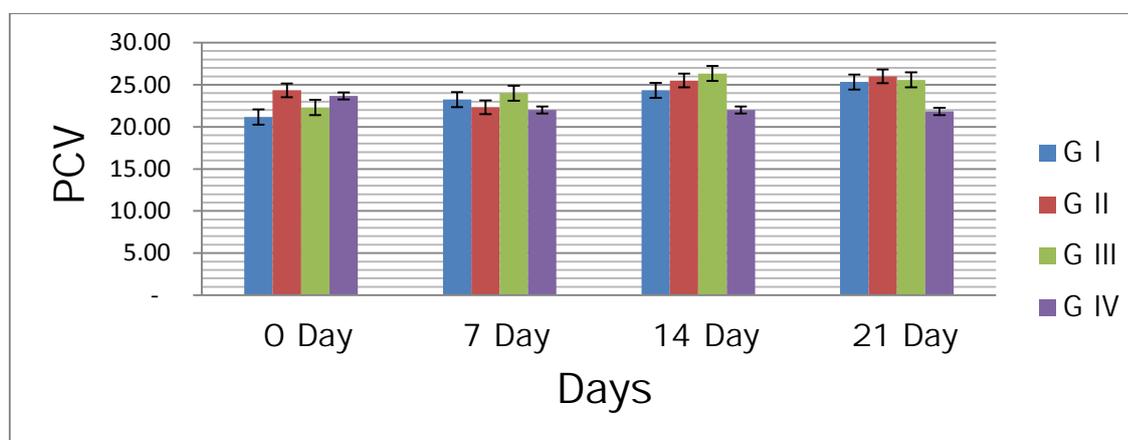

Fig. 4.9: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on MCV (fL), MCH (pg) and MCHC (pg) in goats suffering from GI nematodosis.

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4.7.   EFFECTS ON BIOCHEMICAL PARAMETERS

There was significant decrease in the values of ALP, SGOT (AST) and SGPT (ALT) in goat serum after treatment with PHF and Ivermectin in comparison to control group which indicated marked hepatic damage in goats infected with GI nematodes. This concurs with the findings presented by **Waghmare *et al.* (2009), Sunandhadevi *et al.* (2017), Ahmed *et al.* (2012)** and **Bhardwaj *et al.* (2015).**

Values of blood glucose were also significantly higher in goats treated with Polyherbal formulation and Ivermectin in comparision to the control group. The low level of glucose before treatment in goats suffering from GI Nematodosis could be because of in appetence/anorexia, decreased intestinal absorption of glucose and reduced rate of conversion of lactic acid to glucose. Goats afflicted with *Haemonchosis* exhibited reduced blood glucose levels. Comparable findings regarding blood glucose were reported by (**Dasgupta and Bandyopadhyay, 2000**) and (**Arora *et al.,* 2001**). Following the treatment, a notable enhancement was observed, likely due to parasite elimination and increased glucose absorption from the gastrointestinal tract.

4.7.1   Glucose

G III showed a significant rise in glucose values at 14 days, while G II showed a continuous increase over the study duration. G I and G IV demonstrated relatively stable glucose values however the values in G IV on 21st day reduced significantly.

Glucose level was low in G I & IV on account of high EPG and high worm load and the difference was statistically highly significant (*P*<0.01). Correlation between EPG and glucose was inversely correlated. These findings could be associated with decreased food consumption and impaired absorption of dietary components due to disruptions in the gastrointestinal system resulting from parasitic infections. These results align with findings of **Bandhaiya *et al.* (2019)** however they are contrary to findings of **Murad *et al.* (2018)** who reported mild fluctuations in glucose levels which were not deemed statistically significant.

Mean values along with SE of glucose of different treatment groups and within the treatment group have been given in table 4.11 and fig. 4.10.

Table 4.11: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Glucose level in goats suffering from GI nematodosis. Normal range 50-80 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	44.31 ± 1.96^{A}	44.83 ± 1.46^{B}	45.17 ± 1.24^{Bb}	46.67 ± 0.98^{Bb}
Group II	46.73 ± 3.2^{A}	53.58 ± 1.79^{A}	55.25 ± 1.21^{Aa}	55.5 ± 1.18^{Aa}
Group III	32.67 ± 2.05^{A}	43.64 ± 1.18^{B}	58.17 ± 1.1^{Aa}	56.33 ± 0.56^{Aa}
Group IV	40.25 ± 2.01^{A}	38.78 ± 1.62^{B}	35.75 ± 1.24^{Bc}	36.67 ± 1.49^{Cc}

Values (Mean $\pm$ SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.10: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Glucose level (mg/dl) in goats suffering from GI nematodosis.

4.7.2 Total Protein

Results suggest that Group III protein values showed a decline, while Group IV exhibited a notable decrease in protein values at the 14-day mark. Groups I & II showed relatively stable protein values throughout the experiment.

Examining the serum protein profile is crucial for evaluating nutritional status, detecting potential metabolic changes, and aiding in the clinical diagnosis of various

diseases. Numerous investigations have documented serum protein values in goats, such as, **Castro *et al.* (1977) and Sharma *et al.* (2001),** who also saw an average of 6.7 & 7.3 gm /dl for total protein, respectively. The same are in agreement with the present study.

Mamta (2012) noted a significant decline in total proteins, total albumin, and globulin in bursate worm-infected goats. This indicates bursate worm-induced hypoproteinemia and hypoalbuminemia as the disease progresses. Similar findings were reported by **Barowicz (1970), Shastry and Ahluwalia (1972), Uppal and Rai (1978),** and **Arora *et al.* (2001).** The drop in serum protein and albumin could stem from gut injury, increased albumin catabolism, and pepsinogen leakage (**Smith and Sherman, 1994**).

Shashank *et al.* (2019) discovered notable decline ($p<0.05$) in serum protein levels among goats afflicted with gastrointestinal nematodosis. Corresponding results were found in studies by **Hassan *et al.* (2012), Qamar *et al.* (2012)** and **Ahmed *et al.* (2015).** Hypoproteinaemia and hypoalbuminemia in affected animals can stem from protein-losing gastro enteropathy associated with nematodiasis (**Soulsby, 1982**), as well as compromised protein absorption due to intestinal mucosal damage during concurrent gastrointestinal infections (**Ahmed *et al.*, 2015**). A substantial amount of serum protein is lost into the gut lumen, with affected animals excreting approximately 210-340 ml of serum protein daily in their faeces (**Bordoloi *et al.*, 2012 and Dargie *et al.*, 1975**).

As per **Rasha *et al.* (2020),** total protein and albumin levels in serum decreased significantly ($P <0.05$) in infected sheep and goats compared to control healthy animals. According to **Lakra *et al.* (2007),** in cases of parasitic infection, a decline in serum protein levels occurs due to protein loss in the damaged intestine. However, the mean values of protein, albumin, globulin, and the A: G ratio under various treatments remained within the normal physiological range. Mean values along with SE of Total Protein of different treatment groups and within the treatment group have been given in table 4.12 and fig. 4.11.
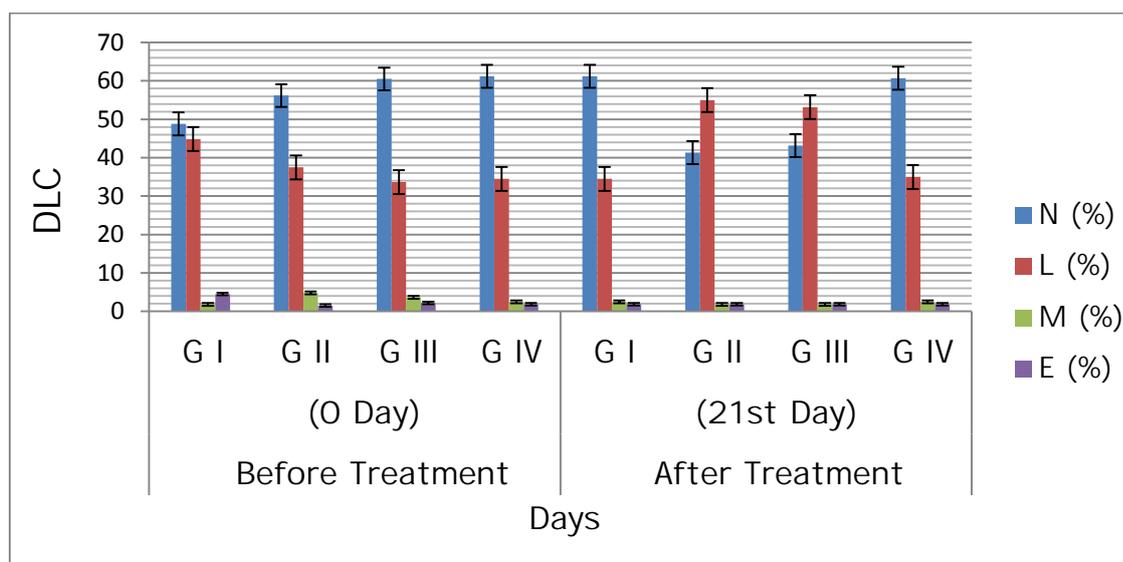
Table 4.12: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Total Protein in goats suffering from GI nematodosis. Normal range; 6.4-7 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	6.28 ± 0.4^{Bb}	6.72 ± 0.17^{B}	6.83 ± 0.14^{Aa}	7.17 ± 0.09^{A}
Group II	7.1 ± 0.27^{Bb}	7.4 ± 0.18^{A}	7.53 ± 0.19^{Aa}	7.17 ± 0.06^{A}
Group III	8.75 ± 0.05^{Aa}	8 ± 0.13^{A}	7.43 ± 0.08^{Aa}	7.09 ± 0.1^{A}
Group IV	7.45 ± 0.2^{Bab}	7.02 ± 0.16^{B}	5.5 ± 0.12^{Bb}	7.38 ± 0.08^{A}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.11: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Total Protein (g/dL) in goats suffering from GI nematodosis

4.7.3 Serum Albumin

No significant variation among the groups was seen (P > 0.05). The serum albumin content in all groups fell within the normal range for the goat species. (2.7–3.9 gm/dl). These results are in consonance with the results of **Katiki _et al._ (2011). Brito _et al._ (2020)** also reported that no statistically significant variation existed amongst the treated groups for total plasma protein (P > 0.05), This suggests that the animals did not exhibit issues with dehydration or other factors that could lead to elevation, such as antigenic stimulation. According to **Rasha _et al._ (2020)** Serum albumin levels

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significantly decreased (P <0.05) in infected sheep and goats compared to control healthy animals. **Jaheed *et al.* (2020),** Stated that the decrease in serum albumin led to a reduction in the A/G ratio, which is consistent with the observations of **Qamar and Maqbool (2012)** as well as **Hosseini *et al.* (2012)**. Comparable results of a decrease in serum protein and albumin, coupled with an increase in serum globulin, were documented by **Bordoloi *et al.* (2012).** Mean values along with SE of serum albumin of different treatment groups and within the treatment group have been given in table 4.13 and fig. 4.12.

Table 4.13: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum Albumin in goats suffering from GI nematodosis. Normal range 2.7-3.9 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	3.14 ± 0.09^A	3.05 ± 0.08^A	3.18 ± 0.07^{Ba}	3.07 ± 0.04^A
Group II	3.13 ± 0.06^A	3.16 ± 0.06^A	3.62 ± 0.08^{Aa}	3.08 ± 0.06^A
Group III	3.02 ± 0.02^A	3.05 ± 0.07^A	3.62 ± 0.06^{Aa}	3.08 ± 0.02^A
Group IV	2.99 ± 0.03^A	3.15 ± 0.05^A	2.48 ± 0.06^{Cb}	3.34 ± 0.07^A

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
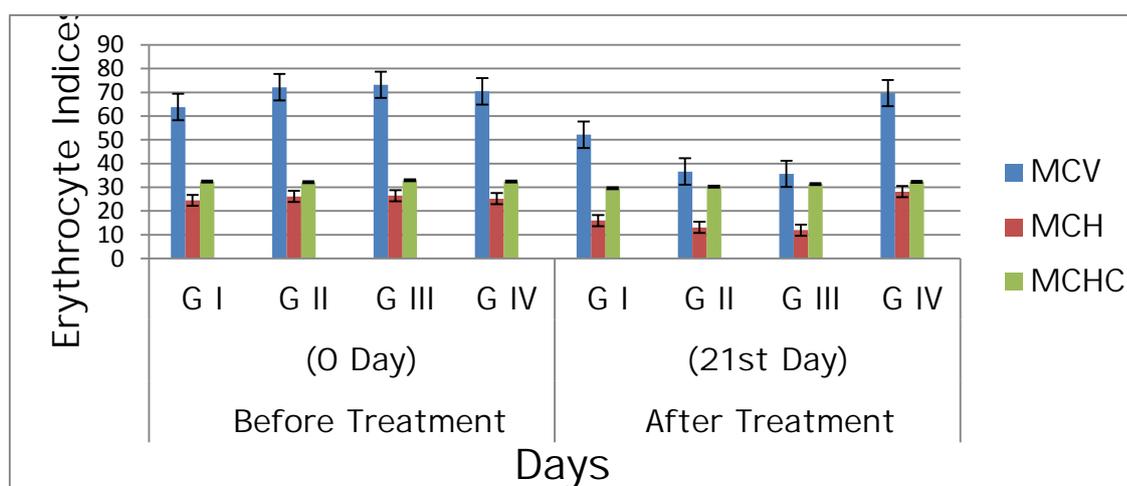

Fig. 4.12: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum Albumin (g/dL) in goats suffering from GI nematodosis.

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4.7.4 Serum Globulin

The globulin values decreased significantly in Group III and remained consistent in Group I & II without any significant variation. Group IV demonstrated fluctuating values, however overall they remained within normal range for goats.

Mamta (2012) decrease in serum globulin in bursate worm infested goats as compared to healthy goats. **Chakratborty and Lodh (1994)** reported increase in serum globulin which could be due to initial immune response to infection and it was also inferred by **Shastry and Ahluwalia (1972).** According to **Rasha *et al.* (2020)** serum globulin level significantly decreased (P <0.05) amongst sheep and goats infected with *H. contortus*, as opposed to the control. Contrary to this **Shashank *et al.* (2019)** reported, there was a significant rise (p < 0.05) in serum globulin levels among goats infected with gastrointestinal nematodes when compared to healthy control goats. An analogous elevation in globulin values was documented by **Diogenes *et al.* (2010)** and **Jas *et al.* (2010).** The parasitic infestation was observed to stimulate the host's immune system by augmenting the production of gamma globulins (**Tarazona *et al.,* 1982**). Table 4.14 and Figure 4.13 present the mean values and standard errors (SE) of globulin levels across various treatment groups, as well as within the treatment group.

Table 4.14: **Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum Globulin in goats afflicted with GI nematodosis. Normal range 2.7-4.1 (MSD Veterinary Manual 2023)**

Drug/Duration	0 day	7th day	14th day	21st day
Group I	3.26 ± 0.38^B	3.69 ± 0.2^A	3.58 ± 0.19^A	3.43 ± 0.12^{Bb}
Group II	4.06 ± 0.27^B	4.03 ± 0.21^A	4.11 ± 0.19^A	4.08 ± 0.09^{Aab}
Group III	5.6 ± 0.09^A	4.52 ± 0.07^A	3.84 ± 0.12^A	4.18 ± 0.07^{Aa}
Group IV	4.35 ± 0.22^B	4.38 ± 0.15^A	3.6 ± 0.06^A	4.48 ± 0.19^{Aa}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
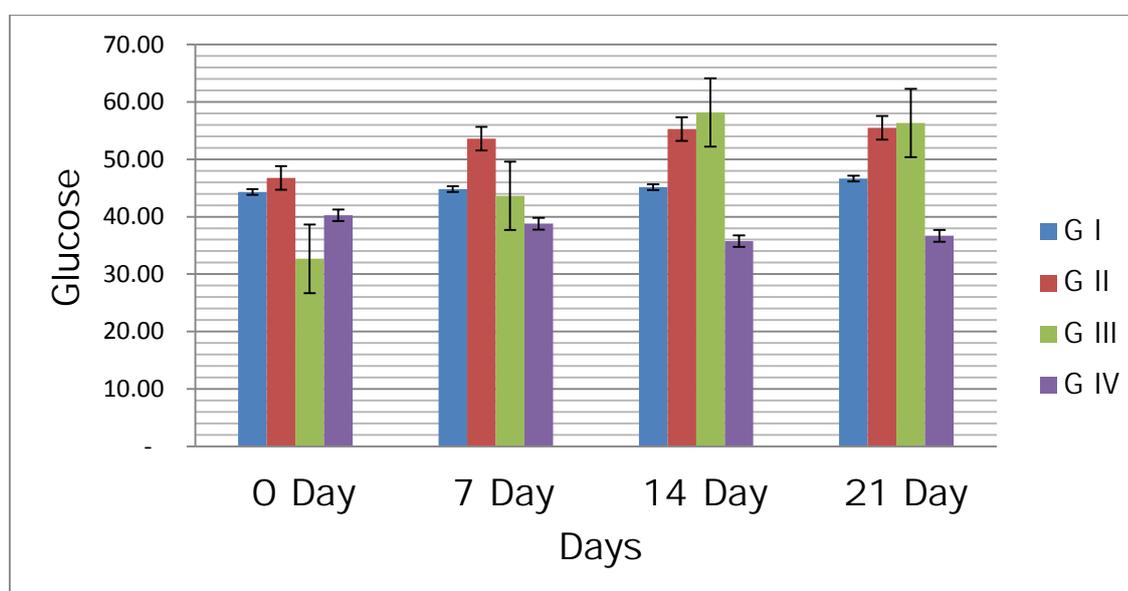
Fig. 4.13: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum Globulin (g/dL) in goats suffering from GI nematodosis

4.7.5 Albumin Globulin ratio (A/G ratio)

Group I showed a consistent decrease, Group II had minor fluctuations, Group III displayed significant changes with an initial increase and subsequent decrease, while Group IV maintained relative stability. Nonetheless, the A/G ratio values for goats remained within the normal range overall.

Castro *et al.* **(1977)** similarly noted an average of 1.1 gm/dl for gamma globulin and 1.2 for the albumin/globulin (A/G) ratio. In a study by **Qamar** *et al.* **(2012)** there was significant decrease in serum proteins and albumin globulin Ratio (A/G Ratio) at ($p < 0.05$) in *Haemonchus* infected animals. **Jaheed** *et al.* **(2020)** reported a decrease in serum albumin levels, leading to a reduction in the A/G ratio, which was consistent with the findings of **Qamar and Maqbool (2012), Hosseini** *et al.* **(2012).** Mean values along with SE of A/G ratio of different treatment groups and within the treatment group have been given in table 4.15 and fig. 4.14.

Table 4.15: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Albumin Globulin ratio in goats suffering from GI nematodosis. Normal range 0.47- 0.94 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	1.59 ± 0.23^A	0.93 ± 0.07^A	0.99 ± 0.07^A	0.94 ± 0.05^A
Group II	0.84 ± 0.05^B	0.83 ± 0.03^A	0.92 ± 0.03^A	0.77 ± 0.03^A
Group III	0.55 ± 0.01^C	0.68 ± 0.02^A	0.97 ± 0.04^A	0.75 ± 0.01^A
Group IV	0.73 ± 0.03^B	0.73 ± 0.02^A	0.69 ± 0.01^B	0.78 ± 0.03^A

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

Fig. 4.14: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Albumin Globulin ratio in goats suffering from GI nematodosis.

4.7.6 Alkaline phosphatase (ALP)

Group I, II and III showed significant decline in values of ALP, while Group IV showed an increase in ALP levels. However, the values of ALP were found within the standard range (93–387 IU /L) for goats which was in agreement with the findings of **Kaneko et al. (1997).** Thus the drugs used for trial did not have any significant effect on the hepatobiliary and/or bone function of the animals.

Chaurasiya et al. (2018), reported no significant variation on enzymatic activity of ALT while AST and ALP were higher (P<0.05) in oak leaves fed groups of

goats suffering from *Haemonchus contortus* infection. The level of ALT, AST and ALP also increased with the advancement of period, but the values were found within the standard range. He attributed this to the secretion of some toxins by *Haemonchus contortus* causing dysfunction of liver cells resulting in increased activities of AST, ALT and alkaline phosphatase. Contrary to these **Brito *et al.* (2020)** reported no significant increase in serum ALP amongst the groups being treated.

Mean values along with SE of ALP of different treatment groups and within the treatment group have been given in table 4.16 and fig. 4.15.

Table 4.16: **Effect of Fenbendazole, Ivermectin and polyherbal drug administration on ALP in goats suffering from GI nematodosis. Normal range 93-387 (MSD Veterinary Manual 2023)**

Drug/Duration	0 day	7th day	14th day	21st day
Group I	272.17 ± 7^B	229 ± 8.96^{Bb}	220.5 ± 8.44^{Bb}	101.33 ± 0.43^{Bb}
Group II	325.17 ± 19.82^A	231.17 ± 15.1^{Bb}	211.67 ± 12.91^{Bb}	85 ± 1.04^{Cb}
Group III	241.17 ± 9.94^B	285.83 ± 7.27^{Bab}	217.5 ± 13.51^{Bb}	87.83 ± 0.59^{Cb}
Group IV	352.67 ± 19.27^A	355.67 ± 16.38^{Aa}	383.83 ± 12.66^{Aa}	363 ± 15.43^{Aa}

Values (Mean $\pm$ SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
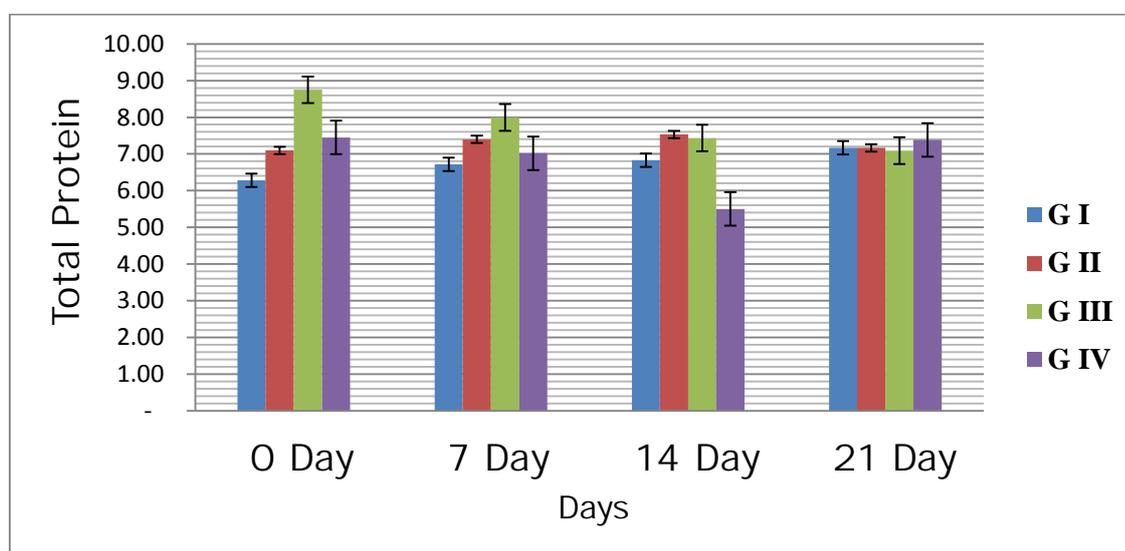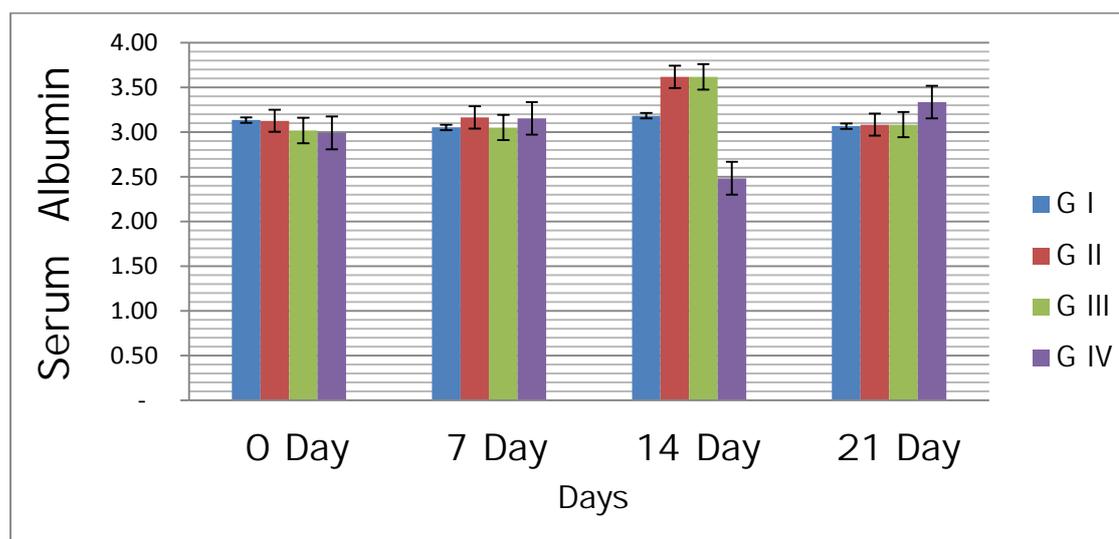

Fig. 4.15: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on ALP (U/L) ratio in goats suffering from GI nematodosis.

4.7.7 SGPT, Alanine amino transferase (ALT)

The values of ALT in Groups I, II and Group III reduced significantly on culmination of treatment on 21st day and were close to the normal range for a healthy goat whereas the values in Group IV showed a steady rise with most significant increase till 14th day. The elevation in alanine aminotransferase values in afflicted animals was a result of the parasites causing traumatic damage to the mucosa of the abomasum and intestine. The nematode larvae were observed to induce damage to the deep layers of the abomasal muscles (**Sharma *et al.,* 2001).** The findings of present study were in agreement with **Jaheed *et al. (*2020)** who also reported that AST and ALT were elevated in goats suffering from *Haemonchus contortus* infection initially, and started declining after 3rd week post treatment. Related results were reported by **Bordoloi *et al.* (2012), Sharma *et al.* (2001)** and **Rashid (2016)** in sheep and goat afflicted with *H. contortus.* The enhancement in serum enzyme values resulting from the polyherbal formulation could be ascribed to its inclusion of diverse phytoconstituents, total phenols, or flavonoids that mitigate lipid peroxidation and exhibit antioxidant activity, as documented by **Jaheed *et al.* (2020).**

Mamta (2012) reported significant increase in AST and ALT values in bursate worm infested goats in comparison to healthy control**.** The same was also reported by **Chakarborty and Lodh (1994)** in goats suffering from *H. contortus* infection. They attributed the increased level of ALT to the pathogenic impact of *H. contortus.*

Bandhaiya *et al.* (2019) reported Significant increases in the values of ALT and AST in *H. contortus*-infected goats, it was noted that the mean levels of ALT and AST were increased in groups with higher worm loads, and a highly significant (P < 0.01) difference was observed in the mean values of ALT and AST. The increase in ALT and AST levels suggested the presence of disruptive activities or changes in membrane permeability, **(Sharma *et al.*, 2001)**. Comparable results were also documented by **Bordoloi *et al.* (2012)** in cases of sheep infected with *H. contortus.*

Shashank *et al.* (2019) reported increase in Alanine Aminotransferase (ALT/SGPT) values in goats suffering from GI nematodosis, and the same were consistent with the outcomes reported b*y* **Sharma *et al.* (2001), Hassan *et al. (*2012), Ratnesh *et al.* (2013),** and **Moudgil *et al.* (2017).**

Mean values along with SE of ALT of different treatment groups and within the treatment group have been given in table 4.17 and fig. 4.16.

Table 4.17: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on ALT in goats suffering from GI nematodosis. Normal range 6-19 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	28.22 ± 1.7^A	29 ± 0.95^A	21.82 ± 0.63^{Bb}	21.82 ± 0.36^{Bb}
Group II	28.07 ± 0.45^A	29 ± 0.28^A	19.33 ± 0.23^{Cb}	22.03 ± 0.27^{Bb}
Group III	28.17 ± 0.87^A	27.5 ± 0.74^A	17 ± 0.56^{Cb}	21.85 ± 0.24^{Bb}
Group IV	25.33 ± 0.84^A	26.5 ± 0.75^A	34.25 ± 2.19^{Aa}	26.5 ± 0.79^{Aa}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences ($P < 0.05$).
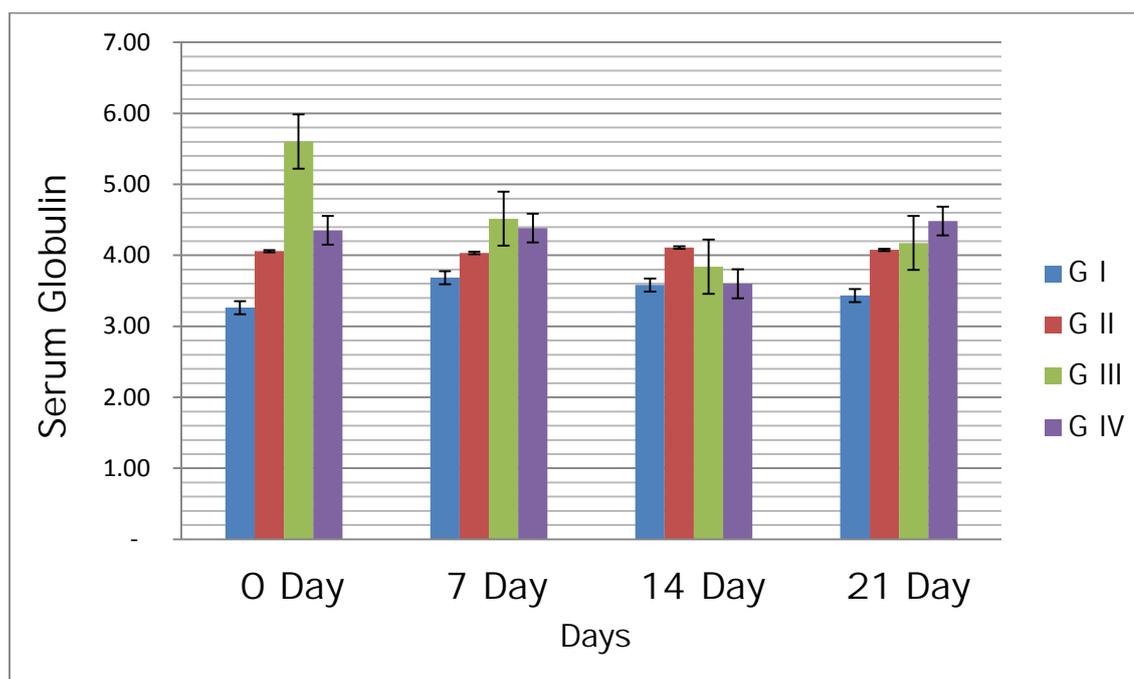

Fig. 4.16: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on SGPT (ALT) (U/L) in goats suffering from GI nematodosis.

4.7.8 SGOT/ Aspartate amino transferase (AST)

Significant reduction in SGOT (AST) levels was seen over the 21day period, with Group II and III demonstrating the most pronounced effect. However, Group IV had a less favourable impact, leading to increased SGOT levels. The results of the current study aligned with those of **Jaheed *et al.* (2020)** who also reported that AST and ALT were elevated in goats suffering from *Haemonchus contortus* infection initially, and started declining after 3rd week post treatment. Parallel results were reported by **Bordoloi *et al.* (2012), Rashid (2016)** and **Sharma *et al.* (2001),** in small ruminants afflicted with *H. contortus*.

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Bandhaiya *et al.* (2019) noted significant increases in the activity of ALT and AST in *H. contortus*-infected goats. It was observed that the mean levels of ALT and AST were elevated in groups with a high worm load, and this difference was highly significant (*P*<0.01). The increase in ALT and AST levels indicated the possibility of disruptive activities or alterations in membrane permeability, as noted by **Sharma *et al.* (2001).** Comparable results were documented in studies conducted by **Bordoloi *et al.* (2012)** concerning sheep afflicted with *H. contortus* infection.

Mean values along with SE of AST of different treatment groups and within the treatment group have been given in table 4.18 and fig. 4.17.

Table 4.18: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on AST in GI nematodosis afflicted goats. Normal range 167-513 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	135.67 ± 5.04[Bb]	117 ± 4.58[B]	111.5 ± 4.25[Bb]	77.33 ± 0.49[Bb]
Group II	189.67 ± 12.41[Aa]	143 ± 7.14[A]	160.67 ± 5.95[Aa]	71.33 ± 0.48[Cb]
Group III	114.33 ± 2.34[Cb]	115.5 ± 2.24[B]	145 ± 5.81[Aa]	69.33 ± 0.48[Cb]
Group IV	113.83 ± 1.67[Cb]	115.83 ± 1.77[B]	137.33 ± 2.34[Aab]	115 ± 2.81[Aa]

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
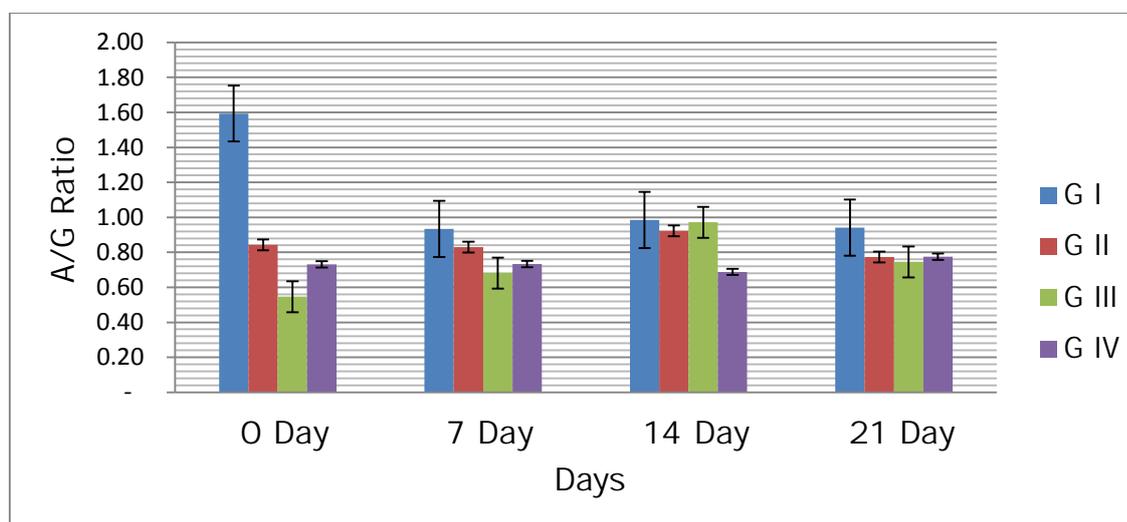

Fig. 4.17: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on SGOT (AST) (U/L) in GI nematodosis afflicted goats

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4.7.9 Urea

The values of urea in Groups I, II and Group III reduced significantly on 21st day post treatment and were close to the normal range for a healthy goat. Most pronounced reduction in urine values were seen in Groups III followed by Group II. However minor fluctuations were seen in Group IV showing initial reduction till 7th day followed by steady rise till 21st day post treatment. Similar to the present investigation, **Brito, *et al.* (2020)** reported that there were no statistically significant differences in the urea values among the goats infected with gastrointestinal nematodes and those treated with condensed tannins (P > 0.05). The values remained within the reference range for goats, except for all groups on day 1. Elevated blood urea levels served as an indicator of a reduction in the glomerular filtration rate. **Gwaze *et al.* (2010)** found that amongst *Trichostrongylus* infected goats in South Africa, 61.4% of goats had urea concentrations within the reference range, and 22.9% had creatinine concentrations within the reference range. Therefore, gastrointestinal nematode infection does indeed cause alterations in these biochemical variables in goats. Mean values along with SE of urea of different treatment groups and within the treatment group have been given in table 4.19 and fig. 4.18.

Table 4.19: **Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Urea in GI nematodosis afflicted goats. Normal range 10-20 (MSD Veterinary Manual 2023)**

Drug/Duration	0 day	7th day	14th day	21st day
Group I	38.73 ± 2.34^A	40.67 ± 2.07^A	35.5 ± 0.91^{Bb}	24.33 ± 0.25^{Bb}
Group II	40.83 ± 1.25^A	40.5 ± 1.03^A	31.33 ± 0.72^{Bb}	20.83 ± 0.46^{Cb}
Group III	57.53 ± 6.43^A	32.35 ± 0.61^A	32.83 ± 0.83^{Bb}	20.67 ± 0.42^{Cb}
Group IV	58.27 ± 5.17^A	46.33 ± 2.99^A	49.83 ± 2.59^{Aa}	51.17 ± 3.77^{Aa}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
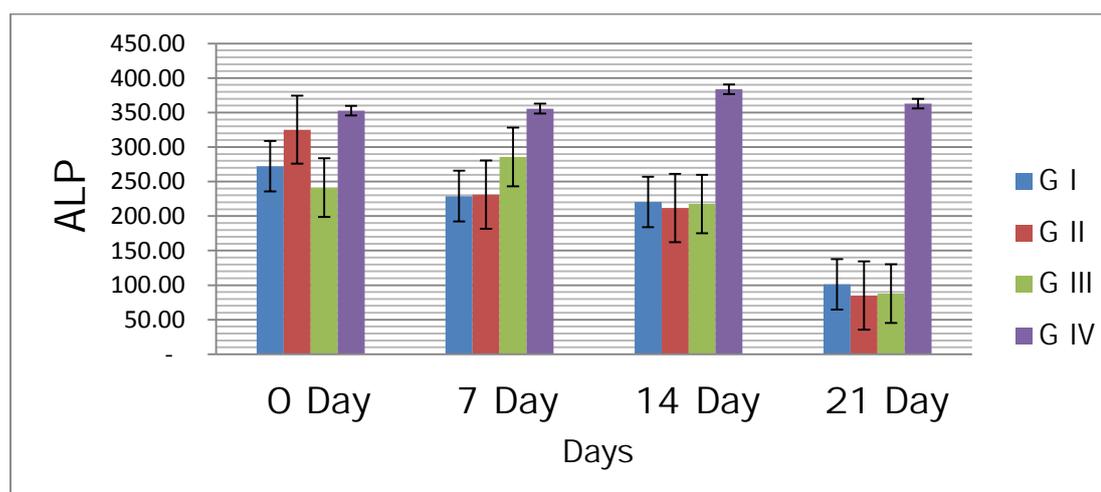
Fig. 4.18: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Urea (mg/dL) in GI nematodosis afflicted goats.

4.7.10 Uric Acid

A significant variation in uric acid levels within and between groups was seen. Uric acid, a metabolic end product of purine metabolism, plays a crucial role in renal and metabolic homeostasis. Perturbations in uric acid levels have been linked to various pathological conditions. In the present research time-dependent fluctuations in uric acid levels across the experimental groups was seen. At the 0-day baseline, all groups exhibited relatively comparable uric acid levels. However, by the 7th day, significant decline was observed in Group I and Group II, suggesting an early impact of fenbendazole and ivermectin administration. In contrast, Group III exhibited a slight increase in uric acid levels at the 14th day, which became more pronounced by the 21st day (p < 0.05). Group IV displayed a non-significant change in the level of uric acid values. The observed variations in uric acid concentrations suggest that the polyherbal formulation may impact purine metabolism, although the exact mechanisms underlying these effects require further investigation. Herbal compounds are known to possess bioactive molecules that can interact with cellular processes, potentially affecting enzyme activities and transport mechanisms related to uric acid production and excretion.

Mean values along with SE of Uric acid of different treatment groups and within the treatment group have been given in table 4.20 and fig. 4.19.

Table 4.20: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on uric acid in GI nematodosis afflicted goats. Normal range 0.13-0.33 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	0.33 ± 0.03^A	0.21 ± 0.01^B	0.23 ± 0.01^{Bb}	0.63 ± 0.03^{Cb}
Group II	0.45 ± 0.05^A	0.28 ± 0.02^B	0.24 ± 0.01^{Bb}	0.79 ± 0.01^{Bb}
Group III	0.31 ± 0.03^A	0.28 ± 0.03^B	0.42 ± 0.06^{Bab}	1.14 ± 0.07^{Aa}
Group IV	0.45 ± 0.04^A	0.42 ± 0.04^A	0.76 ± 0.1^{Aa}	0.52 ± 0.04^{Cb}

Values (Mean $\pm$ SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
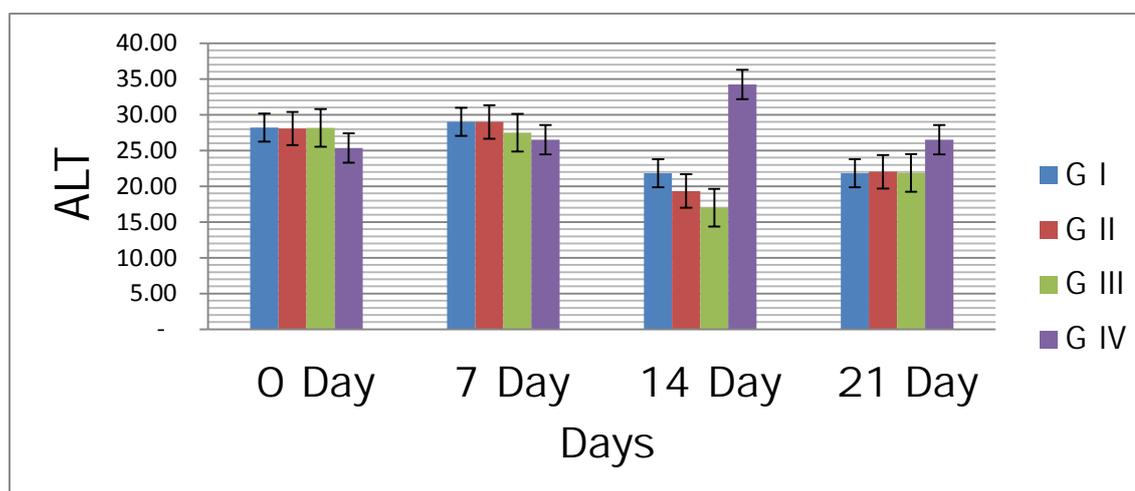

Fig. 4.19: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Uric acid (mg/dL) in GI nematodosis afflicted goats.

4.7.11 Serum creatinine

In this current investigation, Group II and III exhibited a significant increase in creatinine values at 14 days, while Group IV showed a substantial increase at 21 days. Group I also experienced alterations in creatinine levels, although the impact was less pronounced compared to other groups. However the serum creatinine values for all groups were within the standard range for goat species. Similar to these **Botura et al. (2011)** also reported that creatinine; values were all inside the normal range. Contrary to these **Biswas et al. (2017)** reported decrease in values of creatinine in animals treated

with herbal anthelmintics. Furthermore, he indicated that treatment with ivermectin, and herbal extracts were not toxic to the liver and kidney. Similar results were reported by **Gupta *et al.* (2005). Brito, *et al.* (2020)** observed that all groups exhibited creatinine concentrations below the reference value. (1.0–1.8 mg/dl), with no statistical difference among the groups ($P > 0.05$). Mean values along with SE of serum creatinine of different treatment groups and within the treatment group have been given in table 4.21 and fig. 4.20.

Table 4.21: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum creatinine in goats afflicted with GI nematodosis. Normal range; 1-1.8 (MSD Veterinary Manual 2023)

Drug/Duration	0 day	7th day	14th day	21st day
Group I	1.08 ± 0.08^{Aa}	0.9 ± 0.07^{A}	1.03 ± 0.04^{Aa}	1.37 ± 0.05^{A}
Group II	0.57 ± 0.04^{Bb}	0.71 ± 0.06^{A}	1.16 ± 0.04^{Aa}	1.25 ± 0.03^{A}
Group III	0.57 ± 0.01^{Bb}	0.63 ± 0.01^{A}	1.12 ± 0.03^{Aa}	1.27 ± 0.03^{A}
Group IV	0.46 ± 0.04^{Bb}	0.75 ± 0.02^{A}	0.68 ± 0.02^{Bb}	1.47 ± 0.05^{A}

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences ($P < 0.05$).
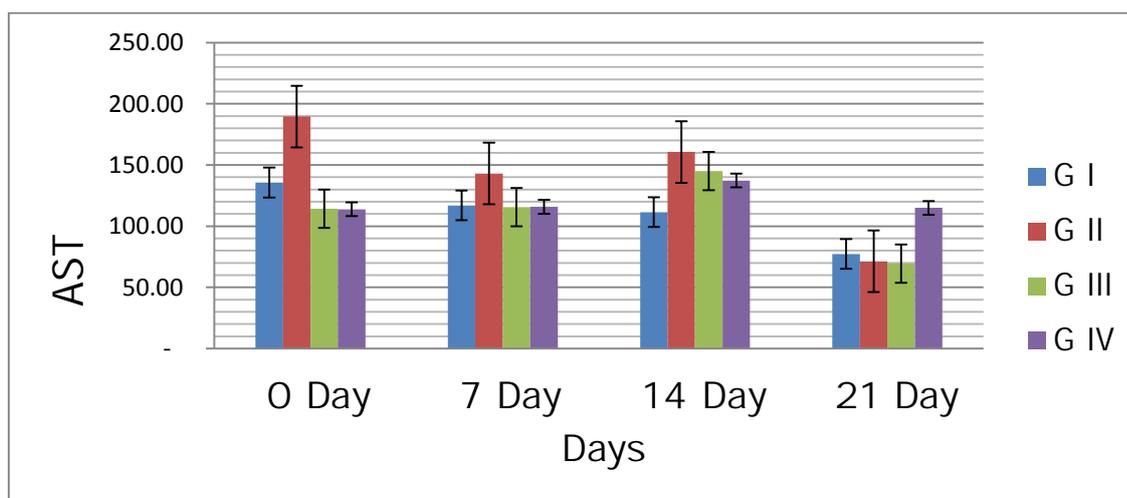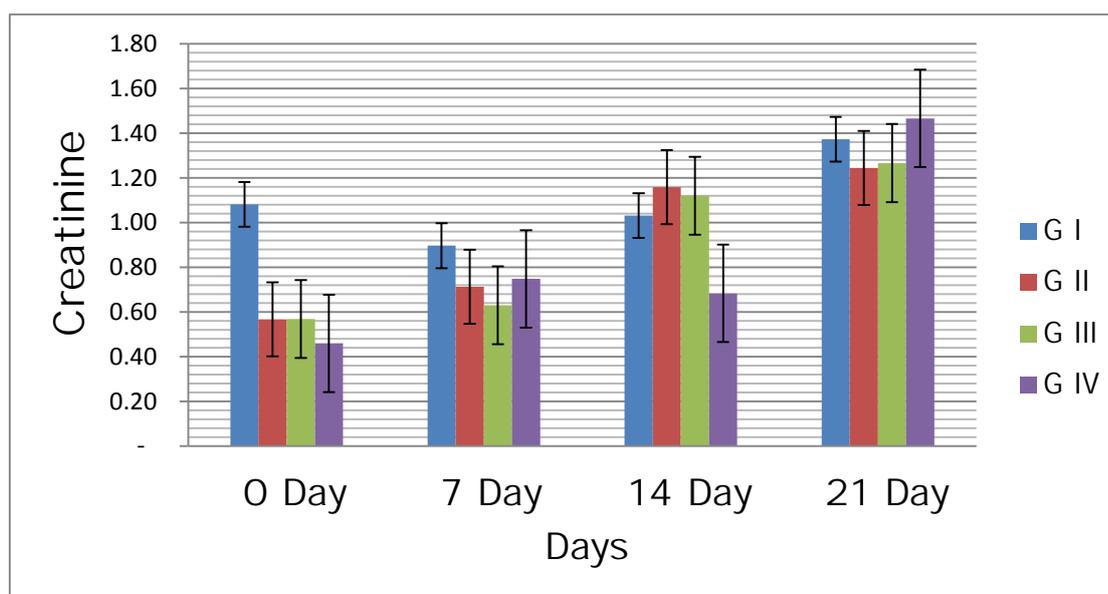

Fig. 4.20: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum creatinine (mg/dL) in goats afflicted with GI nematodosis.

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4.7.12 Serum electrolytes Potassium (K+), Sodium (Na+) and chloride (Cl⁻)

Electrolytes are crucial for cellular metabolism, muscle contraction, nerve transmission, and enzymatic reactions **(Piccione *et al.* 2007).**

Electrolytes play a crucial role in various essential metabolic functions within the human body. Among these vital ions, sodium, potassium, and chloride stand out as particularly important and are commonly measured electrolytes. These ions are primarily obtained from the diet, absorbed in the GI tract, and eliminated through the kidneys. Understanding and monitoring electrolyte levels, especially sodium, potassium, and chloride, are crucial for maintaining proper physiological function and overall health**.**

According to **Kaneko *et al.* (1997)** and **Dakkak *et al.* (1981),** Chloride (Cl⁻) is a significant extracellular anion that plays a key role in regulating the balance of extracellular fluid distribution. Disturbances in chloride levels can occur due to multiple factors. Reduced chloride levels may result from insufficient dietary intake, prolonged episodes of vomiting, decreased renal reabsorption, or certain forms of acidosis and alkalosis. Conversely increased chloride values can be observed in conditions such as dehydration, kidney failure, specific types of acidosis, excessive dietary or parenteral chloride intake, and salicylate poisoning.

Sodium is the dominant cation in extracellular fluid which plays a pivotal role in maintaining water balance and osmotic pressure within fluid compartments. A decrease in serum sodium levels, known as hyponatremia, can be linked to various conditions, including severe polyuria, metabolic acidosis, Addison's disease, diarrhoea, and renal tubular disease. Conversely, an increase in serum sodium levels, termed hypernatremia, can be associated with Cushing's syndrome, severe dehydration from primary water loss, certain brain injuries, diabetic coma post-insulin therapy, and excessive sodium salt treatment.

Potassium, the primary intracellular cation, is essential for maintaining cellular function. Hypokalemia, characterized by low serum potassium levels, can result from potassium deficiency, excessive potassium loss due to prolonged diarrhoea or vomiting, and increased secretion of mineralocorticosteroids. Conversely, hyperkalemia, denoting

elevated serum potassium levels, can lead to issues like oliguria, anuria, and urinary obstruction.

In all the groups there was non-significant decrease in sodium and chloride levels after 21 days of treatment compared to their initial values at 0 day while values of potassium had a reverse course, though all the values of Serum electrolytes in goats suffering from GI nematodosis were found in the normal range.

Therefore, it can be inferred that the medications, including the herbal anthelmintics, fenbendazole, and ivermectin did not directly impact the electrolyte balance of the goats. These medications primarily target parasites and may not have affected the electrolyte levels. Also when medications are used within recommended doses, the impact on electrolyte balance may be minimal and medications might not have caused significant gastrointestinal disturbances, such as diarrhoea, which can lead to electrolyte imbalance.

Mean values along with SE of Serum electrolytes of different treatment groups and within the treatment group have been given in table 4.22 and fig. 4.21.

Table 4.22: **Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Serum electrolytes in goats afflicted with GI nematodosis. Normal ranges are; Sodium (142-155), Potassium (3.5-6.7) and Chloride (99-110.3) (MSD Veterinary Manual 2023).**

Serum electrolytes	Before Treatment (0 Day)				After Treatment (21st Day)			
	G I	**G II**	**G III**	**G IV**	**G I**	**G II**	**G III**	**G IV**
Sodium	148.83 ± 2.21^A	153.67 ± 4.27^A	151.33 ± 2.4^A	144 ± 2.17^A	133.83 ± 0.58^{Bb}	144.67 ± 1.27^{Aa}	150.33 ± 0.84^{Aa}	131.33 ± 1.06^{Bb}
Potassium	4.12 ± 0.04^A	4.01 ± 0.01^A	4.2 ± 0.05^A	4.15 ± 0.07^A	4.62 ± 0.04^A	4.74 ± 0.04^A	4.48 ± 0.05^A	4.23 ± 0.08^B
Chloride	106.17 ± 0.19^A	106 ± 0.26^A	103.83 ± 1.29^A	104 ± 1.71^A	98.33 ± 1.12^B	105.67 ± 0.34^A	96.33 ± 0.8^B	95.67 ± 1.62^B

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
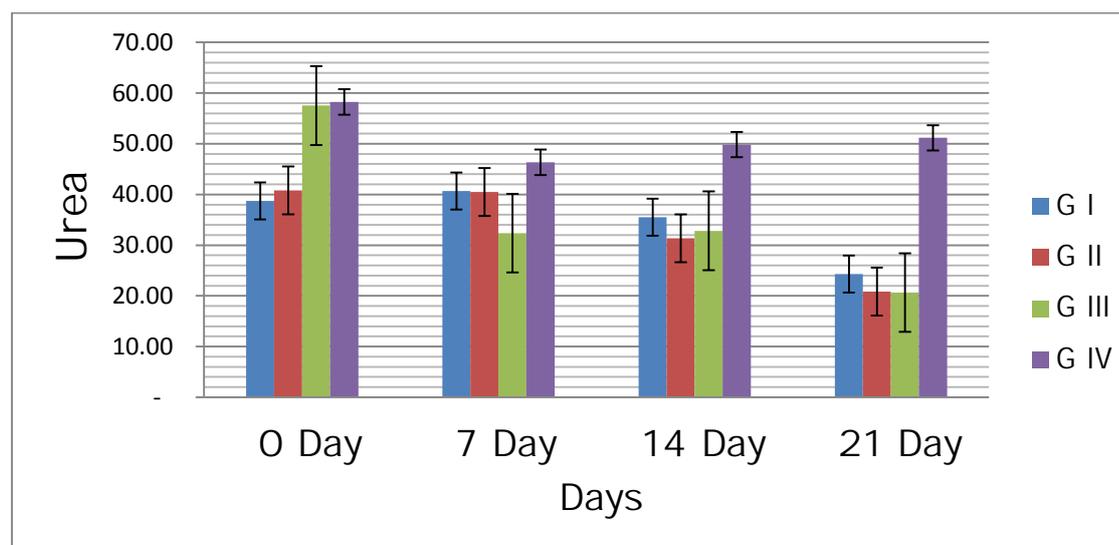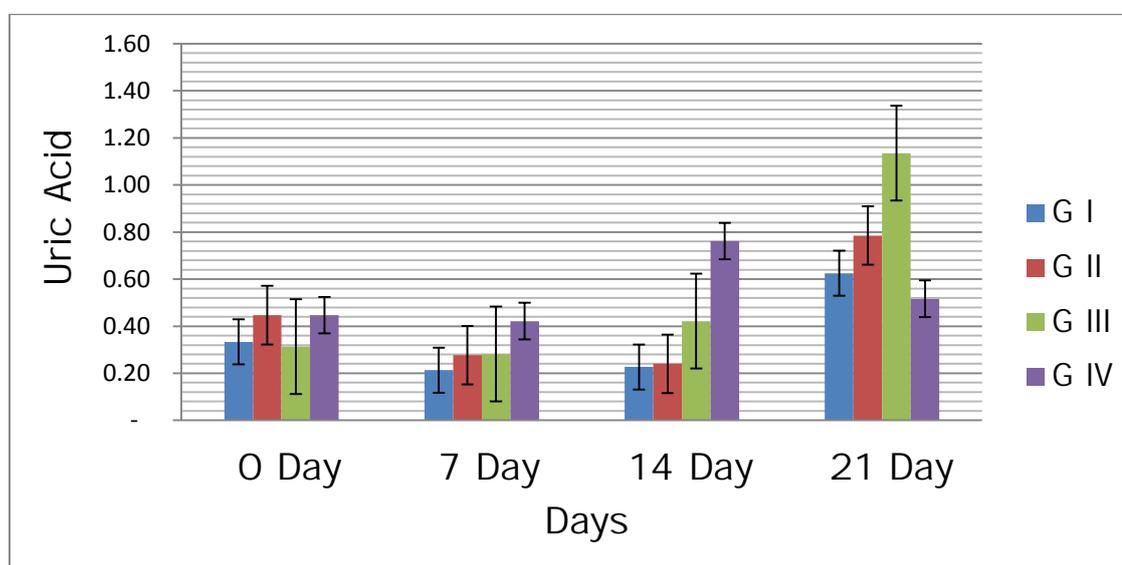
Fig. 4.21: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on serum electrolytes (Unit mmol/L) in goats afflicted with GI nematodosis.

4.8   EFFECTS ON OXIDATIVE STRESS RELATED PARAMETERS

Antioxidants play a crucial role in delaying or inhibiting the oxidation of substances. The balance between the free radicals accumulation and their neutralization by antioxidants is a natural process in the body. However, environmental stressors can diminish the antioxidant capacity of the blood, resulting in oxidative stress. To counteract cellular oxidants and prevent their accumulation, the body relies on antioxidant enzymes (**Kumar *et al.*, 2011**).

Cells continuously generate minute quantity of free radicals during their normal metabolism (**Simioni *et al.*, 2018**). Although, low levels of free radicals are essential in many biochemical processes and involved in homeostasis but excessive production of reactive oxygen species (ROS) like free radical results in oxidative stress. The crucial reactive oxygen species (ROS) comprise hydroxyl radicals, superoxide anions, and hydrogen peroxide, which are generated as by-products of oxidative metabolism. Additional sources of ROS include NADPH oxidase and xanthine oxidase (**Mahmood *et al.*, 2020**). Highly reactive superoxide anion is the mediator in oxidative chain reaction and precursor of most ROS (**Belhadj Slimen *et al.*, 2016**). According to

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Verma *et al.* (2020) the superoxide anion is produced primarily in mitochondrial electron transport chain in complex I and III**.** ROS are scavenged by antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) **(Celi, 2011; Kurutas, 2016 and Simioni *et al.*, 2018).** CAT (catalase) functions to detoxify hydrogen peroxide (H_2O_2) generated during various metabolic processes, as well as under stressful conditions, by catalyzing its reduction to water (H_2O) and oxygen (O_2), **(Kalmath and Swamy, 2020).** SOD (superoxide dismutase) catalyses the dismutation of superoxide radicals into oxygen and hydrogen peroxide. **(Khan *et al.*, 2020).**

An increased generation of ROS and free radicals, along with decline in body's antioxidant defence mechanisms results in oxidative stress (**Trevisan *et al.,* 2001; Williams *et al.,* 2004**). Various external factors like exposure to oxygen, resuscitation, heat, trauma, ultrasound, infections, radiation, and toxins, can trigger an upsurge in free radicals and ROS, eventually leading to oxidative stress (**Halliwell *et al.,* 1992** and **Kumar *et al.,* 2011**).

Infections caused by helminths can also contribute to ROS generation and heightened oxidative stress. The cellular antioxidant defence system functions under both normal and pathological conditions, safeguarding against the deleterious effects of ROS and maintaining cellular homeostasis (**Urban C. *et al.,* 2009**). Key components of this defence system include endogenously produced enzymatic antioxidants like SOD, GSH-Px & CAT. Assessing the concentrations of GSH-Px, SOD, and CAT in the bloodstream offers valuable insights into the antioxidant status of the body. (**Bisla *et al.,* 2003**). Excessive production of pro-oxidants due to oxidative stresses causes an imbalance of antioxidant defence mechanism (**Giorgio *et al.,* 2020**) and damages biological molecules (**Juan *et al.,* 2021**). Oxidative stress causes multiple diseases in ruminants (**Ayemele *et al.*, 2021**, **Huang *et al.,* 2021**).

Administering anthelmintics at the start of treatment could contribute to the chemical stress encountered by the animals (**Dimitrijevíc *et al.,* 2012).**

In the current investigation there was significant increase in Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and catalase and decrease in MDA

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in goats after treatment with Polyherbal formulation and Ivermectin. It is in agreement with findings of **Adenkola et al. (2018)**, and **Jaheed et al. (2020)** who stated that decrease in oxidative stress related parameters during infection was associated with stress caused by the disease. Also in the current investigation it has been revealed that level of GSH-Px, SOD and CAT are low during stress caused by GI nematodosis whereas MDA levels were high. The same are in agreement with findings of **Hu et al. (2016)** who reported that activities of superoxide dismutase (SOD) along with glutathione peroxidase (GSH-Px) decreased in serum; however, the concentration of malonaldehyde (MDA) increased during stress. **Alborzi1 et al. (2023)**, reported that low levels of SOD and CAT activity in infected animals might be linked to oxidative stress which caused a marked increase in lipid peroxidation and MDA levels.

Haematological values of GSH, SOD and CAT are the representative of antioxidant status of body (**Bisla et al., 2003**). The excessive accumulation of ROS enhances the function of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase while inhibiting antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. The addition of the polyherbal formulation improved the total antioxidant capacity (TAC) by lowering the total oxidant status (TOS) and malondialdehyde (MDA) levels in comparison to the control group. The activities of SOD, GSH-Px and CAT activity were enhanced in supplemented groups. **Váradyová et al. (2018)** indicated that herbal nutraceuticals have the potential to offer natural antioxidants, thereby aiding in the reduction of oxidative stress in lambs infected with nematodes.

4.8.1 Glutathione peroxidase (GSH-Px)

Groups I, II and III exhibited a significant increase), in GSH-Px levels after 21 days of treatment compared to their respective baseline levels at 0 day. However, Group IV (control group) did not reveal significant change in GSH-Px levels over the same treatment period.

Esmaeilnejad et al. (2012) reported that with increase in severity of infection in goats afflicted with GI nemtodosis, a significant decrease ($p<0.05$) was seen in the activity of GSH-Px, which is in accordance with the results of **Deger et al. (2009)** and

Bicek *et al.* (2005). The same has been corroborated in this study. **Jaheed *et al* (2020)** and **Rasha *et al.* (2020),** reported that the serum biochemistry of infected animals demonstrated a notable decrease in total antioxidant capacity. Glutathione peroxidase values exhibited a significant decrease in goats affected by GI nematodosis, while malondialdehyde followed an opposing trend. Mean values along with SE of Glutathione peroxidase of different treatment groups and within the treatment group have been given in table 4.23 and fig. 4.22.

Table 4.23: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Glutathione peroxidase in serum of goats afflicted with GI nematodosis.

Drug/Duration	0 day	7th day	14th day	21st day
Group I	42.83 ± 0.79[A]	40.42 ± 0.86[A]	47.17 ± 1.09[Bb]	115.67 ± 1.32[Cc]
Group II	42.83 ± 1.24[A]	42.42 ± 1[A]	63.5 ± 0.79[Aa]	138.5 ± 0.43[Bb]
Group III	42.33 ± 0.96[A]	40.58 ± 1.07[A]	63.5 ± 0.6[Aa]	145.5 ± 0.77[Aa]
Group IV	40.25 ± 0.96[A]	40.58 ± 1.15[A]	42 ± 1.2[Bb]	44.33 ± 0.66[Dd]

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).
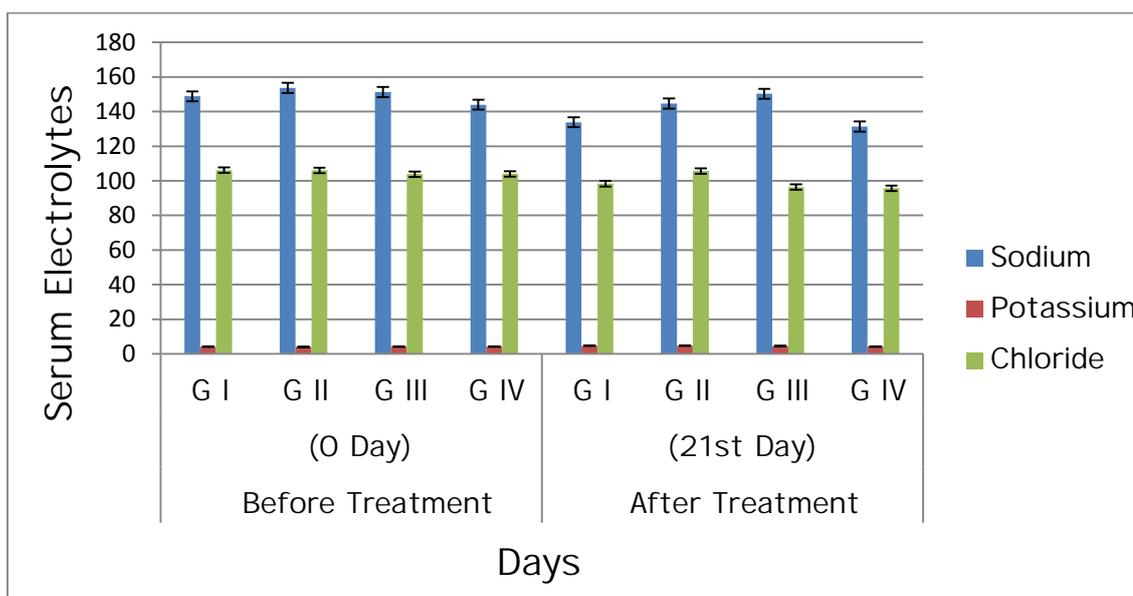

Fig. 4.22: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Glutathione peroxidase (u/ml) in serum of goats afflicted with GI nematodosis.

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4.8.2 Superoxide dismutase (SOD)

SOD which serves as main line of defence against ROS, plays a critical role in detoxifying superoxide radicals (**Gonzales** *et al.,* **1984** and **Alborzi1** *et al.***, 2023).** SOD is assumed as the first defence against pro-oxidants for its capacity to regulate peroxidation (**Halliwell** and **Chirico, 1993).** A noteworthy increase ($p < 0.05$) in serum SOD levels was noted in goats treated with the polyherbal formulation, Ivermectin, and Fenbendazole on the 21st day following treatment. On the 21st day post-treatment, the control group exhibited an insignificant alteration in SOD values when compared to the values observed on the initial day (0 day). Group III showed the most prominent effect on SOD values from 0 days to 21st day, showing a consistent increase over time probably because of the synergistic activity of the phytochemicals of five extracts used in preparation of polyherbal formulation rich in flavonoids which possess antioxidant properties that help mitigate oxidative stress-related diseases (**Spiegler** *et al.***, 2017; David** *et al.,* **2016; Barrau** *et al.,* **2005**). These flavonoids synergistically augment the activity of condensed tannins, as supported by research (**Klongsiriwet** *et al.,* **2015**). Efficacy of polyherbal formulation as seen in the current investigation is covenant with findings of **Sunandhadevi** *et al.* **(2017), Khanolkar** *et al.* **(2018) and Rahal** *et al.* **(2022).**

Mean values along with SE of Superoxide dismutase of different treatment groups and within the treatment group have been given in table 4.24 and fig. 4.23.

Table 4.24: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Superoxide dismutase in serum of goats afflicted with GI nematodosis.

Drug/Duration	0 day	7th day	14th day	21st day
Group I	33 ± 0.35^A	32.9 ± 0.4^A	34.67 ± 0.2^{Bb}	84.67 ± 0.2^{Bb}
Group II	33.5 ± 0.35^A	33.33 ± 0.42^A	41.33 ± 0.89^{Aa}	94.33 ± 0.55^{Aa}
Group III	32.67 ± 0.42^A	30.67 ± 0.43^B	43.67 ± 1.09^{Aa}	95.67 ± 0.4^{Aa}
Group IV	32.83 ± 0.29^A	33.25 ± 0.43^A	32.33 ± 0.39^{Bb}	34.17 ± 0.4^{Cc}

Values (Mean $\pm$ SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences ($P < 0.05$).

Fig. 4.23: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on SOD (u/ml) in serum of goats afflicted with GI nematodosis.

4.8.3 Catalase (CAT)

Groups I, II and III exhibited a significant increase), in CAT levels after 21 days of treatment compared to their respective baseline levels at 0 day. However, Group IV (control group) did not show any significant change in CAT levels over the same treatment period. The findings of present study are in agreement with **Bicek _et al._ (2005), Rasha _et al._ (2020), Deger _et al._ (2009)** and **Jaheed _et al_ (2020). Esmaeilnejad _et al._ (2012)** also reported that catalase activity in the infected goats was inversely proportional to the gravity of parasitemia.

Mean values along with SE of Catalase of different treatment groups and within the treatment group have been given in table 4.25 and fig. 4.24.

Table 4.25: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Catalase in serum of goats afflicted with GI nematodosis.

Drug/Duration	0 day	7th day	14th day	21st day
Group I	23.67 ± 0.6[A]	23.33 ± 0.46[B]	25.83 ± 0.27[Bb]	56.67 ± 0.29[Bb]
Group II	26.5 ± 0.66[A]	23.83 ± 0.56[B]	32 ± 0.62[Aa]	69.83 ± 1.1[Aa]
Group III	24 ± 0.6[A]	22.25 ± 0.34[B]	34.33 ± 0.66[Aa]	74.67 ± 0.82[Aa]
Group IV	26.1 ± 0.63[A]	26.5 ± 0.57[A]	23.92 ± 0.64[Bb]	30.17 ± 0.55[Cc]

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences ($P < 0.05$).

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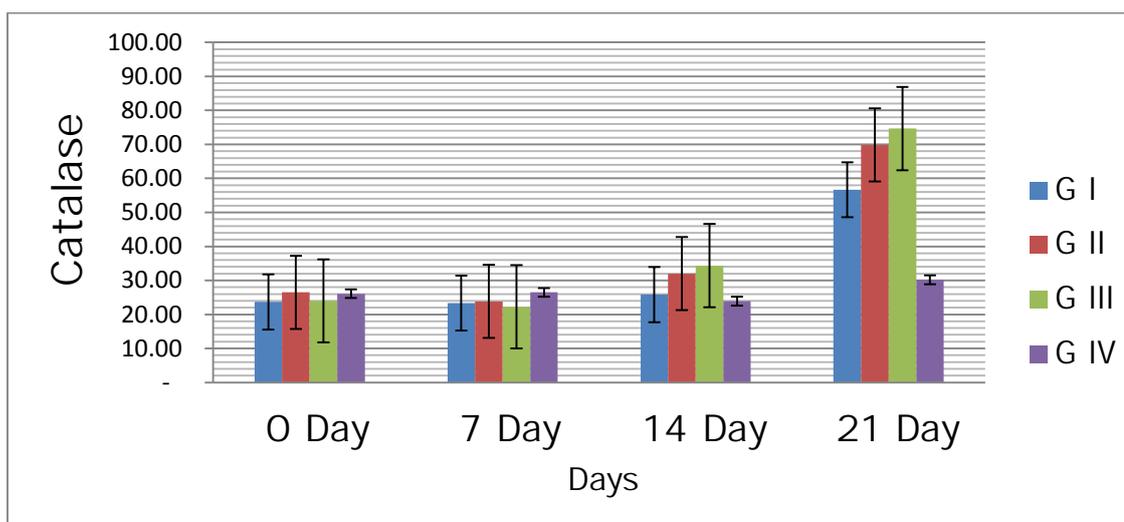
Fig. 4.24: Effect of Fenbendazole, Ivermectin and polyherbal drug administration on Catalase (u/ml) in serum of goats afflicted with GI nematodosis.

4.8.4 Malondialdehyde (MDA)

An important indicator of cellular and tissue oxidative stress is lipid peroxidation, where lipid peroxides derived out of polyunsaturated fatty acids (PUFA) break down to form compounds like malondialdehyde (MDA). The quantization of MDA is a widely used method to assess lipid peroxidation levels **(Simsek *et al.*, 2006).** MDA serves as an indicator of escalated oxidative stress, as it reflects the end result of lipid peroxidation in body tissues or biological fluids (**Halliwell and Chirico, 1993).**

In the present study, a significant effect ($p < 0.05$) on the overall MDA values was observed in all four treatment groups. Group III exhibited the most prominent effect on reducing MDA levels, whereas the control group IV, showed increase in the values of MDA post the treatment period of 21days. The results align with the study conducted by **Hu *et al.* (2016)**.

Charinya and Sunthamala (2023) also found that MDA values increased in the blood of stressed goats. Oxidative damage increases manifold due to Stress resulting in MDA and free radical generation, leading to oxidative stress. In contrast to the control group, **Rasha *et al.* (2020)** found a significant reduction in serum SOD, CAT, and GSH-Px levels during severe infection**.** On the other hand, a noteworthy elevation in serum lipid peroxidation (MDA) levels was observed in infected sheep and goats. This finding aligns with previous studies by **Dede *et al.* (2000)** who found an

increase in the level of MDA in Akkaraman sheep afflicted with *Fasciola spp.,*
Trichostrongylidae, and *Eimeria spp.*, while **Simsek *et al.* (2006)** and **Dimitrijevi´c *et*
al. (2012)** discovered elevated MDA levels in sheep infested with *Dicrocoelium*
dendriticum and *Strongyloides papillosus*, respectively.

   Jaheed *et al.* (2020) documented that infected animals exhibited a notable
reduction in total antioxidant capacity as observed in their serum biochemistry and values
of malondialdehyde increased significantly in goats afflicted with GI nematodosis.

   Mean values along with SE of malondialdehyde of different treatment groups
and within the treatment group have been given in table 4.26 and fig. 4.25.

**Table 4.26: Effect of Fenbendazole, Ivermectin and polyherbal drug
administration on MDA in serum of goats afflicted with GI
nematodosis.**

Drug/Duration	0 day	7th day	14th day	21st day
Group I	11.25 ± 0.36^A	11.58 ± 0.33^A	10.83 ± 0.34^{Bab}	8 ± 0.39^{Bb}
Group II	11.1 ± 0.3^A	11.1 ± 0.28^A	9.9 ± 0.22^{Bb}	4.8 ± 0.08^{Cc}
Group III	11.25 ± 0.39^A	11.73 ± 0.44^A	9.75 ± 0.22^{Bb}	3.97 ± 0.2^{Cc}
Group IV	10.48 ± 0.3^A	10.83 ± 0.26^A	12.83 ± 0.39^{Aa}	12.5 ± 0.15^{Aa}

Values (Mean $\pm$ SE) that share a common superscript (capital letters in columns and small letters in
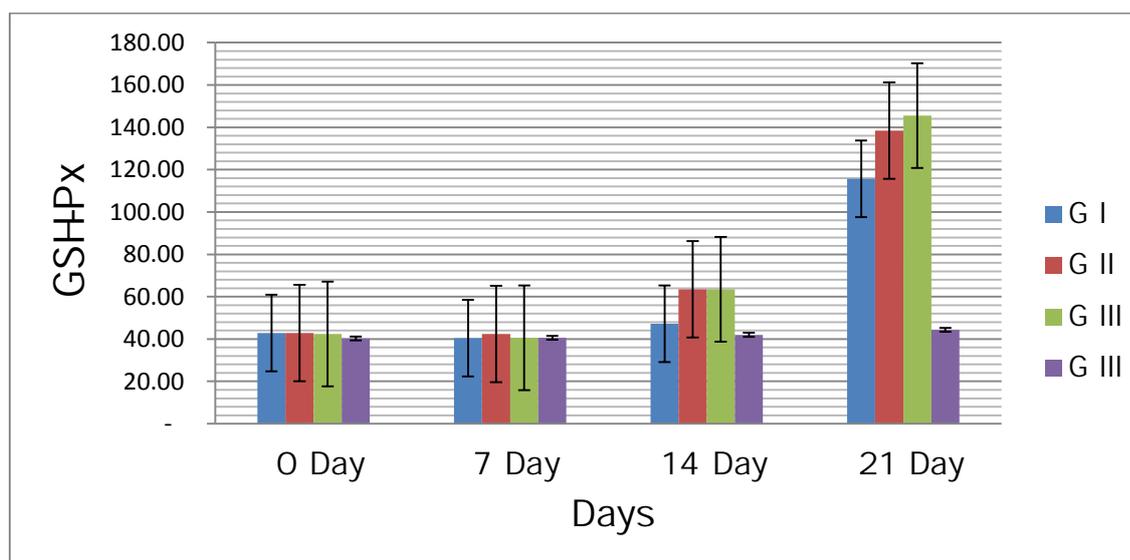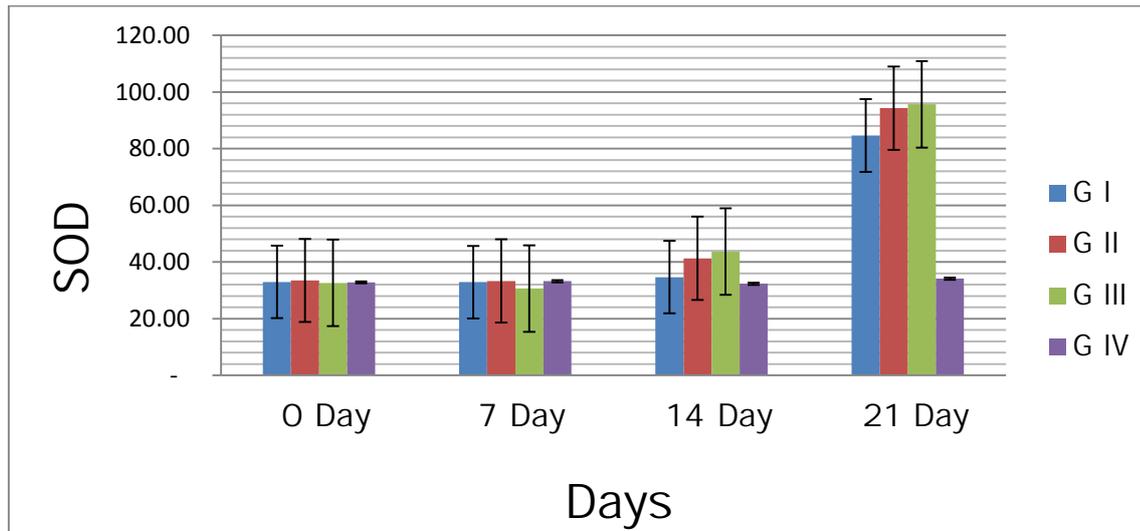rows) indicate no significant differences ($P < 0.05$).
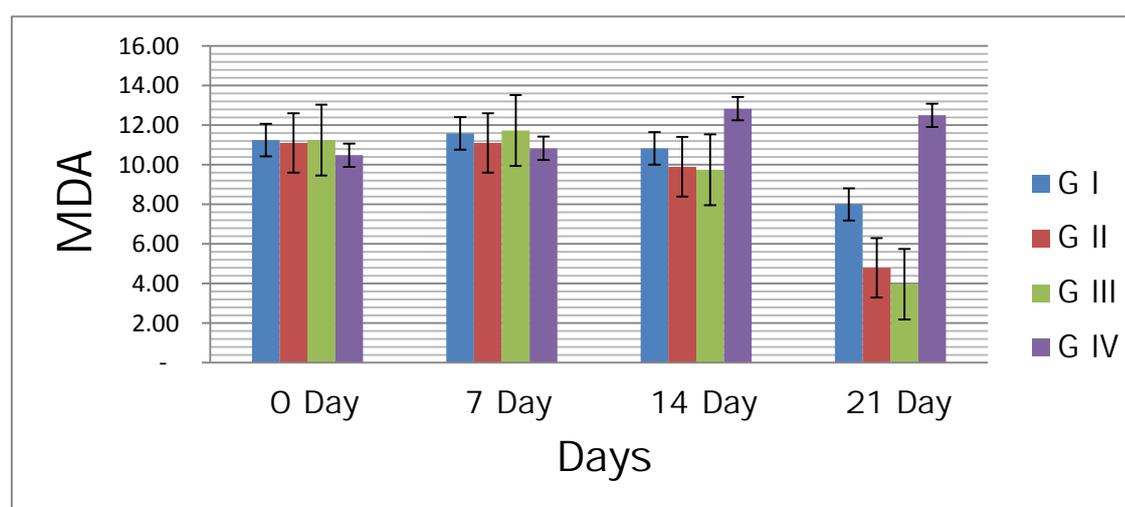
**Fig. 4.25: Effect of Fenbendazole, Ivermectin and polyherbal drug administration
on MDA (nmol/ml) in serum of goats afflicted with GI nematodosis.**

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4.9 THERAPEUTIC AND ANTHELMINTIC RESISTANCE STUDY

Based on history of regular deworming by the farmers and high EPG seen during faecal examination, anthelmintic resistance (AR) to commonly used anthelmintics was suspected in the goats selected for the research work. Numerous parasites with veterinary significance possess genetic characteristics that facilitate the emergence of anthelmintic resistance (AR). Global reports have indicated the emergence of resistance among various nematode species against all major classes of anthelmintic drugs (**Wanyangu, 1996, Kaplan, 2004, Papadopoulos, 2008, Makvana and Singh, 2009, Pena *et al.,* 2014, Adediran *et al.*, 2015**). The issue of anthelmintic resistance has escalated into a significant concern within the field of veterinary medicine, posing a dual threat to agricultural productivity and the well-being of animals.

Sharma *et al.* (2015) reported that, ivermectin treated group revealed 100% decline in faecal egg count. Nevertheless, in the fenbendazole-treated group, a reduction percentage of 57.44% and 70.87% on day 7 and 14 post treatment respectively in faecal egg count were observed, suggesting the low efficacy of fenbendazole against strongyle worms. **Khajuria (2010), Bogan *et al.* (1987), Yadav (1990), Hong *et al.* (1996), Singh *et al.* (2002), Waruiru *et al.* (2003), Sissay *et al.* (2006), Saddiqui *et al.* (2006), and Rialch *et al.* (2013)** have collectively documented heightened resistance of *H. contortus* to benzimidazole groups in comparison to other gastrointestinal nematodes. The development of fenbendazole resistance in goats could potentially be attributed to its prolonged and widespread usage against nematodes over many years (**Meenakshisundaram *et al.*, 2014; Varadharajan and Vijayalakshmi, 2015**).

4.9.1 Egg per gram (EPG)

The mean EPG of goats suffering from GI nematodosis were compared with the control group IV. The mean EPG values on 0 day before treatment in Group I (2016.67 ± 32.77), Group II (2191.67 ± 62.23), Group III (2133.33 ± 52.88) and Group IV (1941.67 ± 65.28) showed no significant variation and difference amongst them was less than EPG of 250. However on 21st day after treatment mean EPG values in Group I (816.67 ± 30.12), Group II (308.33 ± 6.27), Group III (200 ± 9.13)

and Group IV (1941.67 ± 23.81) showed significant variation (P < 0.05) and difference between the groups with highest and lowest EPG was more than EPG of 1740. Significant improvement in the mean EPG in both treatment groups G II (675 ± 13.69) and G III (641.67 ± 12.27) were observed on 14th day post treatment compared to G I and G IV.

A significant difference (P<0.01) in EPG values between the 0 day and the 7th day after administering of polyherbal formulation (PHF) was evident in Group III. This result suggests that the PHF exhibited its effectiveness from the 7th day itself. The EPG values decreased significantly from 7th day to 14th day, and even until the 21st day, indicating that the PHF had efficient anthelmintic activity. These results align with the findings of **Zacharias *et al.* (2008)** and **Biswas *et al.*, (2017).**

On the 14th day after treatment, the EPG counts in group III showed a notable and statistically significant reduction (641.67 ± 12.27) compared to group I (Fenbendazole), where the count remained higher (1550 ± 25.28). The findings regarding the polyherbal formulation are consistent with previous studies by **Khan and Kishor (2014), Garg *et al.* (2004), and Kumar *et al.* (2008).**

Mean values along with SE of EPG of different treatment groups and within the treatment group have been given in table no. 4.27 and fig. 4.26.

Table 4.27: Effect of Fenbendazole, Ivermectin and polyherbal formulation on EPG in goats afflicted with GI nematodosis

Drug/ Duration	0 day	7th day	14th day	21st day
G I	2016.67 ± 32.77[A]	1783.33 ± 30.12[A]	1550 ± 25.28[Bb]	816.67 ± 30.12[Bb]
G II	2191.67 ± 62.23[A]	1741.67 ± 46.07[A]	675 ± 13.69[Cc]	308.33 ± 6.27[Cc]
G III	2133.33 ± 52.88[A]	1725 ± 28.26[A]	641.67 ± 12.27[Cc]	200 ± 9.13[Dc]
G IV	1941.67 ± 65.28[A]	1958.33 ± 68.6[A]	2050 ± 60.09[Aa]	1941.67 ± 23.81[Aa]

Values (Mean ± SE) that share a common superscript (capital letters in columns and small letters in rows) indicate no significant differences (P < 0.05).

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Table 4.29: Anthelmintic efficacy of Fenbendazole, Ivermectin and polyherbal formulation against GI nematodes in goats based on FECRT % at 21st day post treatment.

Drug	Group-I (Fenbendazole)	Group-II (Ivermectin)	Group-III (P.H.F)	Group -IV (Control)
No. of animals	6	6	6	6
Min & Max EPG (0 day)	1850-2400	1850-2800	1800-2600	1550-2600
Min & max EPG (21st day)	600-1100	250-350	100-250	1800-2200
M pre Tr. FEC (± SEM)	2,016.67±80.27	2,191.67±152.4	2,133.33±129.5	1,941.67±159.9
M post Tr. FEC (± SEM)	816.7 ± 74	308.3 ± 15.3	200 ±22.3	1941.7 ± 58
FECRT% (21st day)	59.05%	85.07%	90.62%	-
95% CL	47.59 - 70.51	81.92 - 88.22	87.66 - 93.58	
Interpretation	Resistant & Least effective	Sub optimal efficacy	Effective	

4.10 COPROCULTURE AND IDENTIFICATION OF LARVAE

Goats commonly host single or multiple genera of helminths, with nematodes being the most frequently found. This observation aligns with previous studies conducted by **Arora** *et al.* **(2010), Sutar** *et al.* **(2010), Sharma** *et al.* **(2014),** and **Khan and Kishor (2014).**

Coproculture on day 0 of pooled faecal samples of all groups revealed the occurence of *Haemonchus contortus* (68-85%), *Trichostrongylus spp.* (8-20%), *oesophagostomum spp.* (5-8%) and *Strongyloides spp.* (2-4%) in animals of all groups. The results are similar to the findings of **Manikkavasagan** *et al.* **(2013), Kanojiya** *et al.* **(2015) and Sastya (2016).** However, it was 94-95%, 2-4%, 1-3% and 0-1% on 7th day post-treatment. Coproculture examination on 14th day post treatment revealed that predominant species found were 97% *H. contortus* and 3% *Trichostrongylus spp*, indicating that *H. contortus* is the primary cause of GI nematodosis in goats of tarai region of Uttarakhand. Prevalence of infective larvae (L3) in coproculture pre and post treatment is depicted in table no 4.30.

Table 4.30: Prevalence of infective larvae (L3) in coproculture pre and post treatment.

LARVAL SPECIES	0 Day	7th Day	14th Day
Haemonchus contortus	68-85%	94-95%	0-97%
Trichostrongylus spp.	8-20%	2-4%	0-3%
Oesophagostomum spp.	5-8%	1-3%	0-1%
Strongyloides spp.	2-4%	0-1%	-
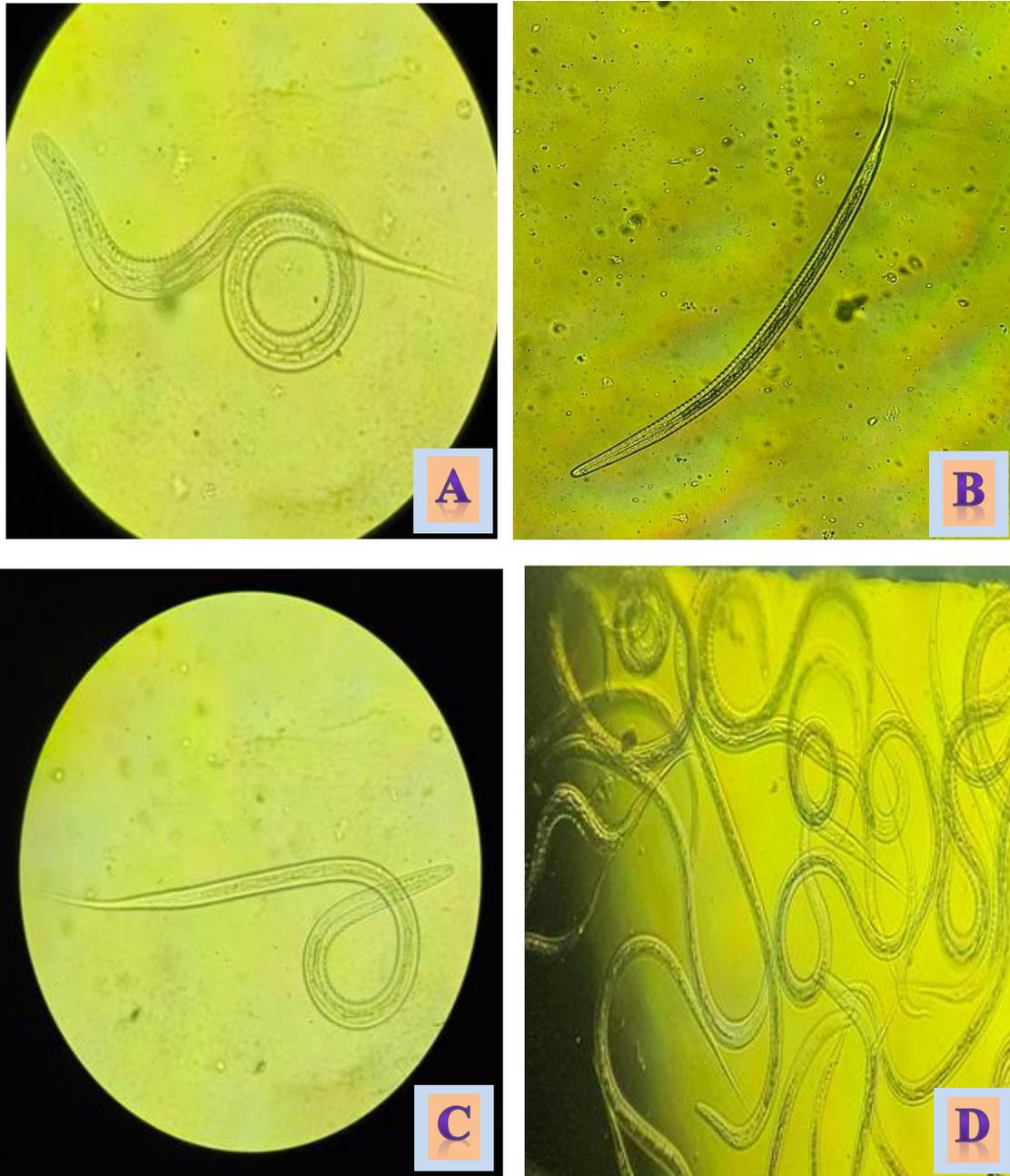
Plate 4.2: **Depiction of larvae (L3) of GI nematodes in goats after coproculture**
(A– *Oesophagostomum spp.*, B– *Haemonchus spp.*, C– *Trichostrongylus spp.*, D- infective larvae (L₃) seen after coproculture)

4.11 PHYTOCHEMICAL ANALYSIS OF EXTRACTS USED IN POLYHERBAL FORMULATION

Five indigenous herbs comprising of Vidanga dried fruits (*Embelia ribes*), Palash seeds (*Butea frondosa*), Neem leaves (*Azadirachta indica*), Pumpkin seeds (*Cucurbita maxima*) and Kali jeeri seeds (*Centratherum anthelminticum*) were taken for use as anthelmintic preparation. Ethanolic extracts were prepared out of above ingredients and they were mixed in following proportions to formulate a polyherbal formulation: Vidanga dried fruits (1000 mg), Palash seeds (1000 mg), Neem leaves (2000 mg), Pumpkin seeds (1500 mg) and Kali jeeri seeds (1000 mg). The mixture was mixed with molten jaggery and wheat bran to form a bolus in order to make it palatable for oral feeding to goats as an anthelmintic. All these extracts were examined physically for their colour, consistency and percentage yield. After that they were examined for the occurrence of various phytochemicals.

4.11.1 Percent yield and physical properties of different extracts

Out of the ethanolic extracts prepared from polyherbal ingredients, highest yield was found in Kali jeeri seeds *viz* 5.78% followed by Neem leaves 5.56% and minimum yield was there in Vadenga dry fruits 2.75 %. The difference in the percentage yield of different extracts may be because of the solubility of various ingredients, method and type of extraction used (**Peach** *et al.,* **1955**).

Physical properties and Percent yield of ethanolic extracts from polyherbal ingredients is shown in table no 4.31 and fig. 4.27.

Table 4.31: Physical properties and Percent yield of ethanolic extracts from polyherbal ingredients

S. No.	Polyherbal ingredients	Colour	Consistency	Dry weight for extraction (gm)	Weight of extract obtained (gm)	% Yield
1	Neem leaves	Greenish black	Sticky	380	21.12	5.56
2	Pumpkin seeds	Greenish black	Oily	530	20.3	3.83
3	Kalijiri seeds	Black	Sticky	850	49.1	5.78
4	Palash seeds	Dark brown	Semisolid	550	16.03	2.91
5	Vadenga Dry fruits	Chocolate brown	Sticky	680	18.7	2.75
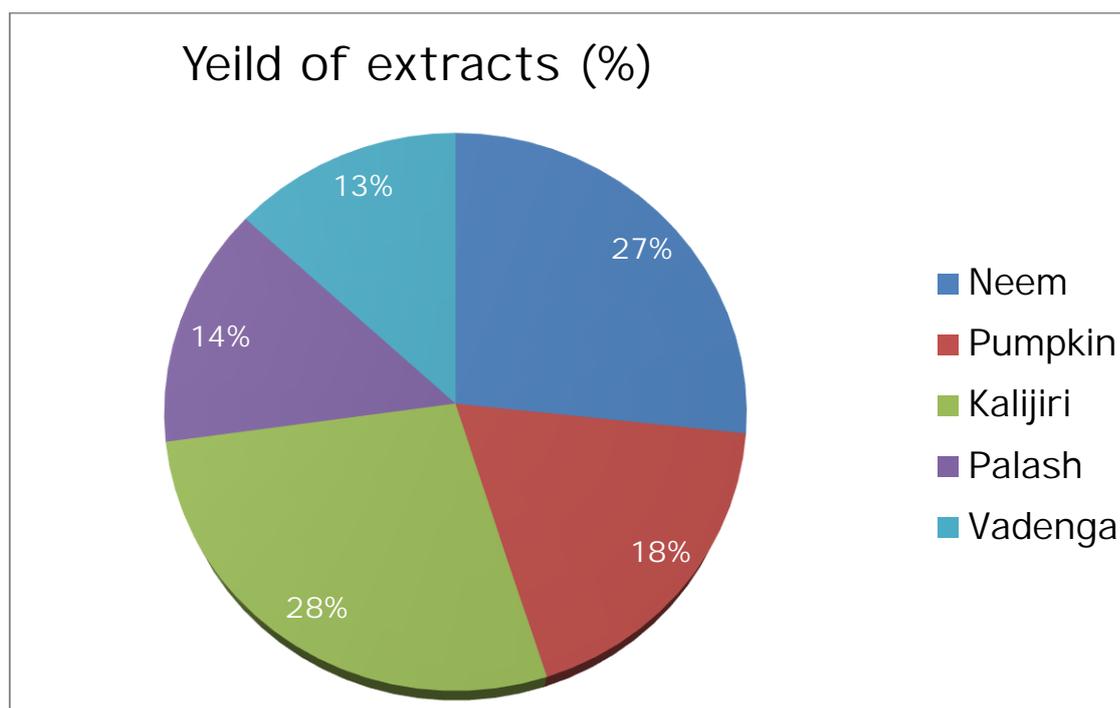
Fig. 4.27: Comparative yield of ethanolic extracts from polyherbal ingredients (%)

4.11.2 Phytochemical analysis of extracts

Different extracts prepared from all five polyherbal ingredients were phytochemically examined for the existence of major phytochemical groups *viz* total phenols, total flavonoids, total antioxidant content, total tannin content and total reducing sugars. The anthelmintic effect of the extract is likely attributed to a range of natural compounds found in the plant, such as sterols, terpenes, polyphenols, flavonoids, tannins, saponins, and alkaloids. These phytochemical constituents are believed to possess potent properties that result in the observed anthelmintic activity of the plant. Nevertheless, further in-depth phytochemical analysis is necessary to identify and characterize each active compound responsible for this effect. Understanding the exact mechanisms of action underlying this activity would require a more comprehensive investigation.

Concentration of major phytochemical groups (Phytochemical assay) present in the polyherbal ingredients is shown in table no 4.32 and fig. 4.28.

Table 4.32: Concentration of major phytochemical groups present in polyherbal ingredients

Conc (mg/100g)	Flavonoid	Phenols	Antioxidant	Tannin	Reducing Sugar
Neem	189.52±0.003	210.89±0.001	167.39±0.022	453.4±0.021	608.36±0.005
Vidanga	195.63±0.002	210.89±0.004	275.50±0.002	370.34±0.016	618.39±0.014
Kalijeeri	478.35±0.011	442.46±0.001	551.12±0.003	481.82±0.025	600.83±0.005
Pumpkin seeds	234.46±0.008	191.32±0.005	563.23±0.01	296.21±0.007	585.22±0.006
Palash seeds	338.45±0.037	228.83±0.002	547.18±0.006	647.93±0.008	621.73±0.005
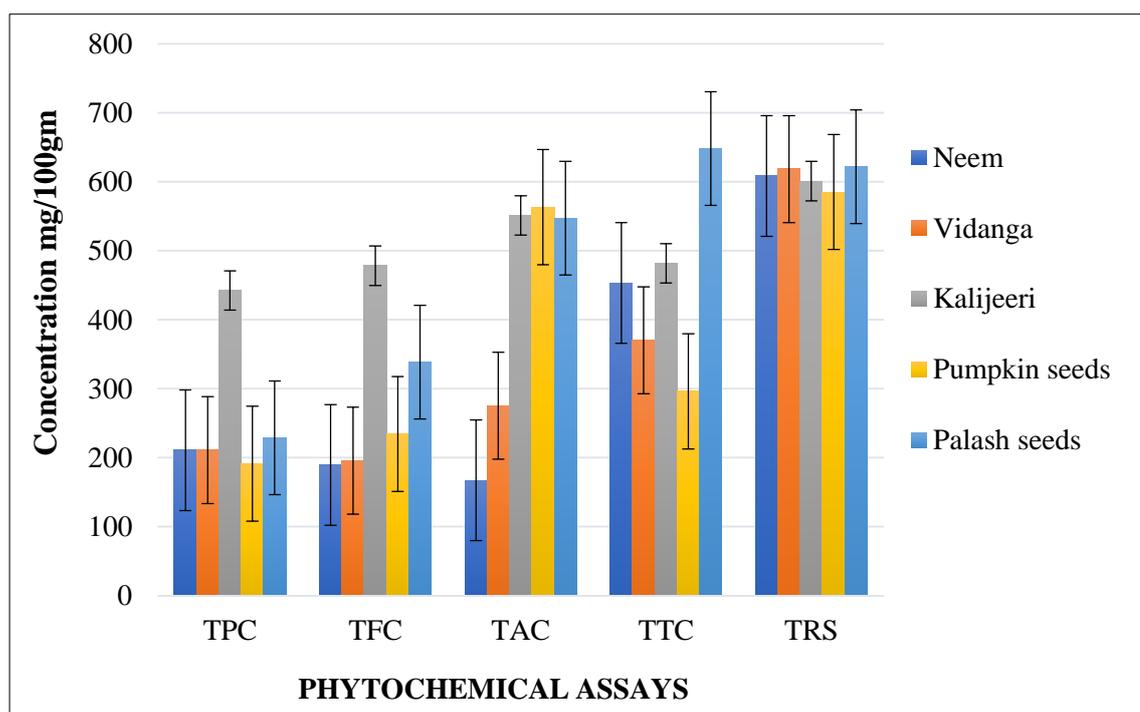
Fig. 4.28. Phytochemical assay of the plant extracts.

(TPC: Total Phenol Content, TFC: Total Flavonoid Content, TAC: Total Antioxidant Content, TTC: Total Tannin Content, TRS: Total Reducing Sugar)
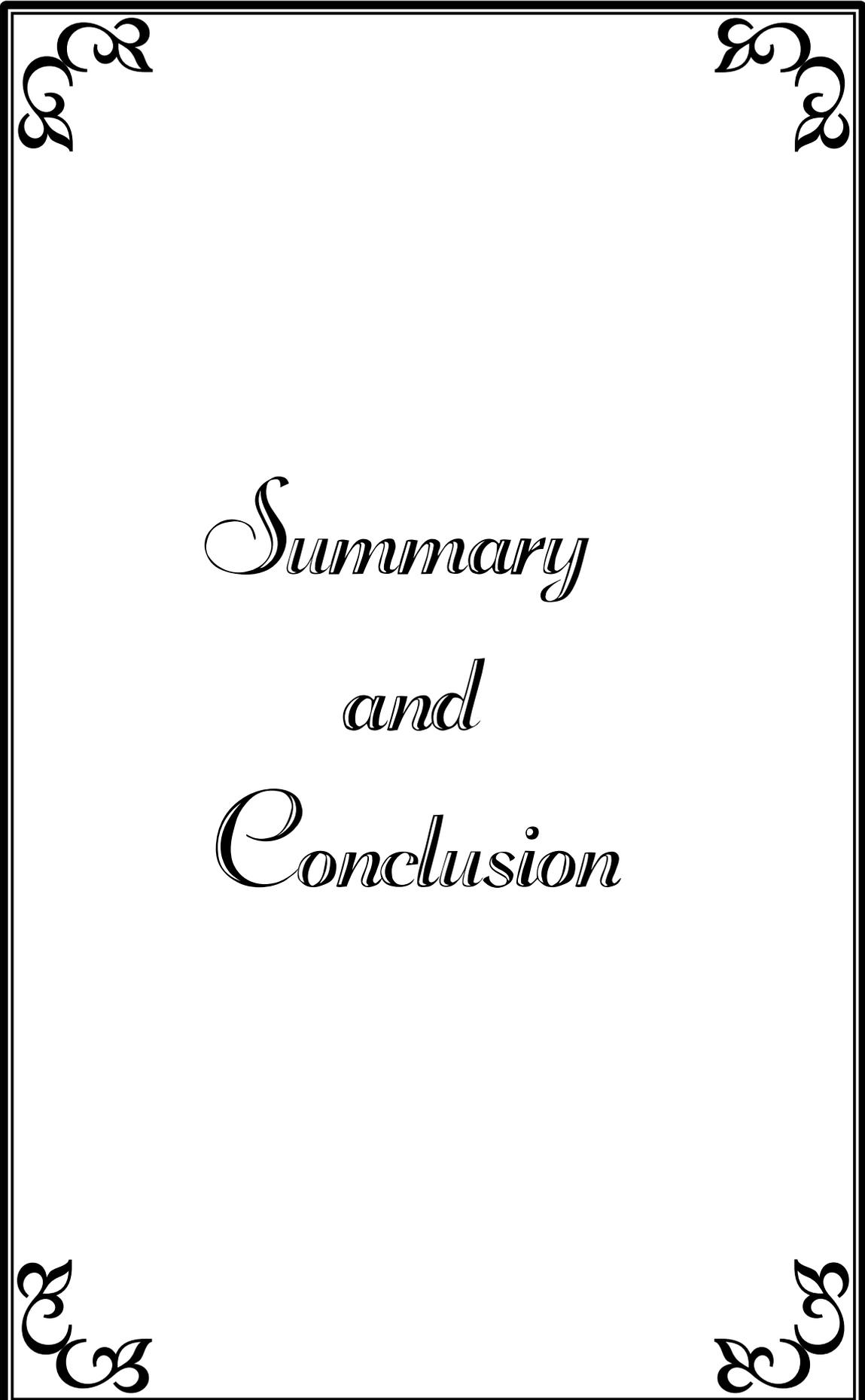
Summary and Conclusion

Chapter 5	SUMMARY AND CONCLUSION

The present study was conducted between Nov 2021 to Dec 2022. The research was conducted at goat farms/herds of Tarai region around Pantnagar. Screening of goats infected with gastro intestinal (GI) nematodes was done using "Faffa Malan Chart" (FAMACHA) technique.

The effect of commonly used anthelmintics *viz* fenbendazole and ivermectin on haematobiochemical parameters and biomarkers of oxidative stress in goats afflicted with gastrointestinal nematodosis was carried out and their efficacy was compared with the polyherbal formulation used in the trial. Based on history of regular deworming by the farmers and high EPG seen during faecal examination, anthelmintic resistance (AR) to commonly used anthelmintics was suspected in the goats selected for the research work**.**

Phytomedicines exhibit a multifaceted therapeutic strategy, harnessing a spectrum of bioactive compounds to synergistically address a range of health conditions **(Spiegler *et al.,* 2017).** The art of polyherbalism introduces a harmonious interaction, affording distinctive benefits beyond those found in solitary herbal preparations. These polyherbal concoctions have showcased remarkable efficacy in managing diverse ailments, even when administered in elevated amounts without compromising safety.

Total 146 goats exhibiting FAMACHA scores ranging from 3 to 5, were examined to determine the prevalence of GI nematodosis. Majority of the goats kept under semi – intensive system of farming had FAMACHA score between 3 to 5 in the Tarai region around pantnagar and the mucous membrane colour of goats varied from whitish to light pinkish in colour.

The phytotherapeutic ingredients and quantities used in making Polyherbal drug are outlined in Table 5.1.

Table 5.1 Phytotherapeutic ingredients and quantities used in making Polyherbal drug.

Ser No	Poly Herbal Ingredients	Dose of ethanolic extracts of the plant materials used
1.	Vidanga dried fruits (*Embelia ribes*)	1000 mg
2.	Palash seeds (*Butea frondosa*)	1000 mg
3.	Neem leaves (*Azadirachta indica*)	2000 mg
4.	Pumpkin seeds (*Cucurbita maxima*)	1500 mg
5.	Kalijiri seeds (*Centratherum anthelminticum*)	1000 mg

A pilot study was conducted on three goats to rule out any adverse effects of the PHF used in the experimental study. Initially *in-vitro* evaluation of polyherbal drug using Adult Worm Motility Assay (AWMA) was carried out. Ethanolic extract of polyherbal formulation (PHF) showed total mortality at 100 mg/ml at the exposure time of 2 hrs. The concentrations of 50mg/ml and 25 mg/ml resulted in total mortality at the exposure time of 4 hrs. The efficacy of the ethanolic extract of polyherbal formulation (PHF) in different dose dependent concentrations revealed that it has good efficacy at the concentrations of 100, 50 and 25 mg/ml at the exposure time of 2 hrs and 4 hrs against the adult round worms of goat origin in the *in vitro* trials.

The selected 24 goats were divided randomly into four equal groups and different coloured tags were placed around the neck for identification. Group I: (n=6) was treated with fenbendazole @ 5mg/kg body weight orally (**Plumb, 2008, Sharma *et al.,* 2015, Papich, 2016).** Group II: (n=6) was treated with ivermectin @ 0.2mg/kg body weight orally (**Lespine *et al.*, 2005, Sharma *et al.*, 2015, Shakya *et al.*, 2018, Myers *et al.*,2020**). Group III: (n=6) was treated with polyherbal formulation @ 6.5 gm/30 kg body wt. orally (Based on safety trial/pilot study, **Parsani *et al.* ,2020, Kalkal and Vohra 2021, Khanolkar *et al.,* 2018**). Group IV: (n=6) was kept as control (untreated). The hematological, biochemical and oxidative stress related parameters were investigated at intervals of 0, 7, 14, and 21 days. Faecal samples were collected directly from rectum on 0 day (Pre-Treatment), 7th day, 14th day and 21st Day (post-treatment) for calculation of EPG and FECRT.

The data of egg count in faeces was analyzed by FECRT % for the anthelmintic efficacy of the polyherbal extracts and conventional anthelmintics on a particular day using the formula given by **Dash *et al.*, 1988.**

$$\textbf{\% efficacy} = 100 \text{ x } \frac{(\text{Pre - treatment EPG - Post - treatment EPG})}{\text{Pre - treatment EPG}}$$

5.1   Haematological analysis

In the GI Nematodosis infected goats there was significant increase in the mean values of Hb, PCV and TEC after treatment with Polyherbal formulation & Ivermectin, as compared to group IV (control). A noteworthy rise in Hemoglobin (Hb), Packed Cell Volume (PCV), and Total Erythrocyte Count (TEC) following treatment signified the eradication of GI Nematodes and the cessation of blood loss. Substantial elevation in Hb, PCV, and TEC could also stem from the correction of iron deficiency and the swift regeneration of erythrocytes. The mean hemoglobin concentration was somewhat higher in the group II and III goats in comparison to group I and IV goats. PCV values in Group I, II and III showed a significant increase on day 21st after treatment compared to the pre-treatment values of day 0. Group IV showed decrease in PCV values which was obviously due to the persisting worm burden being the untreated control group.

On 21st day post treatment, Group III had significant increase in values of TEC. Conversely the Goats in group IV showed non-significant effects on erythrocyte counts compared to the other groups

For all groups (I, II, III, and IV), TLC values generally decreased over time. Group III had the most significant decrease in TLC values compared to the other groups. Group II also showed a substantial decrease in TLC values over time. Group I and IV demonstrated non-significant decrease in TLC values.

The percentage of Neutrophils increased in G I (from 48.83% to 61.17%). G IV had non-significant change, whereas decrease in the values was seen in group II (56.17% to 41.33%) & III (60.50% to 43.17%). The percentage of Lymphocytes showed significant decrease in the values in G I – (44.83% to 34.50%) whereas significant increase in values was seen in G II (37.50% to 55.00%) and G III (33.67% to 53.17%). No substantial alteration was noted in the values within Group IV.

5.2   Biochemical analysis

There was significant decrease in the values of ALP, SGOT (AST) and SGPT (ALT) in goat serum after treatment with PHF and Ivermectin in comparision to control group which indicated marked hepatic damage in goats infected with GI nematodes. It is in agreement with the reports of **Waghmare *et al.* (2009), Bhardwaj *et al.* (2015), Sunandhadevi *et al.* (2017)** and **Ahmed *et al.* (2012).**

Values of blood glucose were also significantly higher in goats treated with Polyherbal formulation and Ivermectin in comparision to group IV(control). Glucose level was low in Group I & IV on account of high EPG and high worm load and the difference was highly significant statistically ($P<0.01$). Correlation between EPG and glucose was inversely correlated The low level of glucose before treatment in goats suffering from GI nematodosis could be because of anorexia, decreased intestinal absorption of glucose and reduced rate of conversion of lactic acid to glucose. **Bandyopadhyay and Dasgupta (2000)**, as well as **Arora *et al.* (2001),** also reported similar findings concerning blood glucose levels. Following treatment, a noteworthy enhancement was observed, likely attributed to the eradication of parasites and increased glucose absorption from the gastrointestinal tract.

The serum albumin levels in all groups were within the typical range for goats. No significant variation among the groups was seen (P > 0.05). Globulin values remained within normal range for goats and the overall values for A/G ratio also remained within normal range for goats.

Group I, II and III showed significant decline in values of ALP, while Group IV showed an increase in ALP levels. However the values of ALP were found within the standard range (93–387 IU L-1) for goats. The values of ALT in Groups I, II and Group III reduced significantly on culmination of treatment on 21st day and were close to the normal range for a healthy goat whereas the values in Group IV showed a steady rise with most significant increase till 14th day. The increase in values of alanine aminotransferase in affected animals was due to traumatic damage of mucosa of abomasum and intestine by the parasites. Significant reduction in SGOT (AST) levels was seen over the 21day period, with Group II and III demonstrating the most

pronounced effect. However, Group IV had a less favourable impact, leading to increased SGOT levels.

The values of urea in Groups I, II and Group III reduced significantly on 21st day post treatment and were close to the normal range for a healthy goat. The serum creatinine values for all groups were within the standard range for goat species.

In all four groups there was non-significant decrease in sodium and chloride levels after 21 days of treatment compared to their initial values at 0 day while values of potassium had a reverse course, though all the values of Serum electrolytes in goats suffering from GI nematodosis were found in the normal range.

5.3   Oxidative stress related parameters

A notable increase ($p < 0.05$) was noticed in the serum concentrations of GSH-Px, SOD, and CAT in goats subjected to the polyhedral formulation treatment, Ivermectin and Fenbendazole on the 21st day post treatment and decrease in MDA levels after treatment was noticed. It has been revealed that level of GSH-Px, SOD and CAT are low during stress caused by GI nematodosis whereas MDA levels were high.

Group III showed the most prominent effect on Oxidative stress related parameters from 0 days to 21st day, showing a consistent increase over time in the values of GSH-Px, SOD and CAT probably because of the synergistic activity of the phytochemicals of five extracts used in preparation of polyherbal drug rich in flavonoids and other phytochemicals which possess antioxidant properties that help in mitigating oxidative stress-related diseases.

5.4 Therapeutic and anthelmintic resistance study

The intensity of EPG of each faecal sample was determined by modified Mc Master Technique **(MAFF, 1971).** The eggs per gram of faeces (EPG) were determined by multiplying the count of nematode eggs in two squares of the McMaster slide by a dilution factor of 50.

Egg per gram (EPG) = Total of eggs in both the chambers x 50

On 21st day after treatment mean EPG values in Group I (816.67 ± 30.12), Group II (308.33 ± 6.27), Group III (200 ± 9.13) and Group IV (1941.67 ± 23.81)

showed significant variation (P < 0.05) and difference between the groups with highest & lowest EPG was more than EPG of 1740. Significant improvement in the mean EPG in both treatment groups G II (675 ± 13.69) and G III (641.67 ± 12.27) were observed on 14th day post treatment compared to G I and G IV.

Table 5.2 Anthelmintic efficacy of Fenbendazole, Ivermectin and polyherbal drug against GI nematodes in goats based on FECRT % at 14th day and 21st day post treatment.

Drug	Group-1 (Fenbendazole)	Group-II (Ivermectin)	Group-III (P.H.F)	Group -IV (Control)
FECRT% (14th day)	21.15%	68.88%	68.40%	-
95% CL	13.72- 28.58%	66.86 -70.89%	60.36 -76.44%	
Interpretation	Resistant	Sub optimal efficacy	Sub optimal efficacy	-
FECRT% (21st day)	59.05%	85.07%	90.62%	-
95% CL	47.59 - 70.51	81.92 - 88.22	87.66 - 93.58	
Interpretation	Resistant & Least effective	Sub optimal efficacy	Effective	

The results showed a substantial reduction of EPG in the groups treated with ivermectin and polyherbal drug on 21st day post treatment, with an efficacy rate of 85.07% and 90.62% respectively. In the current research resistance to fenbendazole was noticed as depicted in Table 5.2.

5.5 Coproculture for larvae identification

Coproculture on day 0 of pooled faecal samples of all groups revealed the occurence of *Haemonchus contortus* (68-85%), *Trichostrongylus spp.* (8-20%), *oesophagostomum spp.* (5-8%) and *Strongyloides spp.* (2-4%) in animals of all groups. It was found to be 94-95%, 2-4%, 1-3% and 0-1% respectively on 7th day post-treatment. Coproculture examination on 14th day post treatment revealed that predominant species found were 97% *H. contortus* and 3% *Trichostrongylus spp*, indicating that *H. contortus* was the primary cause of GI nematodosis in goats of tarai region of Uttarakhand.

5.6 Phytochemical study of extracts

Qualitative chemical analysis of extracts of *A. indica* (leaves), *B. frondosa* (seeds), *E. ribes* (berries), pumpkin seeds (*Cucurbita maxima*) and Kali Jeeri (*Centratheram anthelminticum) was* done to detect total flavonoid, phenols, antioxidants, tannins and reducing sugars by standard methods.

- The plant material with the maximum concentration of flavonoids was Kalijeeri (478.35 mg/100g), while the minimum was in Neem (189.52 mg/100g).

- Kalijeeri also had the highest phenol concentration (442.46 mg/100g), while Palash seeds had the lowest (228.83 mg/100g).

- Pumpkin seeds contained the highest amount of antioxidants (563.23 mg/100g), and Neem had the lowest (167.39 mg/100g).

- Palash seeds had the highest tannin content (647.93 mg/100g), while Pumpkin seeds had the lowest (**296.21** mg/100g).

- Palash seeds had the most reducing sugars (621.73 mg/100g), while Pumpkin seeds had the least (**585.22** mg/100g).

5.7 CONCLUSION

- The polyherbal formulation employed in the current study demonstrated effectiveness in both *in vitro* and *in vivo* trials. Due to the synergistic effects of combining multiple herbs polyherbalism presents numerous advantages that are lacking in single herbal formulations and it enhances the therapeutic effects significantly.

- Based on Haematobiochemical examination and FECRT it was seen that the polyherbal formulation was effective in the cases of GI nematodosis in goats, and can be a good alternative to chemical anthelmintics for controlling GI nematodosis in goats. The EPG was significantly reduced after treatment on day 21 with polyherbal drug and Ivermectin. Treatment with fenbendazole showed resistance and thus it was the least effective in present trial.

- In Groups treated with polyherbal drug and Ivermectin, there was noteworthy improvement in the post treatment values of haematological parameters (Hb, PCV,

TEC and TLC), serum biochemicals (total protein, ALP, AST, ALT, urea and glucose), serum electrolytes (sodium, potassium, chloride) and Oxidative stress related parameters of goats as compared to pre-treatment values. Post treatment values of these haemato-biochemical, electrolyte & Oxidative stress related parameters were almost comparable with values of healthy goats on day 21.

- Implementing planned grazing, utilizing early diagnosis through the FAMACHA technique, and incorporating herbal anthelmintics can collectively mitigate drug resistance and contribute to reducing mortality among goats affected by gastrointestinal nematodosis.

- *Haemonchus contortus* is the primary cause of GI nematodosis in goats of tarai region of Uttarakhand.

- Excessive utilization of commonly employed anthelmintics could lead to the emergence of drug resistance, giving rise to significant public health concerns and causing economic losses within production industries. Deworming with chemical anthelmintics should only be done when necessary and should be part of a comprehensive parasite control program that promotes responsible use of anthelmintics to prevent the emergence of drug resistance in parasites.

- The study on anthelmintic efficacy of various plant products in goat or sheep remains insufficient and requires further research on various polyherbal combinations to assess their efficacy as potent dewormers.
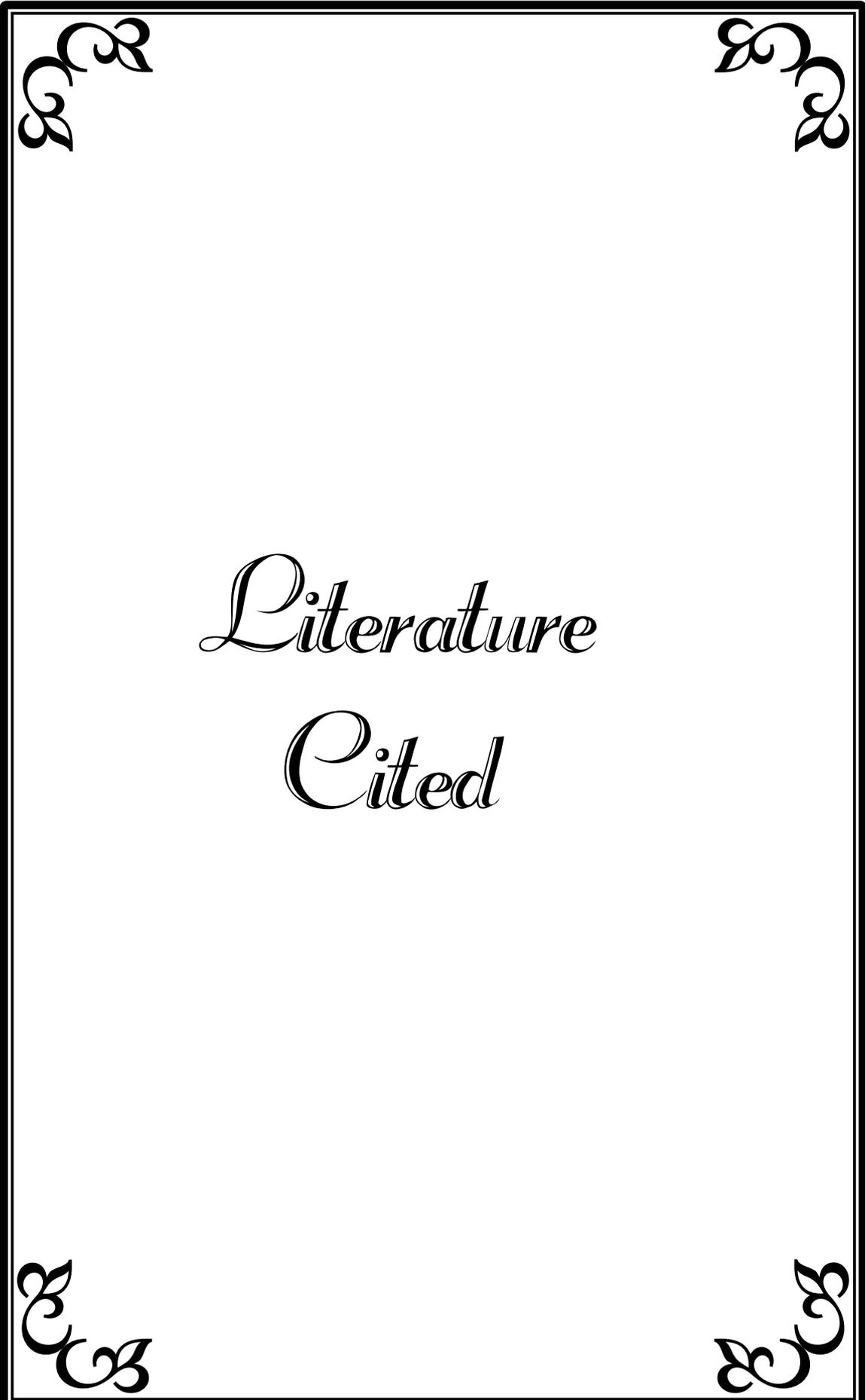
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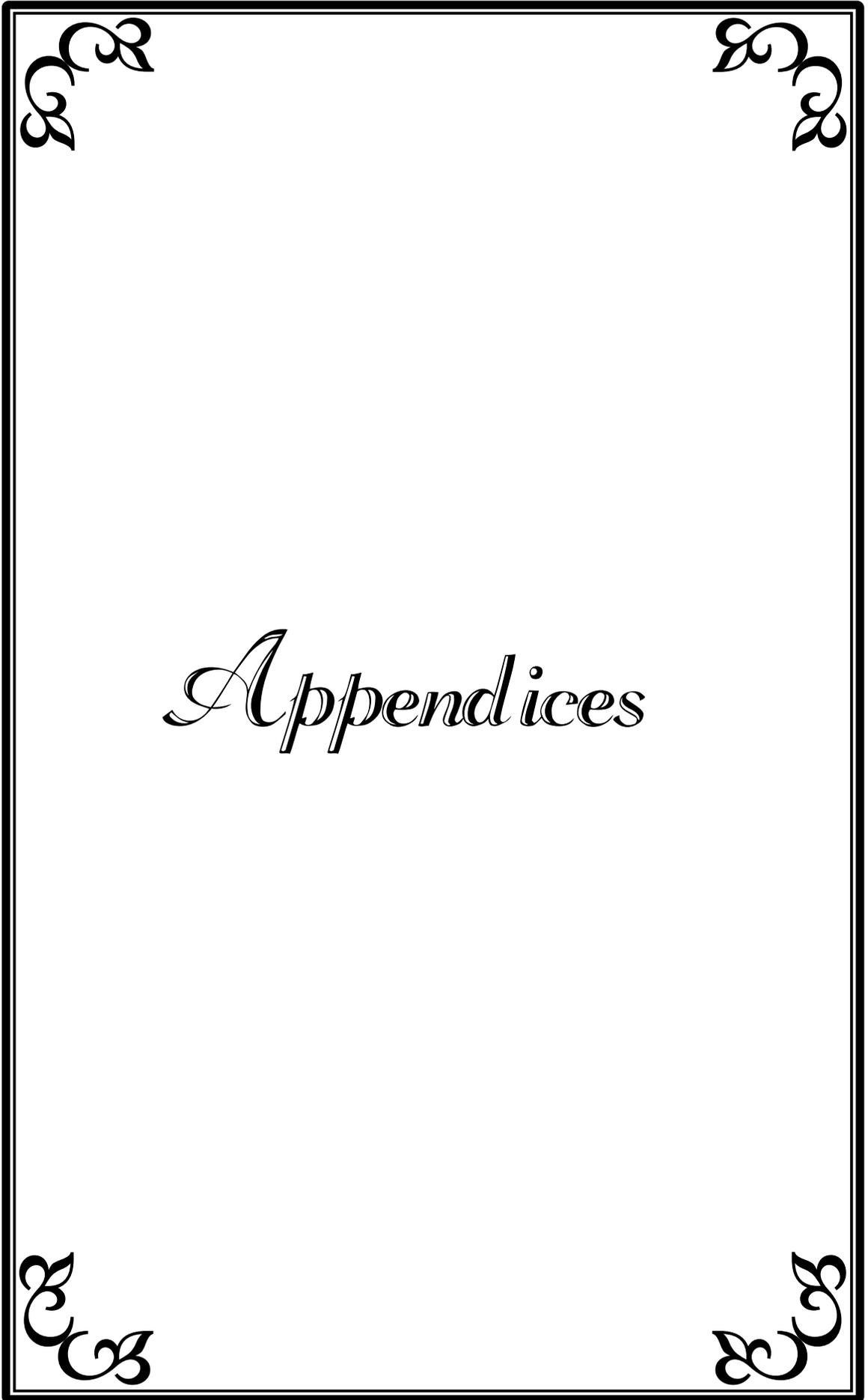
Appendices

Uttarakhand Seeds & Tarai Development Corporation Ltd.

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Telephone : Haldi Exch. 05944 - 230222, 224, 225, 226, 233, 234, 288, 347, 351, 442, Pantnagar Exch. 05944 - 233595
Fax : 05944 - 233830, 230240 Gram : 'BEEJNIGAM' Haldi (UK)
Website : pantnagarseeds.com, www.ukstdc.com
E-mail : tdc@uaseedsandtdc.com, gm@uaseedsandtdc.com

Letter N0 : ukseeds/03/2023/_____,
Dated : May 22, 2023

CERIFICATE

Certified that the following Seed samples received from COL(Dr.) A.S. Rathor of GBPUA&T Pantnagar, Uttarakhand at UKS&TDC, Haldi, Pantnagar are authentic & verified by experts of this institution.

SN.	Name Of Seed Sample	Remarks
1	Vidanga (Embelia Ribes)	} VERIFIED
2	Pumpkin Seeds (Cucurbita maxima)	
3	Palash Seeds (Butea frondosa)	
4	kalijeeri Seeds (Centratherum anthelminticum)	

(UDAIRAJ SINGH)
Seed Production Officer

टेलीफोन / Phone : (0135) 2755476
(0135) 2753433

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दिनांक / Dated........19-05-2023

प्रमाणपत्र / CERTIFICATE

प्रमाणित किया जाता है कि कर्नल ए.एस. राठौर, जी.बी.पी.यू.ए. एण्ड टेक., पंतनगर, उत्तराखण्ड से प्राप्त निम्न पादप नमूना निम्नानुसार (वानस्पतिक नाम) इस कार्यालय के विद्यार्थी पादपालय में परिग्रहण किया गया है :

Certified that the following plants sample received from **Col. A.S. Rathor, GBPUA & Tech, Pantnagar, Uttarakhand** is accessioned at herbarium (BSD Student Herbarium) of this office:

पादप का नाम / Plant name	कुल / Family	परिग्रहण सं. / Acc. No.
1. *Butea monosperma* (Lam.) Kuntze	Fabaceae	1358
2. *Azadirachta indica* A. Juss.	Meliaceae	1359
3. *Cucurbita maxima* Duchesne	Cucurbitaceae	1360

(एस.के. सिंह / S.K. Singh)
वैज्ञानिक–ई / कार्यालयाध्यक्ष / Scientist-E/HOO

<div align="center">

CURRICULUM VITAE

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Educational Qualification

Sr. No.	Examination Passed	Institution	Year	Percentage/ CGPA
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2	Post-graduation (MVSc.)	Rajasthan University of Veterinary & Animal Sciences, Bikaner	2020	8.18
3	B.V.Sc & A.H	Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur	1995	6.58
4	National Diploma in Equine Husbandry Medicine & Surgery	Indian Veterinary Research Institute, Bareilly	2014	7.33
5	Intermediate level (12th)	CBSE (Alwar Public School, Alwar)	1988	59%
6	Matriculation level (10th)	ICSE (Dr Graham's Homes Kalimpong)	1986	74.66%
7	e-Post Graduate Program in Epidemiology (1 Yr)	Indian Institute of public Health – Delhi	2021	A Grade

Specialization: Major: Veterinary Medicine   **Minor:** Veterinary Parasitology

Thesis Title: "Studies on clinico-haematobiochemical and therapeutic efficacy of
    polyherbal formulation against resistant gastro intestinal nematodes in goats"

Publications: 5 Research Articles and 19 abstracts

Papers published from thesis: 01

Papers published other than thesis: 02

Conferences/Seminars/Workspace/Training Attended:

- ISVM 2023- National conference on Advancements in research and innovations in mitigation of disease of livestock companion, wild animals and poultry"

- iAbAsSd-2023-Innovative approaches in basic & applied sciences for societal development

- Biotechnology conference (2023) - Biotechnological intervention in animal production and management at Uttarakhand council for biotechnology.

- Entrepreneurship development programme held at CVASc, Pantnagar-2021**.**

List of papers presented in conference/seminar during degree programme:

- Poster presentation titled "Benzimidazole resistance in *Haemonchus contortus* in goats of Uttarakhand" at Biotechnology conference held at Uttarakhand council for biotechnology (2023).
- Poster presentation titled Evaluation of Anti-Oxidative properties of *Dalbergia sissoo* and *Aegle marmelos* in calves affected with colibacillosis at ISVM 2023
- Poster presentation titled Importance of one health & emergence of anthelmintic resistance at iAbAsSd-2023
- Poster presentation titled *In vitro* antibacterial activity of extracts of *dalbergia sissoo* & *aegle marmelos* in colibacillosis in calves at CAAAHSSE-2021
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Award/Honours/Achievements:

1st in poster presentation (ISVM 2023),

Fellowship of SERS at   iAbAsSd-2023,

Pashudhan Praharee Louis Pasteur Award 2022 on world rabies day

Best Thesis Award at GRISAAS-2021,

Outstanding Achievement Award at ICAAAS-2021

Place: Pantnagar

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Major	:	Veterinary Medicine	**Department**	:	Veterinary Medicine
Minor	:	Veterinary Parasitology			
Thesis title	:	"STUDIES ON CLINICO-HAEMATOBIOCHEMICAL AND THERAPEUTIC EFFICACY OF POLYHERBAL FORMULATION AGAINST RESISTANT GASTRO INTESTINAL NEMATODES IN GOATS"			
No. of pages	:	143	**Advisor**	:	**Dr. Prakash Bhatt**

ABSTRACT

The present study was conducted at goat farms/herds of Tarai region around Pantnagar. Total 146 goats exhibiting FAMACHA scores ranging from 3 to 5, were examined to determine the prevalence of GI nematodosis. Out of 146 goats, 103 were diagnosed with GI nematodosis. From these 103 goats, 24 goats of either sex, aged between 9 months to 5 years, were selected with egg counts exceeding 200. They were randomly assigned to four equal groups. Group I received oral fenbendazole treatment at 5mg/kg body weight, Group II received oral ivermectin at 0.2mg/kg body weight, Group III received an oral polyherbal formulation at 6.5g/30 kg body weight, and Group IV served as the untreated control. A pilot study was also conducted earlier on three goats to rule out any adverse effects of the polyherbal formulation used in the experimental study.

The haematological, biochemical, and oxidative stress related parameters were investigated at intervals of 0, 7, 14, and 21 days. Faecal samples were collected directly from rectum on 0 day (Pre-Treatment), 7th day, 14th day and 21st Day (post-treatment) for calculation of EPG and FECRT.

There was significant improvement in the mean values of Red Blood Cells, Haemoglobin, and Packed Cell Volume after treatment with polyherbal formulation and ivermectin, as compared to control. ALP, AST, and ALT levels within Groups I, II, and III reduced significantly by the 21st day, aligning with the healthy goat's standard range. Whereas the values in Group IV showed a steady rise. The increased enzyme activity in affected animals aligns with detrimental effects caused by traumatic abomasal and intestinal damage, directly linked to parasitic infestation. Serum concentrations of GSH-Px, SOD, and CAT were notably elevated on the 21st day post-treatment in goats treated with the polyherbal formulation, ivermectin, and fenbendazole, along with decreased MDA levels, indicating decreased oxidative stress after treatment. It was revealed that levels of GSH-Px, SOD and CAT were low during stress caused by GI nematodosis whereas MDA levels were high.

Assessment of EPG and FECRT (%) values on the 21st day post-treatment revealed efficacy rates of 85.07% for ivermectin, 90.62% for the polyherbal formulation, and 59.05% for fenbendazole. Significant improvement in the mean EPG in both treatment groups G II and G III was seen on 14th day post treatment compared to G I thus indicating resistance to fenbendazole.

Coproculture on the 14th day post-treatment indicated that 97% of identified species were *H. contortus*, establishing it as the primary cause of GI nematodosis in goats from the Tarai region of Uttarakhand.

(Prakash Bhatt)
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गौण विषय	:	पशु रोगजन्यता विज्ञान			
शोध शीर्षक	:	""बकरियों में प्रतिरोधी गैस्ट्रो इंटेस्टाइनल नेमैटोड्स के खिलाफ पॉलीहर्बल फॉर्म्युलेशन के क्लिनिको-हेमेटोबायोकैमिकल और चिकित्सात्मक प्रभाव पर अध्ययन"			
पृष्ठ संख्या	:	143	सलाहकार	:	डॉ प्रकाश भट्ट

सारांश

यह अध्ययन पंतनगर के आसपास के तराई क्षेत्र की बकरी फार्मों/झुण्डों में किया गया था। कुल 146 बकरियों की फैमाचा स्कोर 3 से 5 तक रही थी, उन्हें गैस्ट्रो इंटेस्टाइनल नेमैटोडोसिस की प्रसारण निर्धारित करने के लिए जांच की गई। 146 बकरियों में से, 103 की गैस्ट्रो इंटेस्टाइनल नेमैटोडोसिस की परिणामांकन हुआ । इन 103 बकरियों में, 9 महीने से 5 वर्ष के बीच की आयु की 24 बकरियों का चयन किया गया, जिनमें अंडांक गिनतियों ने 200 की अधिकता पार की। उन्हें चार समान समूहों में यादृच्छिक रूप से विभाजित किया गया। समूह I को 5 मि.ग्रा./कि.ग्रा. शरीर वजन पर मुखांडी उपचार दिया गया, समूह II को 0.2 मि.ग्रा./कि.ग्रा. शरीर वजन पर मुखांडी उपचार दिया गया, समूह III को 6.5 ग्रा./30 कि.ग्रा. शरीर वजन पर मुखांडी पॉलीहर्बल तंतुवा प्राप्त हुआ, और समूह IV को अअपचिकित नियंत्रण समूह के रूप में उपयोग किया गया। प्रयोगात्मक अध्ययन में तंतुवा के उपयोग से किसी भी प्रकार के विपरीत प्रभाव को निष्कलंक करने के लिए पहले तीन बकरियों पर पायलट अध्ययन किया गया।

रक्तलोहितक, बायोकैमिक, और ऑक्सिडेटिव स्ट्रेस संबंधित पैरामीटरों का अनुसंधान 0, 7, 14, और 21 दिन के अंतरालों पर किया गया। विमानी नमूने सीधे मलाशय से 0 दिन (पूर्व-उपचार), 7वें दिन, 14वें दिन और 21वें दिन (पोस्ट-उपचार) पर लिए गए थे, ताकि ईपीजी और एफईसीआरटी की गणना की जा सके।

पूर्वाचार में तंतुवा के प्रयोग के किसी भी प्रतिकूल प्रभाव को बाहर करने के लिए 21वें दिन पर पूर्व-उपचार, 7वें दिन, 14वें दिन और 21वें दिन के बाद खून की जीवाणुविज्ञान, बायोकैमिक और ऑक्सिडेटिव स्ट्रेस संबंधित मानक रेंज के साथ समर्थ बकरियों में लाभदायक सुधार था। I, II और III समूहों के भीतर ALP, AST और ALT स्तर 21वें दिन पर संक्षिप्त हो गए, जो स्वस्थ बकरियों की मानक सीमा के साथ मेल खाते हैं। जबकि चरण IV के मान ने स्थिर रूप से बढ़ने की दिशा दिखाई। प्रभावित पशुओं में बढ़ी हुई एंजाइम गतिविधि पैरासिटिक संक्रमण से संबंधित पीड़क अबोमसल और आंतों के क्षति के कारण होने वाले हानिकारक प्रभावों के साथ मेल खाती है। पॉलीहर्बल तंतुवा, इवरमेक्टिन और फेनबेंडाजोल के साथ उपचार के बाद बकरियों में ग्लूटाथियोन पेरोक्साइडेज, सुपरऑक्साइड डिस्म्यूटेस और कैटलेस के उच्च स्तर दिखाई दिए, साथ ही घटे हुए ऑक्सिडेटिव स्ट्रेस की घटना, जो उपचार के बाद सूचित करते हैं। यह प्रकट हुआ कि जीआई नेमैटोडोसिस के कारण होने वाले तनाव के दौरान जीएसएच-पीएक्स, एसओडी और कैट के स्तर कम थे जबकि एमडीए स्तर अधिक थे।

उपचार के 21वें दिन पर ईपीजी और एफईसीआरटी (%) मूल्यों की मूल्यांकन ने दिखाया कि इवरमेक्टिन के लिए 85.07%, पॉलीहर्बल तंतुवा के लिए 90.62% और फेनबेंडाजोल के लिए 59.05% की प्रभावकारिता दरें थीं। प्रायः उपचार समूह G II और G III में 14वें दिन पर औसत ईपीजी में सुधार दिखाया गया था, जिससे फेनबेंडाजोल के प्रति प्रतिरोध की सूचना मिलती है। 14वें दिन पर कोप्रोकल्चर ने इसे स्पष्ट किया कि पहचाने गए प्रजातियों में से 97% हो. कॉन्टोर्टस थे, जो उत्तराखंड के तराई क्षेत्र की बकरियों में जीआई नेमैटोडोसिस के प्रमुख कारण के रूप में स्थापित किया गया।

(प्रकाश भट्ट)
सलाहकार

(आशोक सिंह राठौड़)
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Effect of fenbendazole and ivermectin on antioxidant status of goats suffering from gastrointestinal nematodes

AS Rathor, Prakash Bhatt, Stuti Vatsya, JL Singh, Rajesh Kumar and SK Dubey

Abstract
A total of 146 goats having FAMACHA score from 3 to 5 were examined for estimating the occurrence of gastrointestinal (GI) nematodosis in Tarai region around Pantnagar. Hundred and three goats were found positive for GI nematodosis. Out of these 103 goats, 18 goats were separated and divided randomly in three groups. The animals of Group I: (n=6) were treated with fenbendazole @ 5 mg/kg body wt. po; Group II: (n=6) were given ivermectin @ 0.2 mg/kg body wt. po. The animals of Group III: (n=6) were the control without any treatment. Using standard procedures, the serum obtained were assayed for estimation of antioxidant values and lipid peroxidation *viz* glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). On 21st day post treatment, goats of group I & II showed significant reduction in lipid peroxidation however values of CAT, SOD & GSH-Px increased significantly. Values of group III showed elevated values of malondialdehyde (MDA) & reduced levels of GSH-Px, SOD and CAT.

Keywords: Ivermectin, GI nematodosis, oxidative stress, free radicals, anti-oxidant

Introduction
Helminth infection of the gastrointestinal tract is a significant contributing factor to decreased goat productivity on a global scale, especially under grazing conditions. Clinically, gastrointestinal parasitic infections in goats present with symptoms such as enteritis, anemia, emaciation, dehydration, and even death.

As per Tariq *et al.*, (2010) [23], gastrointestinal (GI) parasitism has emerged as primary constraint and major threat to the small ruminant industry, leading to production losses and death in severe cases. The host-parasite relationship with nematode parasites results in substantial damage at the site of attachment, manifesting as enteritis, anaemia, emaciation, dehydration, and death. These changes significantly impact the growth, body weight, yield, and reproductive performance of the animals, resulting in economic losses for the farmers (Sharma *et al.,* 2014) [21]. As per Brunsdon (1988) [1], the GI nematodes cause severe losses in pasture-based livestock farming.

In the sub-clinical form, worms continuously feed on blood (Maiti *et al.,* 1999) [14], causing anemia and hypoproteinemia. Haematological analysis and Serum biochemistry have proved to be the reliable and essential indicators for ascertaining the health status of animals. It also provides insight into the severity of infestation and extent of damage inside the host body, (Otesile *et al.,* 1991) [16]. Imbalance between production of free radicals and antioxidant defences of the body leads to oxidative stress and its estimation has become vital in animal production and management. This assessment has become a supporting tool to evaluate metabolic and nutritional status of infected animals, (Mohebbi-Fani *et al.,* 2012) [15].

The body's antioxidant status plays a critical role in maintaining overall health by serving as an essential defence mechanism against free radicals, which can cause damage to the biological system (Padh, 1991) [18]. Antioxidant enzymes like GSH-Px, SOD and CAT are vital in combating the harmful free radicals (ROS: Reactive oxygen species). SOD changes superoxide radicals into water & hydrogen peroxide, while CAT converts hydrogen peroxide into oxygen and water and GSH-Px helps in destruction of organic peroxides and hydrogen peroxide. Charinya and Sunthamala (2023) [3], reported increase in MDA values in blood in stressed goats. Stress can increase oxidative damage, which leads to MDA and free radical generation, indicative of oxidative stress. Rasha *et al.*, (2020) [19], reported that serum SOD, catalase and

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GSH-Px level significantly decreased in heavy infection, in comparison to control whereas significant increase in Serum lipid peroxidation (MDA) level was seen in infected sheep and goats.

Many physiological disturbances are caused by the gut parasites in host body like weight loss, metabolic disturbances, haematological alterations and increased susceptibility to oxidative stress, Khan, *et al.,* (2015) [11].

The Present research was planned in order to study the effect of commonly used anthelmintics on oxidative stress biomarkers in goats suffering from gastrointestinal nematodosis.

Material and Methods
Screening and experimental group formation
A total of 146 goats having FAMACHA score from 3 to 5 were examined to determine the prevalence of helminthosis in the Tarai region around Pantnagar through target selective treatment (TST).Out of these 146 goats, 103 were found positive for GI nematodosis. From these 103 goats 18 goats of either sex (n = 18; 9 months to 5 yrs of age weighing between 10-30 kgs) having egg counts (>200) were separated and divided randomly in three groups. The animals of Group I: (n=6) were treated with fenbendazole @ 5 mg/kg body wt. po; Group II: (n=6) were given ivermectin @ 0.2 mg/kg body wt. po. The animals of Group III: (n=6) were the control without any treatment. The FAMACHA system is an affordable and validated method for evaluating the extent of anemia, primarily attributed to *H. contortus* infection. Van Wyk and Bath (2002) found that when farmers used and carried out treatments based on FAMACHA score instead of PCV determinations, there was 58% reduction in the number of treatments required at 10 farms in comparison to previous year.

The present study was conducted at the Dept. of Veterinary Medicine, (CVASc) and Biochemistry Dept. (CBSH), Pantnagar. Approval was taken from Institutional Animal Ethical Committee of college vide proposal No IAEC/C.V.A.Sc./VMD/487 dt 25/10/2021 & dt 28/09/2022.

Collection and analysis of biochemical parameters
Five millilitres of jugular vein blood was collected in serum vacutainers with clot activators under aseptic condition from each of the goats on 0 day (before treatment), and days 7, 14 and 21 (after treatment) using sterile syringe and needles. Blood after collection was kept at room temperature to facilitate clotting and was centrifuged for separation of serum. Eppendorf tubes were used to transfer the serum samples maintained at 4 °C till they reached the laboratory for estimation of various antioxidant parameters.

Assessment of antioxidant status
The present investigation focused on the assessment of lipid peroxidation (LPO) and enzymatic antioxidant indices, including GSH-Px, CAT, and SOD, in the serum of goats. To conduct the analysis, we utilized the Activity Assay kit from Real Gene Labs, Ghaziabad, India, following the instructions provided in the user manual. The measurements were taken on day 0 and day 7, 14, and 21.

Estimation of malondialdehyde (MDA) in the blood serum was done for assessment of lipid peroxidation. This marker serves as an indicator of oxidative damage caused by lipid peroxidation.

Principles of Detection
a) Glutathione peroxidase (GSH-Px) is a crucial peroxidase enzyme widely distributed in the body and its main role is to change reduced glutathione (GSH) into oxidized glutathione (GSSG), thereby converting harmful hydrogen peroxide into non-toxic hydroxyl compounds. The activity of GSH-Px was assessed based on the decrease in absorbance at 412nm, resulting from the formation of a characteristic compound when DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) reacts with GSH.

b) Superoxide dismutase (SOD) is an omnipresent enzyme found in animals, microorganisms, plants and cultured cells. It catalyses the conversion of superoxide anions into hydrogen peroxide (H_2O_2) and oxygen (O_2), thus playing a crucial role in the biological antioxidant system. To evaluate SOD activity, we utilized the xanthine and xanthine oxidase reaction system, where superoxide anions (O_2-.) are generated. These anions reduce nitrogen blue tetrazolium to form blue formazan, which absorbs light at 560nm. By measuring the intensity of the blue colour, we can determine the SOD activity, as higher activity leads to a lighter blue colour due to the inhibition of formazan formation. The enzyme activity of SOD in reaction system is defined as a unit of enzyme activity (U/ml) when the inhibition percentage is 50% in the above xanthine oxidase reaction system.

c) Catalase is a ubiquitous enzyme found in various organisms, including animals, plants, microorganisms, and cultured cells. Its primary function is to scavenge hydrogen peroxide (H_2O_2), making it an essential component of the reactive oxygen scavenging system. H_2O_2 exhibits a distinct absorption peak at 240nm. By decomposing H_2O_2, catalase reduces the absorbance of the reaction solution at 240nm over time. Catalase activity can be determined by calculation of the rate of change in absorbance. One unit of enzyme activity is defined as the amount of enzyme which catalyses degradation of one μmol of H_2O_2 in the reaction system per minute every millilitre of serum (plasma).

d) Lipid peroxidase is produced when unsaturated fatty acids of lipids gradually decompose into various complex compounds like malondialdehyde (MDA) due to the activity of oxygen free radicals. Lipid oxidation values are often measured by detecting the level of MDA. A red product with highest absorption peak of 532nm is formed due to condensation of MDA with thiobarbituric acid (TBA). Through colorimetry, lipid peroxide content in the sample can be calculated. Concurrently absorbance at 600nm is measured, and difference in absorbance between 532nm and 600nm is used to calculate the MDA content.

Statistical analysis
All results were expressed as mean ± standard error of the mean (S.E.M) for statistical analysis. Two-way ANOVA and post-hoc Tukey's test were employed to determine any significant differences among the groups at the same sampling time. Statistical significance level for comparisons was set at $p<0.05$, meaning that P values less than 0.05 were considered significant.

Results & Discussion
Significant rise in the levels of serum GSH-Px, CAT and SOD were observed in goats treated with Ivermectin and

Fenbendazole on the 21st day post treatment. Conversely non-significant change in values of GSH-Px, SOD, and CAT in comparison to values on 0 day were seen in the untreated control group on the 21st day post treatment. MDA indicates increased oxidative stress as it represents the final outcome of lipid peroxidation in body tissues or biological fluids (Halliwell and Chirico, 1993) [7]. Previous studies by Dede *et al.,* (2000) [4] reported increased MDA levels in Akkaraman sheep infected with *Trichostrongylidae, Fasciola spp.* and *Eimeria spp.*, while Simsek *et al.,* (2006) [22] and Dimitrijevi´c *et al.* (2012) [5] found increased MDA levels with *Dicrocoelium dendriticum* and *Strongyloides Papillosus* infestations in sheep, respectively. However, the treatment with Ivermectin and Fenbendazole resulted in a lower MDA level compared to the positive control group, indicating improved antioxidant status.

In goats suffering from GI nematodosis, the level of lipid peroxidation decreased significantly ($p<0.05$) on the 21st day after treatment with fenbendazole and ivermectin, while it showed a significant increase in the untreated control group on same day.

Estimation of MDA allows the indirect detection of the level of lipid peroxidation and free oxygen radicals. In this experiment, the MDA levels exhibited an opposite trend to those of GSH-Px, SOD, and CAT. Similarly, various studies (Rashid, 2016; Heidarpour, *et al.,* 2012; Kolodziejczyk, *et al.,* 2006) [20, 9, 13] have reported increased lipid peroxidation during helminth infections. Jaheed *et al., (*2020) [10], reported that, significant decrease in total antioxidant capacity was found in serum biochemistry of infected animals. Values of GSH-Px decreased significantly in goats suffering from GI nematodosis whereas values of malondialdehyde increased significantly.

Effect of Fenbendazole and Ivermectin drug administration on oxidative stress related parameters in goats suffering from GI nematodosis

Groups	Days	Glutathione peroxidase (mu/ml)	Superoxide dismutase (U/ml)	Catalase (u/ml)	MDA (nmol/ml)
Group-1 (Fenbendazole)	0	42.833 A	33.000 A	23.667 A	11.250 A
	7	40.417 A	32.900 A	23.333 B	11.583 A
	14	47.167 B b	34.667 B b	25.833 B b	10.833 B ab
	21	115.667 C c	84.667 B b	56.667 B b	8.000 B b
Group-II (Ivermectin)	0	42.833 A	33.500 A	26.500 A	11.100 A
	7	42.417 A	33.333 A	23.833 B	11.100 A
	14	63.500 A a	41.333 A a	32.000 A a	9.900 B b
	21	138.500 B b	94.333 A a	69.833 A a	4.800 C c
Group-III (Control)	0	40.250 A	32.833 A	26.100 A	10.483 A
	7	40.583 A	33.250 A	26.500 A	10.833 A
	14	42.000 B b	32.333 B b	23.917 B b	12.833 A a
	21	44.333 A	34.167 C c	30.167 C c	12.500 A a

Capital letter (A, B, C etc.) Indicates significant difference among the drugs ($p<0.05$)

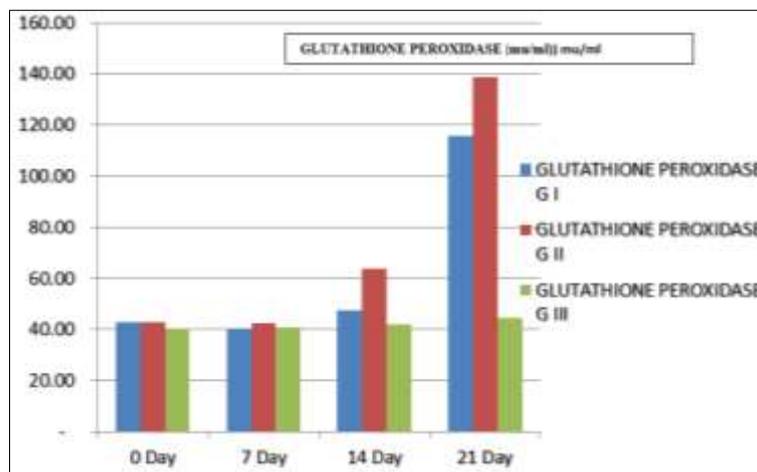Small letter (a, b, c etc.) Indicates significant difference among the days ($p<0.05$)

Fig 1: Effect of Fenbendazole and Ivermectin drug administration on Glutathione peroxidase in goats suffering from GI nematodosis.
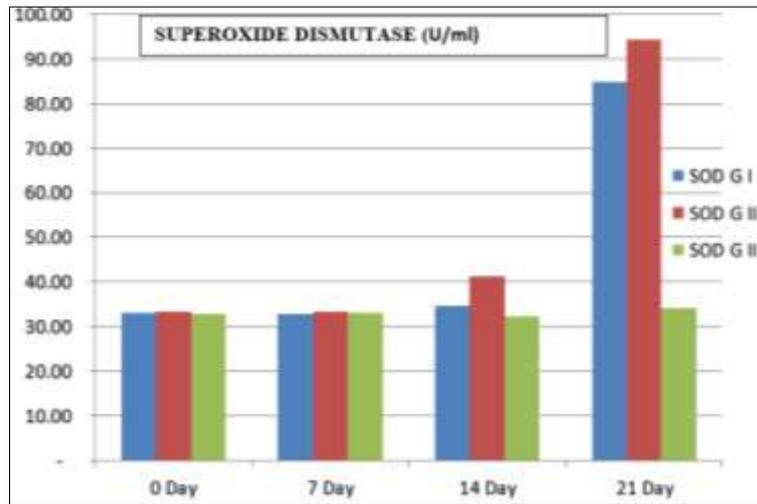
Fig 2: Effect of Fenbendazole and Ivermectin drug administration on Superoxide dismutase in goats suffering from GI nematodosis
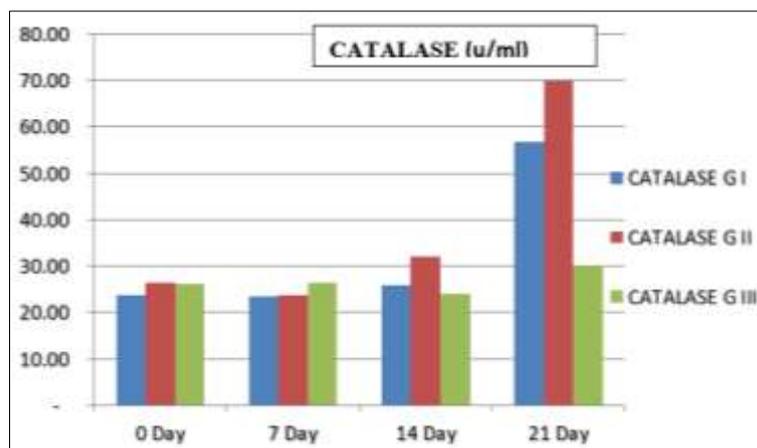

Fig 3: Effect of Fenbendazole and Ivermectin drug administration on Catalase in goats suffering from GI nematodosis
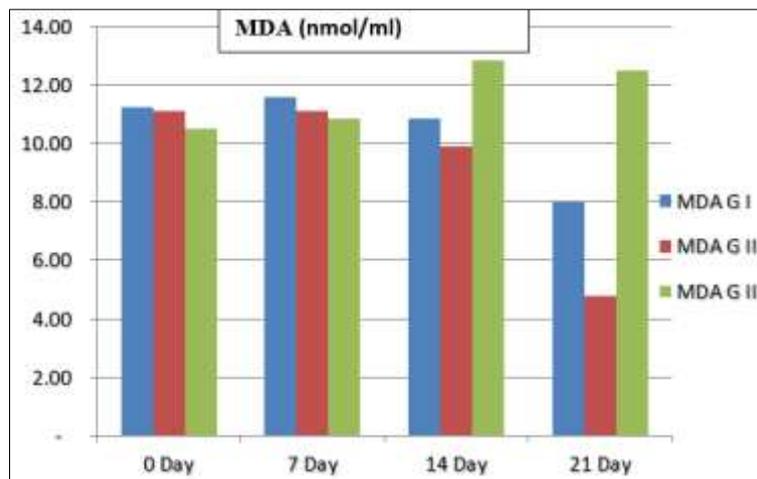

Fig 4: Effect of Fenbendazole and Ivermectin drug administration on malondialdehyde in goats suffering from GI nematodosis.

Conclusion
The present study reveals that nematodosis in goats leads to protein oxidation and oxidative stress. However, treating the infected goats with anthelmintics, reduces the worm load, and improves their antioxidant status. Alteration in serum biochemistry of the infected animal's had strong correlation with the amount of damage and infection severity, Esmaeilnejad *et al*., (2012) [6].

These findings suggest that there is an increase in the level of free radicals coupled with oxidative stress in goats infested with gastrointestinal nematodes. Additionally, the administration of anthelmintics during the early stages of treatment may add to the chemical stress experienced by the animals, Dimitrijevi´c *et al.,* (2012) [5].

Charinya and Sunthamala (2023) [3], reported increase in MDA values in blood in stressed goats. Stress increases the oxidative damage, which leads to generation of MDA and free radicals which is indicative of oxidative stress. Rasha *et al*., (2020) [19], reported that serum SOD, CAT and GSH-Px level significantly decreased in heavy infection, compared to

untreated control whereas MDA level increased significantly in the infected sheep and goats.

After 21 days post-treatment, when the worm burden decreased and the chemical stress from anthelmintics subsided, the serum levels of GSH-Px, SOD, and CAT showed significant increase in goats of both Group I and Group II, in comparison to the untreated control group (Group III). Furthermore, MDA values in Group I and II revealed a significant decrease, indicating that goats treated with anthelmintics were able to achieve and maintain adequate antioxidant compensation, thereby reducing MDA levels.

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Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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