TOXICOPATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE

T H E S I S

Submitted
In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
VETERINARY PATHOLOGY

BY
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(INDIA)

2021

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"TOXICOPATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE"** or part there of has not been submitted for any other degree or diploma of any University, not the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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WAGHMARE NILESH ASHOK has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled **"TOXICOPATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE"** submitted by him is the result of research work is sufficient to warrant its presentation to the examination in the subject of **VETERINARY PATHOLOGY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part there of has not been previously submitted by him for a degree of any other University.

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This is to certify that the thesis entitled **"TOXICOPATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE"** submitted by **WAGHMARE NILESH ASHOK** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY PATHOLOGY** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Place: Akola **(Waghmare Nilesh Ashok)**

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LIST OF ABBREVIATIONS

Abbreviation		Full form
%	-	Per cent
/	-	Per
@	-	At the rate of
<	-	Less than
µg	-	Microgram
µ	-	Micron
ALT	-	Alanine aminotransferase
AST	-	Asparate aminotransferase
b. wt.	-	Body weight
BUN	-	Blood urea nitrogen
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals
CRD	-	Completely Randomized Design
Cumm	-	Cubic millimetre
DLC	-	Differential leucocyte count
ELISA	-	Enzyme-linked immunosorbent assay
EOC	-	Ethanolic extract of *Oxalis corniculata*
ESR	-	Erythrocyte sedimentation rate
et al.	-	Et alia (and others)
etc.	-	Etcetera
Fig.	-	Figure
fL	-	Feltometer
Gm	-	Gram
gm/dL	-	Gram per decilitre
H&E	-	Hematoxyline and Eosin
Hb	-	Haemoglobin
hrs	-	Hours
i.e.	-	That is
IAEC	-	Institutional Animal Ethical Committee
IM	-	Intramuscular
IP	-	Intra peritoneal
U/L	-	International Unit per litre
IV	-	Intravenous
Kg	-	Kilogram
LD_{50}	-	Lethal dose

MCH	-	Mean corpuscular haemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
Mg	-	Milligram
mg/dL	-	Milligram per decilitre
mg/kg	-	Milligram per kilogram
mg/L	-	Milligram per litre
ml	-	Milli litre
mmol/L	-	milli molar per litre
MOC	-	Methanolic extract of *Oxalis corniculata*
NS	-	Non Significant
PCV	-	Packed cell volume
Pg	-	Picogram
PO	-	Per orally
ppm	-	Part per million
RBC	-	Red blood corpuscles
Reg. No.	-	Registration Number
SC	-	Subcutaneously
SGOT	-	Serum glutamate oxalatacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
TEC	-	Total erythrocyte count
TLC	-	Total leucocyte count
U/L	-	Unit per litre
viz.	-	Namely
WBC	-	White blood corpuscles
WHO	-	World health organization
wt.	-	Weight
^{0}C	-	Degree celcius

CHAPTER I

INTRODUCTION

Since the start of human civilization, peoples are using many herbs and herbal products which can be confirmed from Bible, The Iliad, The Rig Vedas, History of Herodotous, etc. Nature is a good source for medicinal drugs/ agents since the beginning of the human beings. It has been recorded that India has a good wealth of traditional knowledge and wealth for Ayurveda (Kumar *et al*., 2013). In India several medicinal plants are routinely utilized for the treatment of numerous diseases or disease conditions without having proper knowledge of their efficacy and safety which may results into toxic effect of such a herb on human as well as on animal health. But without the right information about efficacy and safety of those traditional medicines sometimes it might be harmful and may even cause death. According to World Health Organization (WHO), about 80% of the world's population living in developing countries, people relies essentially on plants for primary health care. Hence, herbal medicine associated pharmacology and pharmaceutical products are required to update frequently (McKay and Blumberg, 2007). Research conducted in previous decades has validated several such claims for use of traditional medicinal plants in human and animal health.

Oxalis corniculata L. plant belongs to Oxalidaceae Family of Genus Oxalis. Oxalises are a large genus of flowering plants in Oxalidaceae family. Oxalidaceae comprising about 8 genera and 900 species and are distributed in worldwide and amongst it 2 genera and dozen of species have been reported in all over the India (Mushir *et al.*, 2015). It is found to be highly distributed in forests and grasslands. This weedy species are commonly found in lawns, gardens, waste areas and green houses. The genus occurs

throughout the planet, apart from the polar areas. Many of the species are referred to as wood sorrels (sometimes written "Woodsorrels" or "Wood-sorrels") as these plant have an acidic taste like Sorrel proper (*Rumex acetosa*), which is simply distantly related. After the color of their flowers the species are also known as a yellow sorrels or pink sorrels. Other species are colloquially called as false shamrocks, and a couple of called sourgrasses. Species of Oxalis are notorious for oxalate accumulation, hence the name given to the genus and genus entirely known as by the term Oxalis. The amounts of oxalate in *O. per-caprae* are reported in ranges of 3.7 – 14.9 % soluble and 5.9 – 16.6 % total, and in *O. conriculata* as 4.1 % soluble and 7% as a total (Libert ant Franceschi, 1987). Within plants, the sap pH is low and therefore the oxalate may occurs as a free acid within the vacuoles and also may be because the acid/potassium salt (Oke, 1969).

The plant *Oxalis corniculata L.* (creeping wood sorrel) also called procumbent yellow sorrel belongs to Oxalidaceae is a common weed of garden, lawns and pastures. *Oxalis corniculata* mainly capable of growing in open, disturbed places but it perform well in shady areas (Holm *et al.,* 1977). The herbaceous plant *Oxalis corniculata* having many of the weedy characteristics is readily self pollinated and produced copious seeds in a short period of time. It is considered as a cosmopolitian weed of tropical countries and temperate zone mainly in gardens, lawns, pastures etc. Spread of this plant is accidental since its oxalic acid containing leaves are used for culinary purpose and for medicinal and herbal values. The leaves of *Oxalis corniculata* are quite edible with a tangy taste. The whole plant is rich in Vit-C. The plant leaves having three major C-glycosylflavans reported are isoorientin, isovitexin and swertisin of creeping oxalis (Mizokami *et al.,* 2008) and is used in wound healing as an antibacterial activity. Traditionally it is utilized in

anaemia, dyspepsia, cancer, piles, diuretic, convulsions (Chetty *et al.,* 2008, Srikanth *et al*., 2018). This plant has been used by tribal people in Orissa as a local medicine to cure the headache, skin diseases and for good digestion. This plant is popular for its medicinal values as a remover of vata and kapha in Ayurveda. In Indian traditional medicine, fresh leaf juice mixed with buttermilk is taken daily to cure jaundice. *Oxalis corniculata* is main ingredient of Unani medicine, "Changeri" is a cooling and appetizing drug used to treat fever biliousness and dysentery. *Oxalis corniculata* shows marked antibacterial activity against *E. coli* (Sreejith *et al*., 2014). But *Oxalis corniculata L.* is a member of the genus oxalises as they contain oxalic acid, giving the leaves and flowers a sour taste which make them refreshing to chew. In very large amounts, oxalate may be considered slightly toxic, interfering with proper digestion and kidney function.

Toxicities caused by *Oxalis corniculata L.* plant in livestock are reported and are found to be toxic to livestock once they eaten a huge quantity of plant. Clinical symptoms like muscle tremors, staggering gait, collapse and sudden death are mainly observed in acute cases (Simmonds *et al*., 2000). Literature scanned revealed very scanty information on experimental toxicity of *Oxalis corniculata* in livestock or in experimental animals showing hematobiochemial and histopathological alterations in visceral organs. Kidneys and gastrointestinal organs are found to be mostly affected in *O. corniculata* toxicity in animals.

Considering the availability of *Oxalis corniculata L.* in most part of the India and its acute, subacute or chronic toxicity in animals which may not be reported due to paucity of literature, hence, the present investigation was carried out to study the subacute toxicity of *Oxalis corniculat L.* plant in mice with following objectives.

1) To study toxicity effect of *Oxalis corniculata* on general performance and hemato- biochemical parameters in mice.

2) To study histopathological alterations in visceral organs during *Oxalis corniculata* toxicity in mice.

CHAPTER II

REVIEW OF LITRATURE

Herbal medicine has become a topic of global importance and plays a major role in the health care system of world's population. Traditional use of plants for treatment of different infections is widely practiced in India as well as in all developing countries. Scientific literature revealed effective medicinal value of different medicinal plants for treatment of different ailments of human as well as in domestic animals. But many of the medicinal plants contain some toxic principles and are responsible for toxicity in human and animals. The literature scanned on plant *Oxalis corniculata* showed many medicinal uses however, there is very little literature available on toxicity of it either in human or in animal species. Hence, the present investigation was carried out to study toxicopathological effect of *Oxalis corniculata* plant in mice.

Literature scanned on toxicological pathology of plant *Oxalis corniculata* and other related species of plants are grouped under following heads.

2.1 Physical characteristics and phytochemical constituents of *Oxalis corniculata*

2.2 Mechanism of toxicity of *Oxalis corniculata*

2.3 General performance and clinical observation

2.4 Hematological and biochemical investigation

2.5 Gross and histopathological observations

2.1 Physical characteristics and phytochemical constituents of *Oxalis corniculata*

Oxalis corniculata is an annual/ perennial creeping woodsorrel growing to 0.1 m (4inches) by 0.3 m (1ft). Flower stage is from June to September. The species is hermaphrodite (has both male and female organs) and is pollinated by insects. The plant is self-fertile and is suitable in light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils (Srikanth *et al.,* 2017).

Taxonomic classification (Srikanth *et al.,* 2017)

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Oxalidales
Family	:	Oxalidaceae
Genus	:	Oxalis
Species	:	*O. corniculata*

Vernacular Names (Srikanth *et al.,* 2017)

Sanskrit	:	Ambashta, Amlalonika, Amlapatrika, Amlika, Amlotaja, Cangeri
Hindi	:	Seh-patti, Tinpatiya, Anboti, Chuka tripati, Bhilmori, Khatari
English	:	Indian sorrel
Urdu	:	Khatt-i-buti
Assamese	:	Changeritenga, Saru tengesi
Bengali	:	Amrul-sak, Amrul shak, Amrul, Tandi chatom arak, Amrool
Kannada	:	Huli-huniche, Hulihunice, Pullam-purachi-sappu, Teltuppi
Tamil	:	Palaikiri, Puliyarail
Telugu	:	Ambotikura, Pulichintha, Pallachintha
Marathi	:	Ambali, Chicha
Malayalam	:	Poliyarala, Puliyaral, Puliyarala, Puliyarila, Pullampurachi
Marathi	:	Umbuti, Ambuti, Bhinsarpati, Aambotee, Ambata chukaa
Oriya	:	Sialthur, Siakthur, Ambo chingari
Arabic	:	Hememdab, Hemda, Homadmad

Phytochemical investigations of *Oxalis corniculata Linn*. have revealed the presence of tannins, palmitic acid, a mixture of 8 oleic, linoleic, linolenic and stearic acids. Methanolic and ethanolic extracts of this plant showed the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavonoids, proteins (12.5%), amino acids and volatile oil (khare, 2007). It also showed the presence of calcium, fiber and tannin. Leaves contain tartaric acid and citric acids, calcium oxalate, flavones (acacetin and 7,4'-diOMe apigenin), glycoflavones (4'-OMe vitexin, 4'-OMeiso-vitexin and 3',4'-diOMe orientin), flavonols (3',4'-diOMe quercetin) and phenolic acids such as p- hydroxybenzoic, vanillic and syringic acids (Sharma and Kumari, 2014). This herb is well known to have an acid taste due to the high content of oxalate in its leaves and stems. Study revealed the presence of three Cglycosylflavones in the leaves namely 6-C-glucosyl luteolin (isoorientin), 6-Cglucosylapigenin (isovitexin) and isovitexin 7- methylether (sertisin) (Srikanth *et al.,* 2017).

2.2 Mechanism of toxicity of *Oxalis corniculata*

Normally after ingestion of the oxalate containing plant, calcium present in the stomach may react with the oxalates to form insoluble salts which cannot be absorbed and eliminated in the feces. This process is more effective in ruminants than simple stomach animals and consequently, ruminants can consume large amounts of *Oxalis corniculata* plant without any apparent signs of toxicity. However, when very large quantities are ingested that exceeds and capacity of digestive tract to convert the soluble oxalates into calcium oxalate, the soluble oxalates get absorbed through intestinal mucosa and are available to interact with blood where it bind serum calcium to form calcium oxalate causing acute hypocalcemia. Calcium oxalate may also crystallize in the brain (Garg, 2002).

Further in the kidney, calcium oxalate may precipitate to form large crystals, resulting in renal tubular damage and renal insufficiency. Oxidative injury caused by generation of free radicals may exacerbate the renal tubular injury damage. (Gwaltney-Brant, 2013).

2.3 Phytochemical analysis of ethanolic extract of *Oxalis corniculata*:

Raghavendra *et al*. (2005) studied phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. *Oxalis corniculata Linn*. was tested for antibacterial activity against three important pathovars of *Xanthomonas* and fourteen human pathogenic bacteria. Powdered leaf material was extracted with different solvents viz., petroleum ether, benzene, chloroform, methanol and ethanol using Soxhlet apparatus. In ethanolic extract they detected the presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins and aminoacids and volatile oils.

Khare (2007) studied indian medicinal plants. In this survey he reported that the *Oxalis corniculata* leaves contain the flavonoids, vitexin, isovitexin and vitexin-2" –O-beta-D-glucopyranoside. The leaves contain 1.47% of lipid (dry weight), a rich source of essential fatty acids and alpha- and beta-tocopherol (1.58 and 6.18 mg/g dry basis, respectively.) They are a good source of vitamin C (125 mg/100 g), carotene (3.6 mg/100 g) and calcium (5.6% of dry material) but contain a high content of oxalates (12% of dry material). The leaves and stem contain tartaric and citric acid; stems contain also malic acid.

Sharma and Kumari (2014) reviewed phytochemistry, pharmacology and therapeutic application of *Oxalis corniculata Linn*. In this reveiw they revealed that the plant *Oxalis corniculata* containing wide ranges of phytochemical constituents like flavonoids, tannins, phytosterols, phenol, glycosides, fatty acids, galacto-glycerolipid and volatile oil. The leaves contain flavonoids, iso vitexine and vitexine-2″-O-beta-D-glucopyrunoside. It is rich source of essential fatty acids like palmitic acid, oleic, linoleic, linolenic and stearic acids.

Srikanth *et al*. (2018) reviewed phytochemistry and pharmacology of *Oxalis corniculata lin*n. In this review they revealed that wide ranges of phytochemical constituents have been isolated from the plant like flavanoids, tannins, phytosterols, phenol, glycosides, fatty acids, galacto-

glycerolipid and volatile oil. The leaves contain flavonoids, iso vitexine and vitexine-2”- O- beta – D- glucopyrunoside. It is rich source of essential fatty acids like palmitic acid, oleic, linoleic, linolenic and stearic acids.

2.4 General performance and clinical observation

Walker (1939) reported mortality occurred in two mobs of travelling sheep of Bellata due to ingestion of *Oxalis corniculata.* The clinical signs observed were depression, slight trembling and staggering gait, recumbent position on their briskets with their necks outstretched along the ground. Dead sheep lay in the same position as live sick sheep.

James (1972) studied oxalate toxicosis in different animals. In this study they found that the sheep having acute toxicity showed rapid and labored respiration, depression, weakness, coma, and almost death. Some animals showed convulsions and some exhibited a mild tetany. The tetany wasnot observed as frequently as would be expected in hypocalcemia.

Singh *et al.* (1995) studied clinicohaematological and biochemical alterations in ethylene glycol induced acute nephrotoxicity in cow calves. 12 healthy male cow calves of about 4-5 month age weighing around 40-50 kg were used. Acute nephrotoxicity was developed in the calves of group A by giving ethylene glycol @ 12 mg/kg b wt for 2 days continuously by oral route where as calves of group B served as healthy control. They found that the treated calves showed progressive depression, hypersalivation, ataxia, incordination, staggering gait, grinding of teeth, recumbancy, coma, convulsion and last death.

Simmonds *et al*. (2000) studied palatability and potential toxicity of australian weeds to goats and found that goats had acute oxalate poisoning due to eating of *Oxalis* spp. Plants in large quantity showed signs of muscle tremors, staggering gait, collapse and sudden death. They further suggested that chronic kidney disease with associated ill thrift is possible when large amounts of oxalate producing plants are eaten over long periods.

Fang *et al*. (2001) studied acute oxalate nephropathy induced by star fruit in rats. Male Sprague-Dawley rats of 180 to 200 g were assigned to four groups namely control, experimental, fasting, and water-deprivation groups. Experimental groups were fed tap water and star fruit juice @ 4 mL/100 g of body weight. No rat in any group died before being killed during this experiment. No abnormal motor disorder, such as seizure, tetany, or hiccups, was observed in any of the group. Weakness and diarrhea was observed in three groups fed sour star fruit juice.

Aslani *et al*. (2011) reported acute oxalate intoxication associated with ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep. Affected animals displayed signs of depression and weakness and were generally found on sternal or lateral recumbency and were unable to rise even with assistance. Some of the recumbent ewes showed S-form deviation of the neck and others turned their heads into their flanks. Body temperature was within the normal range and respiration was dyspneic with excessive sero-mucus dripping from external nares. Some animals died suddenly without observable clinical signs.

Reddy (2012) evaluated safety and potential toxicities of methanolic extract of *Oxalis corniculata l.* (MOC)whole plant by conducting acute and chronic toxicity studies in rat. Study on acute toxicity of extract was found to be safe when given @ 2000 mg/kg body weight orally. General behavior, adverse effects and mortality was observed for up to 14 days. However no signs of tremor, convulsions, salivation, diarrhea, lethargy, sleep and coma were observed. In chronic toxicity study, MOC was administered orally @ 100, 200 and 400 mg/kg once in a week for 6 weeks. The body weight of the rats was slightly increased. But there were no changes observed in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system. The onset of toxicity and signs of toxicity were also not observed. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of chronic study.

Singh and Prakash (2014) studied toxicity assessment of *Oxalis corniculata* and *Phyllanthus Fraternus* plants and reported LD50 values as 1300 mg/kg BW and 1125 mg/kg BW, respectively. The death percentage of rats fed with different doses of ethanolic extract of *Oxalis corniculata* and *Phyllanthus fraternus* was found to be maximum (100%) @ 2000 mg/kg BW, while lowest mortality rate (25%) varied i.e. 800 mg per kg BW for *O. corniculata* and 400 mg per kg BW for *P. fraternus* .The 50% lethal dose (LD50) values of *O. corniculata* and *P. fraternus* were found @ 1300 mg/kg BW and 1125 mg/kg BW orally, respectively. There were no positive signs of toxicity viz. inappetence, depression, aggressiveness, respiratory distress, body weight loss, death when given @ 200 and 400 mg/kg BW.

Alebachew *et al*. (2014) studied toxicological evaluation of methanol leaves extract of *Vernonia bipontini Vatke* in blood, liver and kidney tissues of mice. Each group of mice was given different doses of methanol leaf extract. However, in long- term toxicity study, mice were administered with 400 mg/kg and 800 mg/kg b.wt. for 45 days. They reported low locomotion, weakness, erection of hairs, and white color of the eyes during acute study. During long term administration of the extracts both treated and untreated groups showed no physical changes in their appearances and signs of toxicity when given @ 400 mg/kg. However, almost all mice treated with 800 mg/kg methanol leaf extract of *V. bipontini V.* showed swellings on left lateral part of abdominal region related to spleen, weakness, frequent defecation, mild diarrhea, and enlargement of spleen when compared with control group. Final body weight in group treated @800 mg/kg b.wt. showed significant decreased in body weight compared to control.

Mugisha *et al*. (2014) studied acute and sub acute toxicity of ethanolic leaf extracts *of Rumex abyssinica Jacq*. (Polygonaceae) and *Mentha spicata L.* (Lamiaceae). Study evaluated acute and sub-acute toxicities of 70% ethanolic leaf extracts of both plants in mice and Wistar albino rats. In acute toxicity study the signs observed were hyperurination, abdominal muscle twitches and convulsions when given @ 12500 mg/kg body weight of *Rumex abyssinica Jacq* and *Mentha spicata L* each. Sub-acute toxicity was evaluated

in Wistar albino rats (6 per group) @ 500, 1000 and 1500 mg/kg for 28 days but no signs of toxicity (physical and behavioral); and no mortalities was recorded. There were no significant changes in the body weights of the treated rats when compared to control group.

Herbert and Dittmer (2016) studied the cases of acute and chronic oxalate toxicity in miniature horses associated with soursob (*Oxalis pes-caprae*) ingestion. Over the period of 11 years (2004–2015) 14 miniature horses in six separate outbreaks presented with clinical signs consistent with acute or chronic oxalate toxicity. All animals had access to *Oxalis pes-caprae* or soursob. Miniature horses with acute oxalate toxicity and hypocalcaemia had muscle fasciculations, tremors and synchronous diaphragmatic flutter these horses responded to treatment with intravenous and oral calcium. Chronic oxalate toxicity was associated with ill-thrift, stiffness, enlarged heads, kyphosis and neurological signs.

Subhani *et al*. (2018) evaluated an adverse effect of *Oxalis corniculata* on growth performance of broiler chicks during aflatoxicosis. Two different levels of *O. corniculata* (@ 250; 500 mg/kg b.wt.) with and without AFB1 (@ 350 ppb) and a control treatment were added to the diet. Dietary treatment initiated at the end of 1st week and sustained for next six weeks. During this experiment they reported that, *O. corniculata* supplementation in the diet during five weeks significantly reduced body weight, cumulative feed intake and feed conversion ratio of the treated groups in dose dependent manner. Dietary incorporation of *O. corniculata* @ 250 mg to 500 mg induced negative effects on overall broiler health performance.

Belsty *et al*. (2019) studied the evaluation of *Rumex nepalensis Spreng* root extract on biochemical and histopathologic parameters of mice liver. Group T1 received distilled water. Groups T2, T3, and T4 received root extract of *Rumex nepalensis* at 250, 500, and 1000 mg/kg/day for 28 consecutive days, respectively. During the experimental trail all male and female mice showed extract-related noticeable changes in their general behavior such as depression, piloerection, loss of appetite, and fast breathing

compared to the control group. After 28 days of experiment they found that the mice treated with 250 and 500 mg/kg/day of the root extract showed no significant changes in body weight in both sexes. Males treated with 1000 mg/kg/day of the extract had significant weight reduction, while the females did not show weight change.

Mezui *et al*. (2019) studied acute and subacute toxicity of *Oxalis barrelieri* (Oxalidaceae) aqueous aerial parts extract. A single dose of 2000 mg/kg was administered to three Swiss albino female mice and effects were observed for 14 days. In sub acute toxicity, the experimental rat six groups of 12 animals (6 males and 6 females) for each dose level of *Oxalis barrelieri* were used. Sub acute toxicity was evaluated after single daily administration of extract at 200, 400 and 800 mg/kg orally for a period of 4 weeks received aqueous extract of *Oxalis barrelieri* daily for 28 days. In acute toxicity, a single dose of aqueous extract of *Oxalis barrelieri* (2000 mg/kg) in mice did not result in any deaths in the first stage also 48 hours later, carrying out a second test did not result in any deaths. After 14 days of observation, no changes were observed in mice regarding: coat color, appearance and body weight. In subacute toxicity, All rats (male and female) treated with *O. barrelieri* extract showed a body weight gain similar to that of control rats. No loss of bodyweight was observed. Male rats had higher weight gain than female rats.

Fentahun *et al*. (2020) evaluated acute and subacute toxic effects of 80% methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) on histopathology of liver, kidney and some blood parameters in Swiss albino mice. Treatment groups were given methanolic extract of *rumex abyssinicus jacq* by intragastric tube at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight once a day for 4 weeks. No death was observed throughout the experimental period. However, gentle signs of toxicity such as depression, erection of the hair, loss of appetite and fast breathing were observed, among those mice treated with the crude extract at both doses as compared to the control group.

2.5 Hematological and biochemical investigation

Basu *et al.* (1974) reported erythrocytosis associated with chronic renal disease. In this case study erythrocytosis was noted in six patients with parenchymal renal disease. Kidney biopsies performed in four patients revealed focal sclerosing glomerulonephritis in two and chronic vascular changes with interstitial scarring in two others. The remaining two patients had heavy proteinuria suggestive of glomerular involvement. Three patients who progressed to chronic renal failure had diminution of erythrocytosis. It is concluded that parenchymal renal disease, primarily of a glomerular or vascular type may provoke erythrocytosis.

Singh *et al.* (1995) studied clinico-haematological and biochemical alterations in ethylene glycol (12 mg/kg b.wt for 2 days) induced acute nephrotoxicity in cow calves. Haematological observations revealed increase in total erythrocyte, platelets and packed cell volume till death period while total leucocyte count was increased up to day 3 which was then decreased on day 4 and day 5. TEC, platelets and PCV were significantly increased. In biochemical study they revealed that urea nitrogen level was increased to 322.00 ± 0.0 mg/dl on day 5. Creatinine and uric acid levels were progressively increased upto 5^{th} day. Calcium and chloride level was reduced from day 1 to 5 and potassium from day 2 to 5. The activities of AST, ALT and alkaline phosphatase enzymes were increased progressively.

Fang *et al*. (2001) studied acute oxalate nephropathy induced by star fruit in rats. Biochemical findings were recorded revealed greater peak serum creatinine level ($P < 0.05$). Mean serum creatinine levels on day 0, 1, 2, 3, 4, and 5 were 0.43 ± 0.03, 1.11 ±0.18, 1.31 ± 0.27, 1.16 ± 0.28, 0.8 ±0.26, and 0.82 ± 0.28 mg/dL, respectively. Pearson's correlation analysis of peak serum creatinine level and kidney weight for the experimental group showed a significant correlation (R 0.75; $P < 0.05$; n=9).

Radostits *et al.* (2007) documented that acute oxalate poisoning in sheep. Packed cell volume was decreased to 15 to 20% whereas blood urea nitrogen increased to 85 mg/dl (30.3mmol/litre) in oxalate toxicity. There may

be accompanying elevation of blood potassium, albuminuria and haematuria occasionally in oxalic nephrosis. Proteinuria is also present.

Aslani *et al*. (2011) reported acute oxalate intoxication associated to ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep. Hematological observations revealed mild elevation of packed cell volume (0.37–0.38 L/L), neutrophils (6.18–8.13×109/L), and plasma total protein (76 to 83 g/L). There was marked hypocalcemia (0.73 to 0.97 mmol/L), relatively high phosphorous (1.52 to 3.55 mmol/L) and glucose (2.42 to 10.77 mmol/L), and marked elevation of BUN (26.95–35.03 mmol/L) and creatinine (314.70–476.47 mmol/L) concentrations. The activity of serum AST, ALT, AP, and GGT were in the normal range but there was high activity of serum CK (440 to 4971 U/l).

Bajaj *et al*. (2011) studied induced oxalate toxicity by *ad libitum* feeding of Napier grass *(pennisetum purpureum)* on health of buffalo calves with deprivation of water. They found increased leukocyte count ($P<0.01$) from 9.68 to 16.92x10/mm3 with significantly decrease in packed cell volume from 31.80 to 26.67 and slight increase in haemoglobin from 10.47 to 10.92 g/dl. Biochemical observations revealed rise ($P<0.01$) in plasma creatinine from 0.81 to 1.79 mg/dl and blood urea nitrogen from 8.90 to 12.88 mg/dl with slight increase in aspartate amino transferase enzyme activity. The significant ($P<0.01$) decrease in plasma calcium level from 9.13 to 6.19 mg/dl along with decrease in inorganic phosphorus level (from 5.72 to 3.94 mg/dl) lead to hypocalcaemia.

Rahman *et al*. (2012) reviewed published data on oxalate poisoning in domestic animals with a special focus on tolerance and performance and suggested that oxalic acid is one of the anti-nutrients found in forages. It can bind with dietary calcium (Ca) or magnesium (Mg) to form insoluble Ca or Mg oxalate, which then may lead to low serum Ca or Mg levels as well as to renal failure because of precipitation of these salts in the kidneys. Rumen bacteria degraded oxalate, hence non-ruminants appear to be more sensitive to oxalate than ruminants. In chronic oxalate poisoning,

filtration of insoluble Ca oxalate by the kidneys causes severe damage to the kidney tubules. If animals do not die from the acute effects of the low blood Ca levels and impaired cellular energy metabolism, death results from kidney failure.

Reddy (2012) conducted an experiment on acute and chronic toxicity studies of methanolic extract of *Oxalis corniculata l.* in rat. In chronic toxicity study, hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) from that of the control group and all the values remained within normal limits throughout the experimental period. No significant treatment related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study.

Alebachew *et al*. (2014) studied toxicological evaluation of extract of *Vernonia bipontini Vatke* in blood, liver and kidney tissues of mice. The chronic effects of methanol leaf extract of *V. bipontini Vatke* revealed no significant difference in hematological and biochemical composition of blood between control and mice treated at a dose of 400 mg/kg of the extract. However, significant changes were observed in mice treated with 800 mg/kg of the plant extract when compared to control group. Red blood cell count (M/UL) significantly decreased in mice treated with 800 mg/kg of the extract. Platelet count (K/UL) also decreased considerably 800 mg/kg of the extract, while a dose of 400mg/kg showed no significant change in the platelet count. Moreover, at 800mg/kg of the extract considerably decreased MCH and MCHC and at 400mg/kg MCHC is significantly decreased. Besides, 800mg/ kg of the methanol leaf extract caused a significant increase in serum AST, ALT, and ALP levels, while 400mg/kg of the extract showed no change. Moreover, blood urea concentration increased non-significantly at 400 and 800 mg/kg of. There was no change in the total WBC (K/UL) and lymphocyte percent at all doses. Changes in Hgb, Hct and Mcv were not significant in mice treated with 400mg/kg of methanol leaf extract of V. bipontini V.

However, these hematological parameters significantly decreased in mice treated with the extract at a dose of 800mg/kg as compared to the control.

Mugisha *et al*. (2014) studied acute and sub-acute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq. (Polygonaceae) and *Mentha spicata* L. (Lamiaceae). *R. abyssinica* showed no significant effect on WBC, Lym, RBC, HGB, MCV and MCHC, although HCT significantly increased ($p < 0.05$) at 1500 mg/kg body weight. Treatment with *M. spicata* ethanolic extract caused significant increase ($p < 0.05$) in the levels of WBC, Lymphocyte and MCHC and cause significant reduction in HCT level. Liver function test revealed dose dependant increase ($p < 0.05$) in ALP and ALT enzymes in the rats treated with both extracts, although the levels were more pronounced with *M. spicata*. Kidney function test showed no alterations in serum creatinine and urea by both plant extracts. A slight insignificant increase and decrease was observed in the levels of urea and creatinine respectively, but the values were within the normal range.

Singh and Prakash (2014) studied toxicity assessment of *Oxalis corniculata* and *Phyllanthus Fraternus* plants and recorded no significant ($p>0.05$) changes in GOT, GPT, ALP, urea, uric acid and creatinine level in plasma in rats. Among these the *O. corniculata* extract displayed higher levels of urea, uric acid and creatinine than *P. fraternus* while there was not much impact on uric acid level with the treatment of plant extracts.

Selçuk *et al*. (2015) reported a case of acute tubule interstitial nephritis due to large amount of Sorrel (*Rumex acetosa*) intake in twelve year old boy. Laboratory examinations revealed mild proteinuria (dipstick), microscopic hematuria, microscopic pyuria, and calcium oxalate crystals on urinalysis; and increased alanine transaminase (ALT) (456 U/L), aspartate transaminase (AST) (79 U/L), creatinine (1.17 mg/dL, 103.43 mmol/L), and urea (51 mg/dL, 8.5 mmol/L) levels. Complete blood count was normal. Urinalysis revealed phosphaturia, glucosuria, and generalized aminoaciduria.

Musale *et al.* (2017) investigated efficacy of calcium borogluconate and lime water therapy in *Anagallis arvensis* intoxicated cattle. In this experiment he recorded Haematological analysis of affected cattle revealed $p<0.01$ decrease in Hb (g %), PCV (%) and TEC (10^6/µl). There was significant ($p<0.01$) neutrophilic leukocytosis with eosinophilia and lymphopenia. Blood biochemical profile indicated significant ($p<0.01$) hypoproteinemia, hypoalbunemia, hypoglobulinaemia, decreased A:G ratio, hypocalcemia and elevated AST and ALT activities. There was highly significant ($p<0.01$) increase in BUN and craetinine in *Anagallis arvensis* toxic cattle.

Mezui *et al*. (2019) studied acute and subacute toxicity of *Oxalis barrelieri* (Oxalidaceae) aqueous aerial parts extract. The satellite group was treated with the extract of *Oxalis barrelieri* (800 mg/kg) for 4weeks. In acute toxicity**,** the higher dose of extract (800 mg/kg),the rate of red blood cells decreased significantly ($p<0.05$) two weeks after treatment in male rat. There was significant increase ($P<0.001$) in ASAT activity in male and female rats two weeks after extract administration, and a reversible significant increase ($P<0.05$) in triglyceride level in male rats only.

Fentahun *et al*. (2020) evaluated acute and sub acute toxic effects of 80% methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) on some blood parameters in Swiss albino mice. Haematobiochemical examination revealed non significant decrease in RBC values in mice treated with @100 mg/kg b.wt. and @ 200 mg/kg b.wt. There was non significantly decrease in both WBC, HCT, HGB, MCH and PLT count @ 100, 200 and @400 mg/kg b.wt. compared to control group. Though statistically not significant ($p > 0.05$) the values of MCV and MCHC and HCT and HGB at the dose of 100 mg/kg body weight/day and RBC at the dose of 400 mg/kg body weight/day were found to be increase as compared to the controls.

2.6 Gross pathological and histopathological observations

Walker (1939) reported mortality occurred in two mobs of travelling sheep of Bellata due to ingestion of *Oxalis corniculata.* On postmortem examination liver was slightly greasy and the gall bladder was distended. Intestines and abomasums showed congestion. Near the pylorus the congestion was more marked. The congestion of the small intestine was more marked than in the abomasum. The mesenteric lymphatic glands were considerably enlarged and oedematous. The serous surfaces of the heart, thoracic and abdominal cavities were apparently normal. The lungs were quite normal and the heart rather flaccid. On microscopic examination the kidneys, which had been macroscopically normal, were pathologically significant. Kidney showed very interesting changes in the cortical zone, glomeruli were contracted, whilst the epithelium of the convoluted tubules was necrotic, and in several places had desquamated. Collection of crystals were apparent throughout the whole of the conical zone and in close proximity to the convoluted tubules. The presence of these crystals was associated with deposition of fibroplastic tissue suggesting that they were definitely exerting an irritant effect. No crystals were discernible in the straight tubules.

James (1972) studied oxalate toxicosis in different animals. The gross pathological changes observed were hemorrhages and edema of the rumen wall, in some instances ascites, and sometimes hyperemia of the abomasal mucosa and also there were few gross changes in acute poisoning; and only mild kidney lesions occur in subacute toxicity. The kidneys were pale and fibrous in chronic poisonings. Histopathology includes calcium oxalate crystals in the wall of the rumen and in the tubules of the kidney. Hemorrhages were observed in the ruminal wall and microhemorrhage were recorded in the medulla oblongata.

Canfield and Dickens (1982) reported marked emphysema of the lungs with bullae forming at the margins in oxalate poisoning in a koala *(Phascolarctos cinereus)*. The bladder wall was thickened and had red streaks with turbid, dark red fluid, containing brown, granular material in the lumen.

The mucosa had petchial and ecchymotic haemorrhages having a incomplete plug of friable, brown, granular material at the urethral bladder junction**.** Both kidneys were enlarged, soft and had a large red and fine light yellow mottling on the surface. On sectioning, the kidney had white yellow corticomedullary streaks, patchy red areas in the cortex and a reddened cortico-medullary junction. Histopathological changes include haemorrhage and oedema throughout the wall and focal infiltrates of mononuclear phagocytes present in all layers of bladder. The kidneys had proximal tubular lumens filled with radially arranged, rhomboidal to triangular unstained crystals. Some of the cells lining the affected tubules were either necrotic or missing. Some tubules in the medulla were dilated with amorphous pink material and crystals composed of concentric rings. Cortico-medullary and engorged medullary vessels were observed.

Suckling (1886) reported a case of poisoning by common sorrel *(Rumex Acetosa)* in boy. The small intestine contained yellowish fluid material, containing stalks of some vegetable partially digested; these stalk were ribbed, and were about the size of sorrel stalks. The lungs were a little congested at the base and along the posterior border. The liver and kidneys were congested. The condition of the heart and the visceral congestion were compatible with and pointed to, death from collapse.

Mckenzie *et al*. (1987) evaluated acute oxalate poisoning of sheep by Buffel grass *(Cenchrus ciliaris)*. On pathological investigation kidneys were swollen with pale cortices and mild liver congestion. Histopathological results showed nephrotic with necrosis of some cortical tubules, dilation of most tubules, hyaline casts and many rosettes of birefringent calcium oxalate crystals in tubular lumens and oedema of the interstitium. Some tubular epithelium was necrotic and hyaline casts were present in some distended tubules. Some mild focal rumenitis and fatty change in the liver were also observed. The second sheep necropsied had fewer crystals but severe dilation of cortical and medullary tubules and Bowman's spaces with extensive interstitial fibrosis, thickened Bowman's capsules and some lymphocytes and neutrophils present.

Fang *et al*. (2001) studied acute oxalate nephropathy induced by star fruit in rats and recorded dilatation of renal tubules, tubular epithelium flattening, and interstitial mononuclear cell infiltration. Numerous refractile oxalate crystals were observed in some tubules. Glomeruli and blood vessels were normal. Under polarized light microscopy, there were numerous calcium oxalate crystals characterized by birefringence and organizing in fan or sheave shaped arrays. In comparison to the experimental group, the fasting and water deprivation groups showed moderate amounts of calcium oxalate crystals and less extensive tubular degeneration.

Gulbahar *et al*. (2002) reported a case of renal oxalosis in a calf and reported gross lesions of pale and soft kidneys which on the cut showed dilated thin and some renal calyces containing numerous small, pale yellow, granular calculi. Microscopically most cortical and medullary tubules were greatly dilated with birefringent crystalline casts, whereas glomeruli were unaffected. The crystals were morphologically consistent with calcium oxalate crystals and stained for calcium oxalate by Pizzolato's technique. The affected tubular epithelium was finely vacuolated and often degenerated. The cytoplasm of a few tubular epithelia contained birefringent crystals. Mild to moderate, focal tubular epithelial hyperplasia was also noted. Some tubules, especiall those in the medulla, contained PAS-positive hyaline casts that were admixed with calcium oxalate crystals, degenerated cellular debris and a few inflammatory cells. Numerous birefringent crystalline deposits were scattered in the interstitium. Diffuse, interstitial fibrosis and small, focal accumulations of lymphocytes were noted in the renal cortex and to a lesser extent in the medulla.

Khan and Glenton (2010) conducted an experiment on experimental induction of calcium oxalate nephrolithiasis in mice administered ethylene glycol, glyoxylate or hydroxyl proline via diet in male and female normocalciuric mice, and in hypercalciuric sodium phosphate co-transporter type 2 a _/_ mice for 4 weeks. All mice on ethylene glycol, glyoxylate or hydroxyl proline became hyperoxaluric and showed calcium oxalate crystalluria. No female, normocalciuric or hypercalciuric mice showed

renal calcium oxalate crystal deposits. In all mice the kidneys showed epithelial injury. Male mice particularly on glyoxylate had more renal injury and inflammatory cell migration into the interstitium around the crystal deposits.

Aslani *et al*. (2011) reported an acute oxalate intoxication associated to ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep and recorded general congestion of subcutaneous tissues, congestion, and some hemorrhages on rumen mucosa and other parts of the gastrointestinal tract, moderate hydrothorax, hydropericardium, pulmonary edema and hemorrhages. Kidneys were mottled with pale areas, swollen, and congested. The most significant histopathological finding observed were heavy deposits of typical oxalate crystals in the cortical and medullary tubules of kidneys. Renal tubular necrosis was characterized by eosinophilic tubular epithelial cells with pyknotic nuclei, and sloughed epithelial cells in lumen. Rumen showed multifocal to diffuse hydropic degeneration and necrosis of ruminal epithelial cells with precipitation of crystalline material and infiltration of inflammatory cells in lamina propria. There was mild to moderate congestion with vacuolar degeneration in the livers. There was no remarkable lesion in other tissues and organs.

Alebachew *et al*. (2014) studied toxicological evaluation of methanol leaves extract of *Vernonia bipontini Vatke* in blood, liver and kidney tissues of mice and recorded dilated sinusoids, nuclear enlargement, lots of binucleated hepatocytes, peripheral cramped chromatin, shrinkages of hepatocytes, fragmentation of hepatocytes in mice treated with 800mg/kg. While no histopathological changes were observed in liver and kidney of mice treated @ 400 mg/kg b.wt. Kidney tissue sections of mice did not show significant histopathological changes at 400 mg/kg, however, at 800 mg/kg kidney sections showed increased cellularity of glomerulus, urinary space obliteration and enlarged macula densa.

Mugisha *et al*. (2014) studied acute and subacute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq (Polygonaceae) and *Mentha spicata* L. (Lamiaceae). *M. spicata* did not cause any significant histopathological changes at various dose used. However, at 1000 and 1500 mg/kg body weight, *R. abyssinica* caused gross histopathological changes in the liver, kidney, lung and small intestinal tissues. Focal cellular necrosis, congestion and haemorrhages were observed in both the liver and kidney. The major lesions in lung included cellular infiltration and tissue degeneration were observed.

Singh and Prakash (2014) studied toxicity assessment of *Oxalis corniculata* and *Phyllanthus Fraternus* plants and reported normal histoarchitecture of liver and kidney tissues of both normal control rat as well as extracts treated rats.

Mitchell *et. al*. (2017) investigated pathology and epidemiology of oxalate nephrosis in cheetahs in North America, southern Africa and France. Histological examination showed small to very large numbers of colorless refractive crystals (average 0.3-81 crystals in three 100_ fields) in cortical and medullary tubules forming rosettes, globules, and acicular fragments that were birefringent with polarized light. Crystals were not uniformly distributed in the renal cortex, with clusters apparently occurring in single or groups of tubules and were not associated with tracts of inflammation. Affected tubules contained small amounts of sloughed necrotic cellular debris mixed with variable amounts of pale amorphous eosinophilic material and, in many cases, were lined by a discontinuous layer of epithelial cells with variable degrees of necrosis characterized by shrunken cells with hypereosinophilic cytoplasm and dark basophilic pyknotic nuclei. Adjacent tubules were variably dilated and lined by regenerative low cuboidal epithelial cells with pale basophilic cytoplasm and large crowded oval nuclei. Variable degrees of mild interstitial fibrosis and small numbers of intratubular cellular casts were present. Crystals and fine mineral deposits variably present on tubular basement membranes in the cortex and medulla stained variably positive with von Kossa, which was interpreted as the presence of calcium

phosphate and/or carbonate salts, since melanin pigment was not visible in crystals or on tubular basement membranes on hematoxylin and eosin stains.

Udobang and Okokon (2017) studied effects of subchronic administration of *Setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract on histopathological indices of rats. No toxic effect was observed at dose of 150 mg/kg but toxic effect was observed at 300 and 450 mg/kg. In liver mild toxic tissue effect was seen due to the presence of thrombosed to congested portal blood vessels and inflammation, with absence of strong indices for hepatotoxicity such as steatosis (fatty changes), necrosis, fibrosis and bile spillage. Lung showed presence of thrombosed to congested blood vessels within the intern alveolar septae and the absence of strong indices for pulmonary damage such as alveolar edema, exudates, hemorrhage, haemosiderin laden macrophages, necrosis and fibrosis was seen. Presence of thrombosed to congested interstitial blood vessels despite absence of strong indices for renal damage such as glomerular necrosis / thickening and tubular necrosis was observed in kidney. Spleen showed preserved normal architecture with variably sized lymphoid follicles and areas of hemorrhage. Histologic sections of brain show the three layers of the cerebellar cortex consisting of the granular layer, the Purkinje cell layer and the molecular layer with unremarkable features. In some areas, the cerebrum showed normal histologic findings. Sections of the heart showed striated muscle fibers admixed with areas of scanty cytoplasm. However, neither inflammation nor necrosis is seen.

Belsty *et al*. (2019) studied the evaluation of *Rumex nepalensis Spreng*. root extract on histology of liver in mice and recorded congestion of portal and central veins, sinusoid dilatation and Kupffer cell proliferation in the 500 and 1000 mg/kg of the root extract treated groups.

Mezui *et al*. (2019) studied acute and subacute toxicity of *Oxalis barrelieri* (Oxalidaceae) aqueous aerial parts extract and observed reversible slight dose dependent structural alteration of the kidney such as renal tissue damage including enlargement of the glomerular chamber and destruction of nephrons and reversible vascular congestion in liver.

CHAPTER III

MATERIALS AND METHODS

The present investigation on "Toxicopathological studies of *Oxalis corniculata* in mice" was carried out to study the toxicity effect of *Oxalis corniculata* on general performance, hematobiochemical parameters and histopathological alterations in visceral organs in mice. The experimental trial was conducted at Small Laboratory Animal House of Department of Veterinary Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences, Akola.

3.1 IAEC approval

The Institutional Animal Ethical Committee (IAEC) 312/GO/ReBi/S/2000/ CPCSEA approved a detailed mentioned experimental protocol before the start of the experiment in mice. The experimental study was conducted by following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, forest and climate changes, Government of India.

3.2 Glass and plastic wares

The glass wares used during present study were of Borosilicated glass which were clean properly as per standard method and were sterilized in hot air oven at 160ºc for one hour. The plastic wares were obtained from Tarson company and were sterilized every time before use as per standard recommended procedure.

3.3 Plant Material

3.3.1 Collection and authentication of plant materials

The weed plant *Oxalis corniculata* was obtained/procured (Plate 3.1) from the campus of Post Graduate Institute of Veterinary And Animal Sciences, Akola and also from Dr. Panjabrao *Deshmukh* Krishi Vidyapeeth, Akola.The whole plant of *Oxalis corniculata* was identified and

authenticated from expert taxonomist Dr. S. P. Rothe, Principal, Maherbanu College of art and Sciences, Akola (Plate 3.2). The plants were dried at room temperature. The shade dried plants were processed for grinding so as to obtain fine powder with the help of pulverizing machine. Obtained powder was then filtered by sieving for removal of large size fibers. Freshly prepared powder was further subjected to ethanolic extraction as per standard methodology as described under.

The fresh whole plant materials were dried in shade in room for about 2 weeks, after which these were grinded to a uniform powder. Ethanolic extracts was prepared by soaking 50 g each of the dry powdered plant materials in 600 mL of 95% ethanol at room temperature for 48 hrs with occasional shaking at every 8 hrs. It was then filtered by Whatmann filter paper (size no.1) and the filtrates were evaporated on rotary evaporator to concentrate in crude extract form at 40 ºC. There, 6.56 g light green residue (13.12% w/w) of *O. corniculata* was obtained (Plate 3.3). The aqueous suspension of this extract was made by dissolving this into distilled water according to dose values (Singh and Prakash, 2014).

3.3.2.1 Phytochemical analysis of plant extract

For different groups of phytoconstituents, qualitative phytochemical analysis was done for the presences of alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates and glycosides as described by Harborne (1998).

3.3.2.2 Experimental chemicals for phytochemical analysis

The following technical/ analytical grade chemicals were used for phytochemical analysis.

Ferric chloride	Lead acetate	Dilute HCl
Mercuric chloride	Potassium iodide	Bismuth subnitrate
Tartaric acid	Iodine	Sodium biocarbonate
Chloroform	Conc. H_2SO_4	Acetic anhydride
Ethanol	Magnesium turnings	α naphthol
Pyridine	Sodium nitroprusside	Glacial acetic acid
Sodium hydroxide	Nitric acid	Copper sulphate

Plate 3.1. Plant of *Oxalis corniculata* used for present investigation

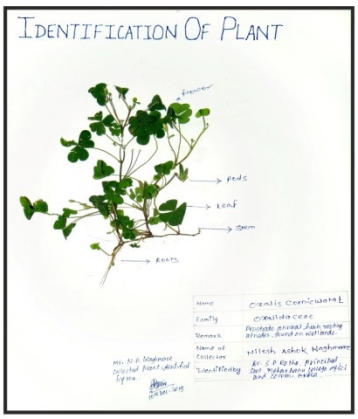


Plate 3.2. Identification of plant by taxonomist

Plate 3.3. Ethanolic extract of *Oxalis corniculata* plant

Plate 3.4. Male Albino mice used for present study

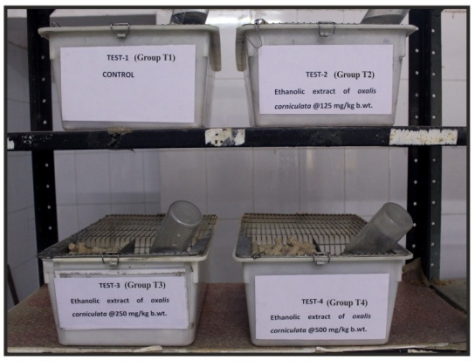


Plate 3.5. Grouping of experimental mice

3.3.2.3 Test for Tannins and Phenols

a) Lead acetate test

Few drops of extract added to few drops of lead acetate solution. Development of precipitate indicates the presence of tannin and phenols.

3.3.2.5 Test for Saponins

a) Frothing test

5 mg of test extract was added in small amount of water and sodium bicarbonate solution. Shake vigorously, formation of froth indicated the presence of saponins.

3.3.2.6 Test for fixed oils and fats

a) Spot test/ Stain test

Little quantity of plant extract is pressed in between to filter papers. Oil stain on the paper indicated the presence of terpenoids.

3.3.2.7 Test for phytosterols

a) Liebermann-Burchard Reaction

5ml of test extract was mixed with 10 ml $CHCl_3$ and 1ml acetic anhydride and few drops of concentrated H_2SO_4 were added. Green ring indicates the presence of steroids.

3.3.2.8 Test for Flavonoids (Shinoda Test)

10– 20 mg dry test extract was dissolved in 5ml of 95% ethanol and 2-3 drops of hydrochloric acid and 0.5g magnesium turnings were added in a beaker. Development of pink magenta color within 3 minutes indicates presence of flavonoids.

3.3.2.8 Test for Carbohydrates (Molisch's Test)

2-3 drops of α-naphthol solution in alcohol was added to 2-3 ml of extract solution and shaken for few minutes then 0.5 ml of concentrated H_2SO_4 was added from the side of test tube. Appearance of violet ring at the junction of two solutions indicates presence of carbohydrates.

3.3.2.9 Test for Glycosides

a) Keller-Kiliani Test

3–5 drops Glacial acetic acid, 2-3 drop 5% ferric chloride and concentrated H_2SO_4 were added to the test tube containing 2 ml of solution of test extract. Appearance of bluish green in the upper layer indicates presence of cardiac glycosides.

3.3.2.10 Test for Gums and mucilage

a) Alcohol test

100mg test extract solution was dissolved in 10 ml distilled water and 25 mL alcohol was added in it with constant stirring. White or cloudy precipitate indicates the presence of Gums and mucilage.

3.3.2.11 Test for Proteins

a) Xanthoprotein Test

2 ml of test extract was dissolved in distilled water and 0.5 ml concentrated nitric acid was added. Development of white or yellow colour indicates presence of protein.

3.3.2.11 Test for Volatile oils

a) Fluorescence test

l0 mL of plant extract was filtered till saturation and exposed to UV light. Bright pinkish fluorescence indicates presence of volatile oils.

3.3.2.12 Test for Alkaloids

10–20mg dry extract was added to 1-2ml dilute hydrochloric acid, shaken well, and filtered. With filtrate, the following tests were performed.

a) Mayer's Test

Mayer's reagent: 1.36gm Mercuric chloride dissolved in 60ml distilled water and 5gm of potassium iodide dissolved in 10ml distilled water. Mix both solutions and dilute upto 100ml volume using distilled water.

To 2-3 mL of test filtrate, 2-3 drops of Mayer's reagent were added. Appearance of cream colour precipitate indicates presence of alkaloids.

3.4 Experimental animals

Total 32 Swiss albino male mice weighing around 35 to 40 gm (Plate 3.4)were procured from CPCSEA approved Small Animal Laboratory House, Department of Pharmacology and Toxicology, PGIVAS, Akola. The experimental animals were then acclimatized for one week with local environment before the start of experiment under hygienic and standard managemental conditions in Laboratory Animal House of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola. All animals were given standard pelleted feed and ad libitum clean drinking water.

3.5 Housing and bedding material

All mice were housed under standard managemental conditions throughout the experimental period of 28 days. Temperature and humidity was maintained at 22^0C and 55%, respectively. They were provide with 12 hr light and 12 hr dark cycle period and kept in polypropylene cages having dimension of 47×34×18 cm layered with rice husk as bedding material and changed on every fourth day during the experimental period.

3.6 Feeding and watering

The standard whitish brown color pellet feed was procured from M/s. Nutrivet Life Science (Laboratory Animal Diets) Sinhagad road, Pune for experiment purpose. Throughout the trial phase of 28 days, the mice were provided with ad-libitum feed and clean drinking. Standard pellet feed congregate the necessity as per the CPCSEA guidelines and was composed of proximate principles given in Table: 3.1.

Table 3.1. Proximate principles of feed

No.	Test parameters	Results	Ranges
1.	Moisture	08.50%	10% Max.
2.	Crude protein	19.10%	17-22%
3.	Crude Fat	04.00%	3-6%
4.	Crude fiber	04.50%	3-7%
5.	Calcium	00.98%	0.95%
6.	Phosphorus	00.67%	0.66%
7.	Total ash	08.00%	8.5% Max
8.	Carbohydrate	57.20%	55-65%
9.	Metabolizable energy	03.00%	2.8-3.2

3.7 Grouping of animals

The mice were examined for normal health check up, weighed and were randomly allocated to four different treatment groups comprising eight male mice in each group (Plate 3.5).

3.8 Animal identification

For easy identification and dosing of mice, picric acid solution prepared in water was used for marking.

Table 3.2. Identification marks of each mice in different groups

Animal Number within cage	Body Mark
1	Head
2	Neck
3	Back
4	Tail
5	Right hindlimb
6	Left hindlimb
7	Right ear
8	Blank

3.9 Route and duration of ethanolic extract of *Oxalis corniculata* administration

The Ethanolic extract of *Oxalis corniculata* was administered per orally by using mice oral gavage daily once for a period of 28 days.

3.10 Experimental design

All experimental mice were acclimatized for a period of 7 days. Mice were then divided equally into four groups, each group comprising of eight mice. Group T1 was served as a normal saline control group, while group T2 was served with ethanolic extract of *Oxalis corniculata* @ 125 mg/kg b.wt. dailyp.o., group T3 was treated with ethanolic extract of *Oxalis corniculata* @ 250 mg/kg b.wt. dailyp.o. daily. While group T4 was treated with ethanolic extract of *Oxalis corniculata* @ 500 mg/kg b.wt. p.o. once daily. The mice of, T1, T2, T3 and T4 were given treatment for 28 days. The details of the experimental schedule is given below.

Table 3.3. Experimental study protocol

Group	No. of Animals	Treatment	Experimental period
T1 (Control)	08	Normal saline by oral gavage	28 Days
T2	08	Ethanolic extract of *Oxalis corniculata* @ 125 mg/kg b.wt. by oral gavage	28 Days
T3	08	Ethanolic extract of *Oxalis corniculata* @ 250 mg/kg b.wt. by oral gavage	28 Days
T4	08	Ethanolic extract of *Oxalis corniculata* @ 500 mg/kg b.wt. by oral gavage	28 Days

3.11 Animal sacrifice

On last day of experiment the animals were sacrificed by anesthetizing in a jar containing cotton wool soaked in diethyl ether inhalation.

3.12 Blood collection

Before euthanasia, blood samples were collected at the end of experiment from all the mice from retro orbital or cardiac puncture by giving appropriate dose of Thiopentone sodium intraperitonially and about 1 to 1.5 mL blood from each mouse was collected. Blood was collected in two aliquot. One aliquot contain anticoagulant (EDTA) and was used for hematological estimation. In second aliquot, serum was collected from anticoagulant free blood for biochemical examination. Blood smear were prepared directly from blood before collection in aliquot for differential leukocyte count.

Parameters studied

3.14 General performance and clinical observations

3.15 Hematological observations

3.16 Biochemical observations

3.17 Gross pathological observations

3.18 Histopathological investigations

3.13 General performance

General performance of mice during experiment was studied on the basis of clinical observations, average weekly feed consumption, average weekly body weight and average weekly body weight gain.

3.13.1 Clinical observations

The mice of all groups were kept under close observations for clinical sign, symptoms, any behavioral changes and mortality during the experimental period of 4 weeks (1^{st} to 4^{th} week).

3.13.2 Average Weekly body weights (gm)

The body weight of all group mice was recorded at weekly interval from 0 to 4^{th} week of experiment and mean weekly body weight was calculated.

3.13.3Average weekly body weight gain (gm)

During 1^{st} to 4^{th} week of experimental period average weekly body weight gain of individual mice was calculated as a difference in body weight attained at the end of week and start of that particular week. After that average weekly body weight gain of each group was calculated.

3.13.4 Average Weekly Feed Consumption (gm)

The mice of all groups were offered 100 gm of feed daily and the daily feed consumption was recorded. At the end of each week, the average weekly feed consumption (gm) was recorded by following formula.

$$\text{Average weekly feed consumption (gm) per mice} = \frac{\text{Feed consumed by group for a week period}}{\text{Number of mice}}$$

3.14 Haematological investigations

Hematological parameter included Hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Total leucocytes count (TLC) and Differential leucocytes count (DLC) were estimated as per the standard methods.

3.14.1Haemoglobin (g/dL)

Blood haemoglobin (Hb) was estimated as per the standard method described by Benjamin (2001) by Sahli's haemglobinometer and values were expressed in g/dL. Average value for each group was calculated.

3.14.2Packed cell volume (per cent)

Packed cell volume (PCV) was estimated by using Wintrobe microhematocrit tube as per standard method described by Benjamin (2001).

3.14.3 Total erythrocyte count ($1x10^6$/cumm)

Total erythrocyte count (TEC) was carried out by hemocytometer and standard blood diluting pipette using RBC diluting fluid as per method described by Benjamin (2001) and values were expressed in 10^6/cumm.

3.14.4Erythrocyte indices

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as per standard equations mentioned by Benjamin, (2001).

a) Mean corpuscular volume (Femtoliter):

Mean corpuscular volume (MCV) was calculated by using following formula and the value is expressed in Femtoliter.

$$\text{MCV (fL)} = \frac{\text{PCV (\%) x 10}}{\text{RBC counts in million/cumm}}$$

b) Mean corpuscular haemoglobin (Picogram)

Mean corpuscular hemoglobin (MCH) was calculated by using following formula and estimated in picogrom.

$$\text{MCH (pg)} = \frac{\text{Hb (g/dl) x 10}}{\text{RBC counts in million /cumm}}$$

c) Mean corpuscular haemoglobin concentration (gm/dL)

Mean corpuscular hemoglobin concentration (MCHC) was calculated by using following formula and the value is expressed in gm/dL.

$$\text{MCHC (gm/dL)} = \frac{\text{Hb (g/dl) x 100}}{\text{PCV (\%)}}$$

3.14.5 Total leucocyte counts ($1x10^3$/cumm)

Total leucocyte count was carried out with improved Neubaure's chamber by using WBC diluting fluid with the help of standard WBC diluting pipette. (Benjamin, 2001)

3.14.6 Differential leucocyte count (DLC)

The thin blood smear from fresh blood was prepared from all the mice of the entire group. After air dry, blood smear was stained by Leishman's stain as describe by Benjamin (2001). Observed under oil imulsion

(100x magnifications) of microscope using oil emulsion total 100 leucocytes were counted and expressed as percentages of each cell.

3.15 Biochemical Parameter

At the end of 4^{th} week of experiment serum samples from each group were collected. Serum samples were preserved at -20^0C until further examination. Biochemical parameters were estimated by using AGD Diagnostic kits supplied by AGD Biomedicals Pvt. Ltd., Akola on Autoanalyzer (AGD AGD Biomedical, Model No. AGD 2020) as per standard methods.

Following biochemical parameters were estimated.

3.15.1 Serum total protein (gm/dL)

Serum total protein level in each group was estimated by Biuret method (Vatzidis, 1977).

3.15.2 Serum albumin (gm/dL)

Serum albumin levels was estimated by Bromocresol Green method (Gustaffson, 1978).

3.15.3 Serum globulin (gm/dL)

Serum globulin levels were estimated as difference between total protein and albumin for each group.

3.15.5 Serum Aspartate transaminase (AST/ SGOT) (IU/L)

Serum asparate aminotransferase (AST) level was estimated as per UV Kinetic IFCC method (Acta, 1976).

3.15.6 Serum Alanine transaminase (ALT/SGPT) (IU/L)

Serum alanine transaminase (ALT) level was estimated as per UV Kinetic IFCC method (Acta, 1976).

3.15.7 Serum Creatinine (mg/dL)

Serum creatinine level was estimated by modified Jaffes method described by Bartels (1972)

3.15.8 Serum BUN (mg/dl)

Serum urea level was estimated by using the standard method mentioned in the biochemical AGD kits and the value is expressed as mg/dl.

3.15.9Serum Calcium (mg/dl)

Serum Calcium was estimated by Colorimetric method by using standard kit (Roe and Kahn,1927).

3.15.10 Serum Phosphorus (mg/dl)

Serum Phosphorus was estimated by Colorimetric method by using standard kit (Roe and Kahn,1927).

3.16 Gross Pathological Observations

A detailed necropsy examination was carried out on each mouse and gross pathological lesions observed on visceral organs were recorded.

3.17 Histopathology

After detailed necropsy examination tissues of liver, kidney, lung, spleen, heart, brain, intestine and stomach were collected in 10% neutral buffer formalin solution. After fixation, tissues were processed for routine histopathological technique. Tissues were processed after washing for 7-8 hours by using series of graded ethanol, cleared in xylene and embedded in paraffin. Paraffin blocks were prepared and sections of 4-6 micron were cut and stained with routine Haematoxylene and Eosin (H and E) stain as per the method described by Luna, (1968)

3.18 Statistical analysis

The obtained data during present investigations was analyzed by applying Completely Randomized Design (CRD) as described by Snedecor and Cochran (1989).

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled "TOXICOPATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE" was an attempt to study the toxic effect of *Oxalis corniculata* on general performance, hematobichemical and histopathological parameters in mice given at different doses for 28 days. The study was conducted during 7th December 2020 – 10th January 2021 under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines.

The experiment was priorly approved by Institutional Animal Ethical Committee of Post Graduate Institute of Veterinary and Animal Sciences, Akola. Thirty two male mice of 4 - 5 weeks weighing around 35-40 gm for the experiment were procured from recognized CPCSEA Small Animal Breeding House.

The Swiss Albino mice were acclimatized for a week period and were randomly divided into four equal groups (i.e. T1, T2, T3 and T4) comprising eight mice in each group. From the first day of experiment, all the groups were served with their respective dietary treatments for a period of 28 days. Group T1 was served as control. While group T2 fed ethanolic extract of *Oxalis corniculata*(EOC) @ 125 mg/kg body weight, group T3 fed with EOC@ 250 mg/kg body weight and group T4 fed with EOC@ 500 mg/kg body weight by oral route for a period of 28 days.

During experimental period of 28 days, all experimental mice were observed daily for clinical signs. The general performance was recorded on the basis of feed consumption, body weight and body weight gain during the experimental period of 28 days. Six mice from each group were humanly sacrificed at the end of 28th day. Blood, serum and tissue samples were collected to study hematological, biochemical levels, gross and

histopathological changes in various organs. The data obtained was analyzed with completely randomized design and are discussed as under.

4.1 Phytochemical analylsis of plant

The result of phytochemical investigations is shown in Table 4.

Table 4.1. Phytochemical investigation of *Oxalis corniculata l.* whole plant

Sr No.	Name of the test	Result
1	Alkaloids	-ve
2	Carbohydrates	+ve
3	Glycosides	+ve
4	Phytosterols	+ve
5	Phenolic compounds/tannins	+ve
6	Flavonoids	+ve
7	Saponins	–ve
8	Protein	+ve
9	Amino acids	+ve
11	Volatile oils	+ve

For phytochemical (qualitative) analysis of *Oxalis corniculata l.*, biological compounds viz. tannins /phenols, terpenoids, flavonoids and glycosides were analyzed. Phytochemical analysis revealed presence of carbohydrates, glycosides, phytosterols, phenolilc compounds/ tannis, favonoides, proteins, amino acids in the plant extract, however it was found negative for alkaloids and saponins. Similarly Raghavendra *et al*. (2005) carried out phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant and detected the presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins and aminoacids and volatile oils. The present findings are in accordance with Khare (2007). In addition to these Sharma and Kumari (2014) recorded presence of calcium, fiber and tannin. Leaves contain tartaric acid and citric acids, calcium oxalate, flavones (acacetin and 7,4'-diOMe

apigenin), glycoflavones (4'-OMe vitexin, 4'-OMeiso-vitexin and 3',4'-diOMe orientin), flavonols (3',4'-diOMe quercetin) and phenolic acids such as p-hydroxybenzoic, vanillic and syringic acids. Srikanth *et al.* (2017) recorded presence of Cglycosylflavones in the leaves namely 6-C-glucosyl luteolin (isoorientin), 6-Cglucosylapigenin (isovitexin) and isovitexin 7- methylether (sertisin).

4.2 General Performance

The general performance of different groups was evaluated on the basis of clinical observations, weekly feed consumption, weekly body weight and weekly body weight gain from the first day to 4th week of an experimental period.

4.2.1 Clinical Observations

All the mice from T1 (control), T2 (EOC @ 125 mg/kg b. wt.), T3 (EOC @ 250 mg/kg b. wt.) and T4 group (EOC @ 500 mg/kg b. wt.) were closely observed daily for any behavioral change for a period of four weeks. All the animals in T1, T2, T3 and T4 groups did not show any clinical signs and symptoms throughout the experimental period of 28 days. No behavioral changes were observed in any of the group. There was no mortality in any of the group during the entire period of experiment. No signs of tremor, convulsions, salivation, diarrhea, lethargy, sleep and coma were observed.

Similar observations of no clinical signs of toxicity during different oxalate toxicities were also observed by Fang *et al*. (2001) during acute oxalate nephropathy induced by star fruit in rats, Reddy (2012) during evaluation of safety and potential toxicities of methanolic extract of *Oxalis corniculata l.*@ 100, 200,400 mg/kg of body weight once in week. The 50% lethal dose (LD50) values of *O. corniculata* was recorded as 1300 mg/kg b.wt. by Singh and Prakash (2014) also recorded no positive signs of inappetence, depression, aggressiveness, respiratory distress, body weight loss, death when given @ 200 and 400 mg/kg BW. Mugisha *et al*. (2014) during acute and sub-acute toxicity of ethanolic leaf extracts *of Rumex abyssinica Jacq.*

(Polygonaceae) and *Mentha spicata* L. (Lamiaceae). Mezui *et al*. (2019) during study of acute and subacute toxicity of *Oxalis barrelieri* (Oxalidaceae) aqueous aerial parts extract also recorded no clinical signs.

Contrary to the present finding, Belsty *et al*. (2019) recorded depression, piloerection, loss of appetite, and fast breathing in mice treated with 250 and 500 mg/kg/day of the root extract of *Rumex nepalensis Spreng*. Fentahun *et al*. (2020) observed gentle signs of toxicity such as depression, erection of the hair, loss of appetite and fast breathing during study of acute and subacute toxic effects of 80% methanol rhizome extracts of *rumex abyssinicus jacq*. (plygonaceae) in mice.

In the present study, no clinical signs of toxicity and mortality were recorded might be due to high LD50 of *Oxalis corniculata* plant. According to the toxicity scale of Hodge and Sterner any compound with an oral LD50 of between 500-1000 mg/kg b. wt. should be considered as practically non toxic. Hence the present doses are found to be non toxic for production of behavior changes in male mice for 28 days.

4.2.2 Average weekly feed consumption (gm)

The average weekly feed consumption per mice in each group during the experimental period of 28 days is illustrated in Table 4.2 and depicted graphically in Fig.4.1.

Total feed consumption of each group at the end of the experiment was found to be numerically increased in control group mice (146.91 g) when compared with other treatment group viz. T2 (141.38 g), T3 (143.32 g) and T4 (125.53 g). The pooled mean values revealed significant differences among control and different treatment groups. Significant increased feed consumption was observed in group T1 (36.73±0.07) but it differ non significantly with group T2 (35.35±0.13) and T3 (35.83±0.45). The significantly decreased feed consumption was observed in group T4 (31.40±2.08) when compared with group T1, T2 and T3.

Table 4.2. Average weekly feed consumption (g) per week in diverse groups

Groups	1st Week	2nd Week	3rd Week	4th Week	Total feed consumption	Pooled mean
T1	36.92	36.65	36.76	36.58	146.91	36.73±0.07[a]
T2	35.11	35.25	35.72	35.30	141.38	35.35±0.13[a]
T3	36.76	36.43	35.00	35.13	143.32	35.83±0.45[a]
T4	37.6	29.8	28.8	29.4	125.53	31.40±2.08[b]
Level of significance						*
CD(0.05)						3.282

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

Significance levels *P≤ 0.05, NS= Non significant

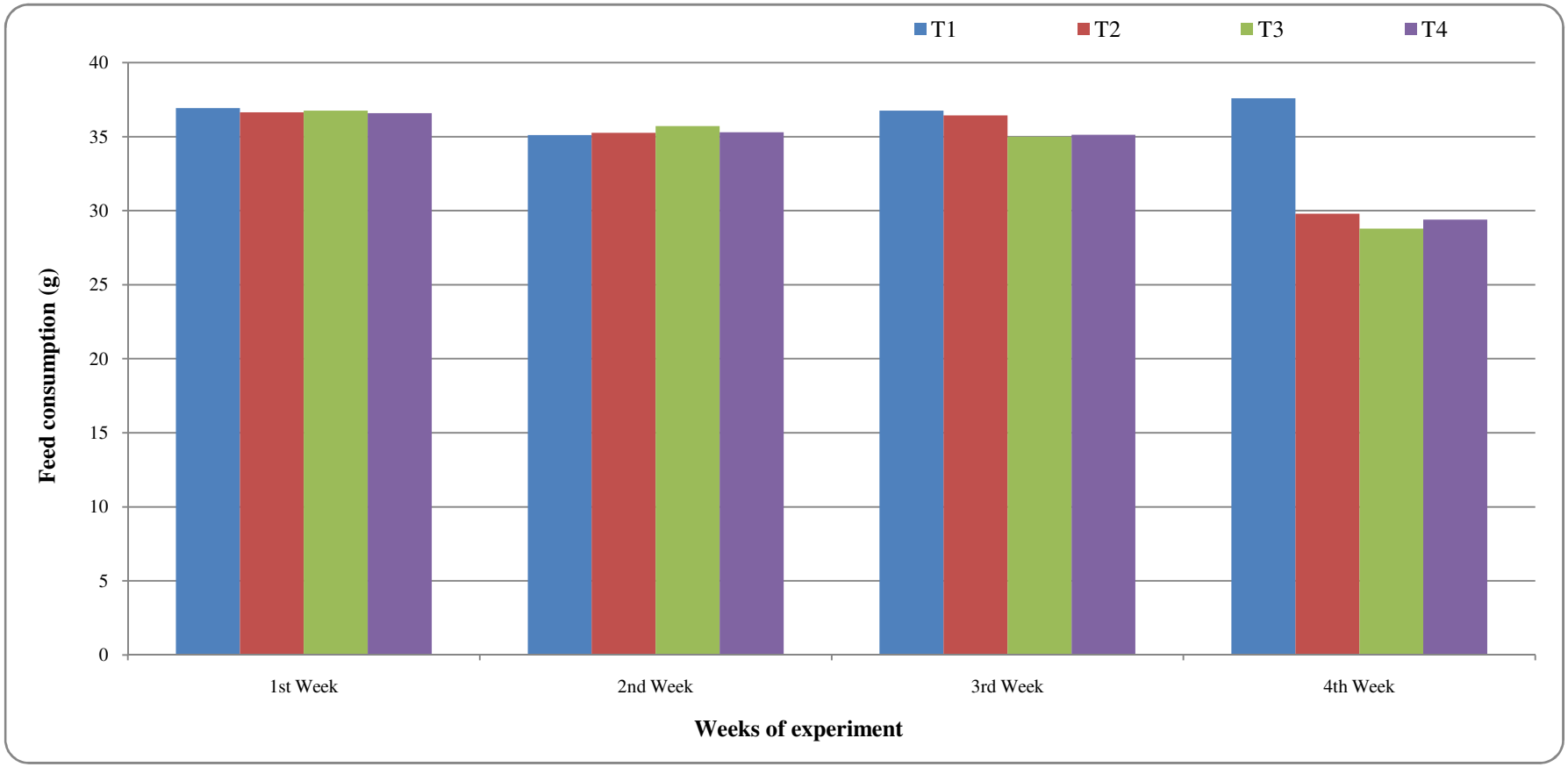


Figure 4.1. Average weekly feed consumption (gm) per week in diverse groups

The above finding of considerable decrease in feed consumption after 3rd week in the ethanolic extract of *Oxalis corniculata* treated group. Similar result of decreased feed consumption in ethanolic extract of *Oxalis corniculata* treated groups was recorded by Subhani *et al*. (2018) in broiler chicks. Plant containing oxalates which damages the kidney and ultimately affects the metabolism and thereby the feed consumption is declined and might be the reason for decreased feed consumption.

4.2.3Average weekly body weight (gm)

The average weekly body weight of mice in T1, T2, T3 and T4 groups was recorded at the end of every week during experiment and are depicted in Table 4.3 and Fig. 4.2.

The average weekly body weight recorded at 0 day of experiment revealed non significant differences within treatment and control group mice. The average weekly body weight recorded in T1, T2, T3 and T4 group showed non significant differences in first two weeks of the experiment. However, at the end of 3rd and 4th week of experiment, average weekly body weight revealed significant differences between control and treatment group mice. Significant higher body weight was observed in control group compared to other treatment groups. Both weeks (3rd and 4th) showed dose dependant decrease in body weight in mice might be due to lower feed conversion efficiency of mice in treated groups. However non significant difference was recorded in group T2, T3 and T4 at 3rd and 4th week of experiment.

Significant dose dependant decreased pooled mean body weight was observed in T4 (38.89±0.32 gm) followed by T3 (39.15±0.48 gm) and T2 (39.70±0.52gm) group when compared with T1 (41.84±0.69gm) group. However, pooled mean of body weight revealed non significant differences within treatment group.

Table 4.3. Average weekly body weight (gm) per mice in different groups during experimental period

Group	0 Day	1st Week	2nd Week	3rd Week	4th Week	Pooled mean
T1	38.55±0.34	39.33±0.48	40.90±0.62	44.60±1.31[a]	46.00±1.62[a]	41.84±0.69[a]
T2	37.68±1.01	38.10±0.97	39.45±1.07	41.65±0.69[b]	42.18±0.75[b]	39.70±0.52[b]
T3	36.13±0.67	37.28±0.43	39.52±0.44	40.72±0.62[b]	42.10±0.89[b]	39.15±0.48[b]
T4	37.47±0.60	38.10±0.69	39.22±0.64	39.67±0.65[b]	39.99±0.63[b]	38.89±0.32[b]
Level ofsignificance	-	-	-	**	**	**
CD(0.05)	NS	NS	NS	2.552	3.086	1.292

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

Significance levels *P≤ 0.05, NS= Non significant

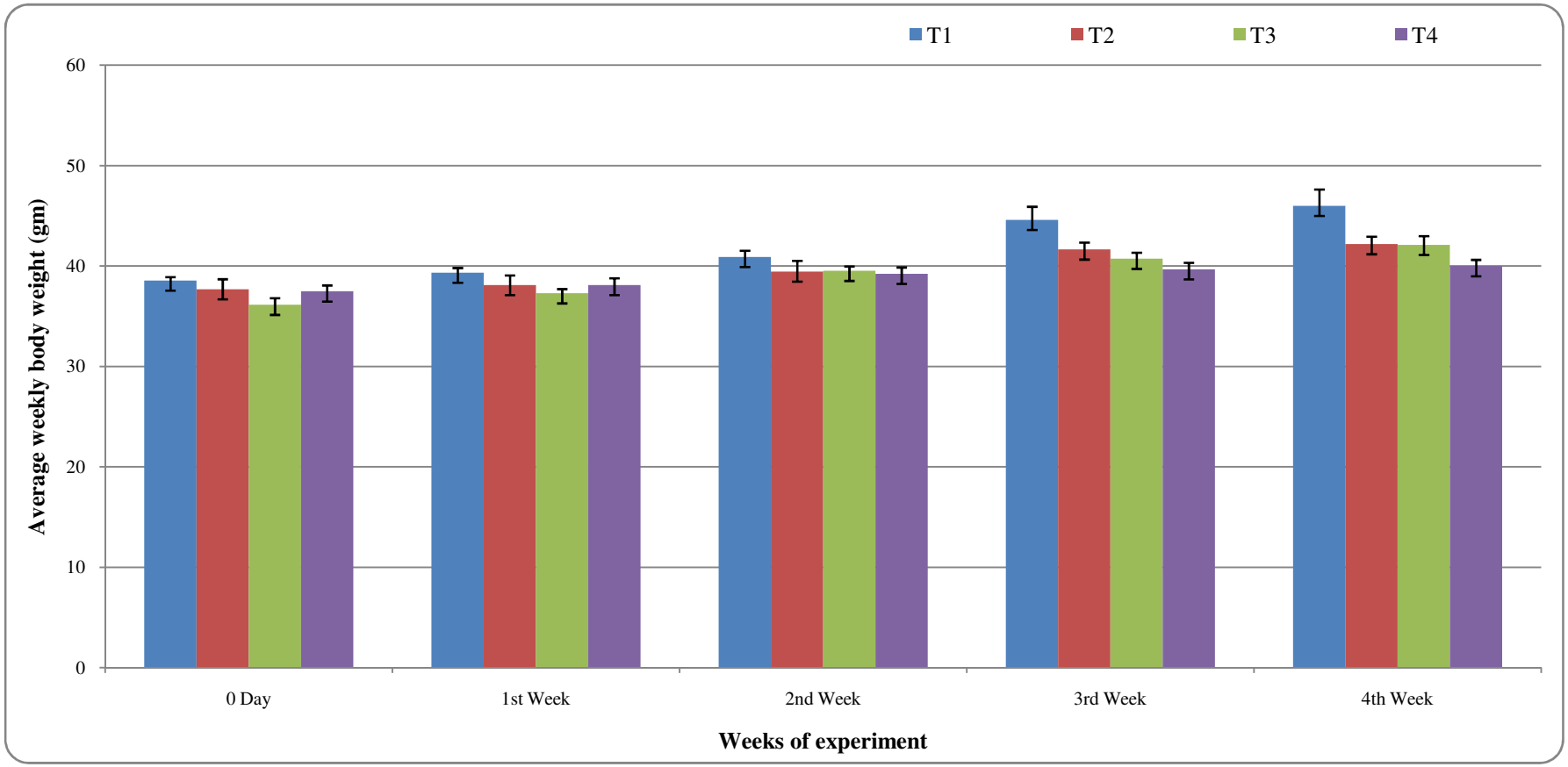


Figure 4.2. Average weekly body weight (gm) per mice in control and different groups

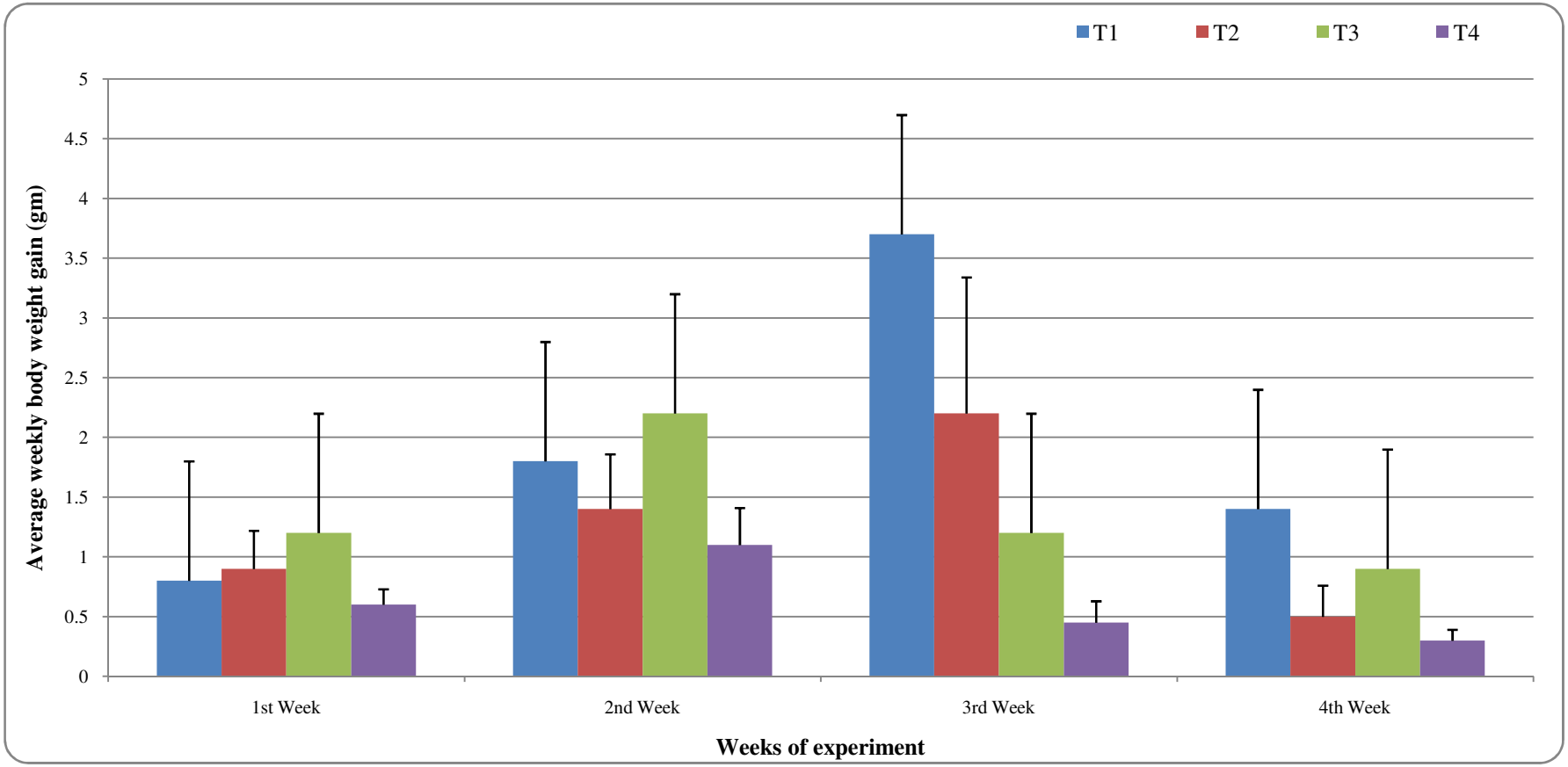


Figure 4.3. Average weekly body weight gain (gm) per mice in control and different groups

Similar but significant decreased average weekly body weight in ethanolic extract of *Oxalis corniculata* treated groups was reported by Alebachew *et al*. (2014) given methanol leaves extract of *Vernonia bipontini* @800 mg/kg b.wt. in mice. Similar to present observations, Subhani *et al*. (2018) observed significant reduced dose dependant body weight in broiler birds given *Oxalis corniculata* @ 250; 500 mg/kg body weight. Belsty *et al*. (2019) recorded non significant changes in body weight of mice given *rumex nepalensis Spreng*. root extract given @ 250 and 500 mg/kg/day in both sexes of mice. Contrary to present findings Mezui *et al*. (2019) studied acute and subacute toxicity of *Oxalis barrelieri* (Oxalidaceae) aqueous aerial parts extract and observed a body weight gain.

According to Alebachew *et al*. (2014) decreased body weight in EOC treated groups might be due to some anti nutritional factors such as phytosterols, tannins and oxalates present in ethanolic extract of *Oxalis corniculata* which reduces the body weight by forming complexes with metals (Ca^{++}, Zn, Mg and Fe) and proteins and reduces minerals and protein bioavailability and might be the reason for dose dependant decrease in body weight.

4.2.4 Average weekly body weight gain (gm)

The average weekly body weight gain in T1, T2, T3 and T4 groups were recorded for four week experimental period and are depicted in Table 4.4 and presented in Fig. 4.3.

The average weekly body weight gain at 1^{st}, 2^{nd} and 4^{th} week revealed non significant differences between control and different treatment group mice. However, numerically dose dependant decreased body weight gain was observed in treatment groups. At 3^{rd} week, group T3 (1.20±0.41gm) and T4 (0.45±0.18gm) showed significant decreased body weight gain, but group T2 (2.20±1.14gm) showed non significant differences with that of group T1 (3.70±1.00gm). But overall observations revealed dose dependant decrease in average weekly body weight gain in mice given *Oxalis corniculata*.

Table 4.4. Average weekly body weight gain (gm) per mice in different groups during experimental period

Group	1st Week	2nd Week	3rd Week	4th Week	Pooled mean
T1	0.80±0.21	1.80±0.43	3.70±1.00[a]	1.40±0.73	1.90±0.38[a]
T2	0.90±0.32	1.40±0.46	2.20±1.14[ab]	0.50±0.26	1.24±0.33[ab]
T3	1.20±0.45	2.20±0.55	1.20±0.41[b]	0.90±0.55	1.38±0.25[ab]
T4	0.60±0.13	1.10±0.31	0.45±0.18[b]	0.30±0.09	0.63±0.11[b]
Level ofsignificance	-	-	*	-	*
CD(0.05)	NS	NS	2.33	NS	0.811

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

Significance levels *P≤ 0.05, NS= Non significant

Significant decreased pooled mean body weight was observed in group T4 (0.63±0.11 gm) followed by group T2 (1.24±0.33gm), T3 (1.38±0.25 gm) and T1 (1.90±0.38gm) suggested significant decreased body weight gain in T4 group.

As earlier described, According to Alebachew *et al*. (2014) presence of anti nutritional factors such as tannins, phytosterols and oxalic acid in plant extract are responsible for inhibition of digestive enzymes which ultimately leads to reduced feed conversion ratio and results in decreased body weight gain.

4.3 Hematological observations

The hematological parameters viz. Hb, PCV, TEC, MCV, MCH, MCHC, TLC and DLC were estimated at the end of experimental trial and are depicted in Table 4.5 and Fig.4.4.

4.3.1 Haemoglobin (g/dL)

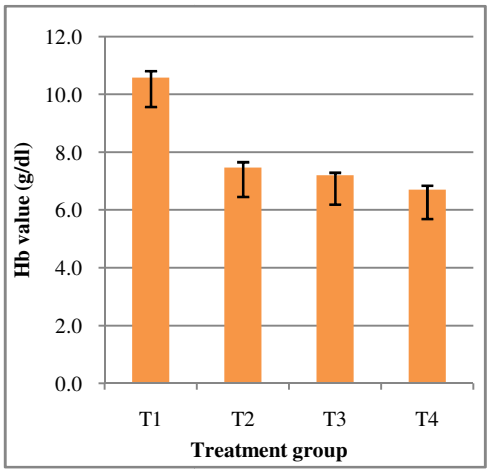
The mean hemoglobin (Hb) value at the end of 28th day of experiment in control and treatment groups differs significantly. The hemoglobin values were observed as 10.60±0.24, 7.50±0.2, 7.20±0.1, 6.7±0.15 g/dL in group T1, T2, T3 and T4, respectively.

Table 4.5. Mean hematological values related to erythrocytes of control and different treatment groups

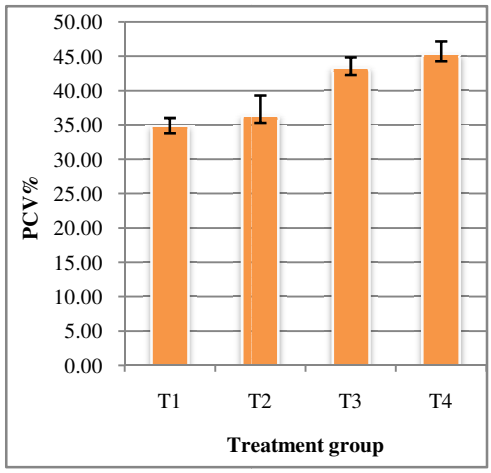
Group	Hb(g/dL)	PCV(%)	TEC(106/cumm)	MCV(fL)	MCH(Pg)	MCHC(%)
T1	10.60±0.24[a]	34.83±1.22[b]	8.00±0.21[c]	43.81±1.53[a]	13.30±0.53[a]	30.57±1.29[a]
T2	7.50±0.20[b]	36.33±2.99[b]	7.20±0.19[c]	50.84±4.78[a]	10.40±10.40[b]	21.42±2.17[b]
T3	7.20±0.10[bc]	43.00±10.56[a]	16.20±0.28[b]	26.17±0.77[b]	4.50±4.50[c]	16.73±0.68[c]
T4	6.70±0.15[c]	45.30±1.91[a]	22.30±0.56[a]	20.42±1.06[b]	3.00±0.13[d]	14.92±0.75[c]
Significance/ NS	**	**	**	**	**	**
CD (0.05)	0.535	5.991	1.014	7.647	0.955	4.007

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

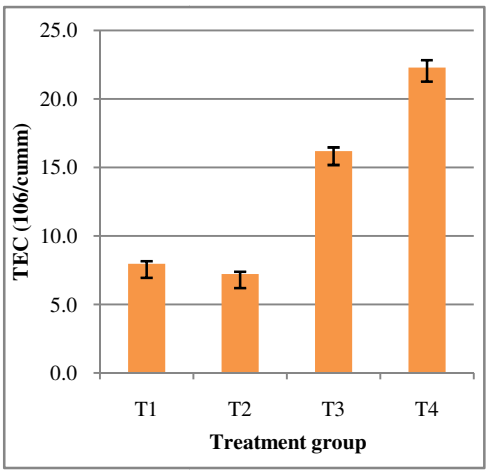
NS= Non-significant **Significant at 1% *Significant at 5%



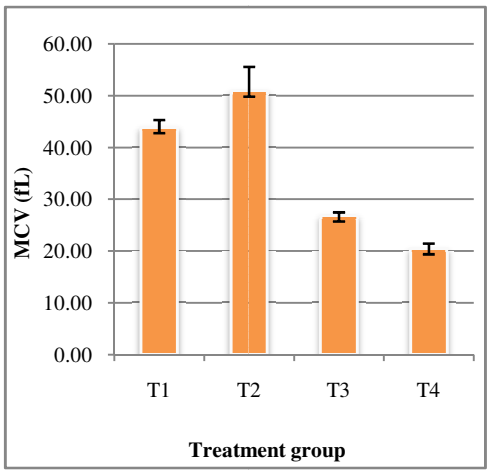
A) Mean Hb level in control and different treatment groups



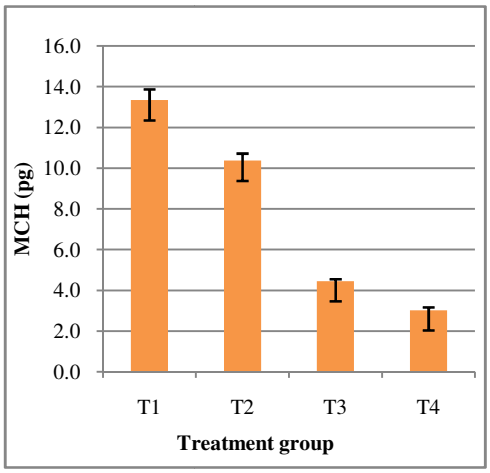
B) Mean PCV values in control and different treatment groups



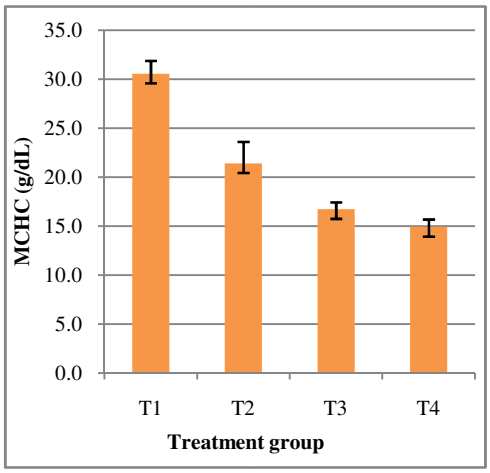
C) Mean TEC values in control and different treatment groups



D) Mean MCV in control and different treatment groups



E) Mean MCH in control and different treatment groups



F) Mean MCHC in control and different treatment groups

Figure 4.4. Haematological values related to erythrocytes in control and different treatment groups (A to F)

The results thus showed dose dependant significant decrease in Hb concentration in T2 (7.5±0.2 g/dL), T3 (7.2±0.1 g/dL) and T4 (6.7±0.15) groups mice treated with ethanolic extract of *Oxalis corniculata* when compared with that of T1 group (10.6±0.24 g/dL).

Similar observations of significant decrease in the hemoglobin in mice was also reported by Alebachew *et al*. (2014) during toxicological evaluation of methanol leaves extract of *Vernonia bipontini Vatke* in mice given @ 800 mg/kg of body weight. However, Fentahun *et al*. (2020) reported non significant decrease in hemoglobin level at @ 100, 200 and @400 mg/kg b.wt of methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae).

Contrary to present findings, Bajaj *et al*. (2011) observed increased hemoglobin level in Napier grass *(pennisetum purpureum)* fed buffalo calves. Reddy (2012) found non significant difference in hemoglobin value at @100, 200,400 mg/kg of body weight of methanolic extract of *Oxalis corniculata* in rat. Mugisha *et al*. (2014) reported non significant difference in hemoglobin count at @ 500, 1000 and 1500 mg/kg b.wt. of ethanolic leaf extracts *of Rumex abyssinica* Jacq. (Polygonaceae) and *Mentha spicata* L. (Lamiaceae).

Alebachew *et al*. (2014) reported significantly decrease in the mean corpuscular hemoglobin in mice given methanol leaves extract of *Vernonia bipontini Vatke* at dose of @800 mg/kg of b.wt. and suggested inhibition of RBC formation, which reduced hemoglobin content. Kidney damage caused by *Oxalis corniculata* or decrease in feed intake due to inflammation of intestine might be the reason for dose dependant decreased hemoglobin level (Benjamin, 2001).

4.3.2Packed Cell Volume (%)

The mean values of packed cell volume (PCV) in control and different treatment groups are illustrated in Table 4.5 and Fig.4.4.

At the end of experiment, the average mean PCV showed significant differences between control and different treatment groups and are

observed as 34.83±1.22%, 36.33±2.99%, 43.00±1.56% and 45.30±1.91% in group T1, T2, T3 and T4, respectively. Significant increased PCV was observed in group T4 (45.3±1.91%) followed by group T3 (43±1.56%) and T2 (36.33±2.99%) when compared with group T1 (34.83±1.22%) indicated the dose dependant adverse effect of *Oxalis corniculata* toxicity in mice. The significant higher value in group T3 and T4 indicated dehydration due to toxicity.

Significant increase in the packed cell volume in mice indicated dehydration and similar result was also reported by Singh *et al.* (1995) in ethylene glycol induced nephrotoxicity in cow calf may be due to severe dehydration as a result of dieresis. Aslani *et al*. (2011) in oxalate intoxication associated to ingestion of eshnan (Seidlitzia rosmarinus) in sheep and Mugisha *et al*. (2014) in acute and sub-acute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq. (Polygonaceae) and *Mentha spicata* L. (Lamiaceae) in rats also reported significant increase in PCV and suggested imbalance in the rate of hematological parameter synthesis and catabolism.

Contrary to the present findings, Bajaj *et al*. (2011) recorded significant decreased packed cell volume from 31.80 to 26.67 in buffalo calves during oxalate toxicity by *ad libitum* feeding of Napier grass *(pennisetum purpureum)*. Fentahun *et al*. (2020) also reported non significant decrease in packed cell volume in mice treated with 80% methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) @ 100, 200 and @400 mg/kg b.wt.

4.3.3 Total Erythrocyte Count (10^6/cu mm)

The mean values of TEC (x10^6/ cu mm) in control and different treatment groups at the end of experiment revealed significant differences and are depicted in Table 4.5 and Fig.4.4.

At the end of 4th week, the mean TEC values in T1, T2, T3 and T4 groups were recorded as 8.00±0.21, 7.20±0.19, 16.20±0.28 and 22.30±0.56 (10^6/cu mm), respectively. The T2 group (7.20±0.19) animals showed non significant TEC count when compared with T1 group (8.00±0.21). Group T3

(16.20±0.28) and T4 (22.30±0.56) showed significant increased TEC count when compared with T1 and T2 group.

Similar findings of significant increased TEC was also recorded by Basu *et al.* (1974) during case study of erythrocytosis associated with chronic renal disease and suggested that it might be due to renal vascular disease or glomerular damage could provoke the release of increased amounts of erythropoietin and lead to subsequent erythrocytosis. Singh *et al*. (1995) in cow calf induced with acute nephorphathy and suggested severe dehydration due to dieresis. Fentahun *et al*. (2020) also observed significant increase in TEC value in methanol rhizome extracts of *Rumex abyssinicus jacq* treated group in mice @400 mg/kg of b.wt.

Contrary to present investigation, Mugisha *et al*. (2014) during acute and sub acute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq and*Mentha spicata* L. (Lamiaceae) and recorded no significant effect on RBC given @ 1500 mg/kg body weight. Alebachew *et al*. (2014) studied toxicological evaluation of extract of *Vernonia bipontini Vatke* in blood and recorded significantly decreased red blood cell count (M/UL) in mice treated with 800 mg/kg of the extract however at 400 mg/kg there was no significant difference.

4.3.4 Erythrocyte indices

The mean values of erythrocyte indices in T1, T2, T3 and T4 groups are presented in Table 4.5 and Fig.4.4.

Erythrocyte indices define the size of erythrocyte and hemoglobin content in erythrocyte. MCV, MCH and MCHC were obtained from the hemoglobin concentration, total erythrocyte count and packed cell volume and are discussed as under.

a) Mean Corpuscular Volume (MCV) (fL)

The mean values of MCV (fL) in T1, T2, T3 and T4 groups revealed significant differences at the end of experiment and are depicted in

Table 4.6 and Fig. 4.5 The mean values of MCV in group T1, T2, T3 and T4 are observed as 43.81±1.53, 50.84±4.78, 26.17±0.77and 20.42±1.06(fL), respectively indicating significant decrease in group T3 and T4. However, group T2 differ non significantly with control group and significant increased values was observed when compared with group T3 and T4. Significantly decreased in mean values of MCV indicated microcytic anemia in EOC treated group.

The present findings of treatment groups are in agreement with Alebachew *et al*. (2014) in mice fed with methanol leaves extract of Vernonia bipontini Vatke at dose of 800 mg/kg of b.wt. Contrary to present investigation, Mugisha *et al*. (2014) reported non significant effect on MCV during acute and sub-acute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq and and *Mentha spicata* L. (Lamiaceae). Fentahun *et al*. (2020) also observed non significant effect on MCV in 80% methanol rhizome extracts of *Rumex abyssinicus jacq* treated group in mice.

b) Mean Corpuscular hemoglobin (MCH) (pg)

The mean corpuscular hemoglobin (MCH) was found to be significantly decreased in treatment groups (T2, T3 and T4) when compared with control group. The MCH values obtained were observed as 13.3±0.53 pg, 10.4±10.40 pg, 4.50±4.50 pg and 3.00±0.13.00 pg in T1, T2, T3 and T4 groups, respectively. Results thus indicated dose dependant decrease in MCH in mice treated with *Oxalis corniculata.*

The above obtained findings are in accordance with Alebachew *et al*. (2014) who also reported significantly decrease in the mean value of mean corpuscular hemoglobin in mice treated with methanol leaves extract of *Vernonia bipontini Vatke* at dose of @800 mg/kg of b.wt. and suggested that it might be due to methanol leaf extract of related species of *V. bipontini V* (V. amygdalina) who possesses the potential of adversely affecting hematological indices. Methanol leaf extract of *V. bipontini V* may induce inhibition of RBC formation, which reduced hemoglobin content.

c) Mean Corpuscular Hemoglobin Concentration (MCHC) (%)

The mean MCHC values in control and different treatment groups estimated at the end of 4th week showed significant difference between control and different treatment groups and were observed as 30.57±1.29 %, 21.42±2.17 %, 16.73±0.68and 14.92±0.75 % in T1, T2, T3 and T4 group, respectively. The dose dependant decrease in MCHC was observed in treatment groups.

Significant decreased MCHC was observed in group T2, T3 and T4 groups when compared with that of control group. The present findings of treatment groups are in agreement with Alebachew *et al*., (2014) in mice fed with methanol leaves extract of *Vernonia bipontini Vatke* at dose of 800 mg/kg of b.wt. Mugisha *et al*. (2014) recorded non significant increase in MCHC value in mice treated with acute and sub-acute toxicity of ethanolic leaf extracts *of Rumex abyssinica* Jacq and and *Mentha spicata* L. (Lamiaceae). Fentahun *et al*. (2020) observed non significant difference in MCHC in mice treated with 80% methanol rhizome extracts of *Rumex abyssinicus jacq* .

Decreased MCV and MCHC values in EOC treated groups indicating microcytic hypochromic anaemia. Microcytic hypochromic anaemia in EOC treated groups might be due to inflammatory condition of intestine which leads to decreased in feed consumption this leads to microcytic hypochromic anaemia (Benjamin, 2001). Basu *et al.* (1974) associated it with chronic renal disease and suggested that renal vascular disease or glomerular damage could provoke different kinds of anemia.

4.3.5Total leucocyte count (10^3/cu mm)

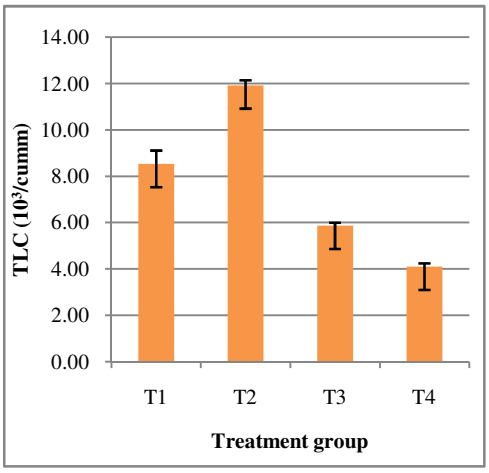
The mean total leucocyte count (TLC) in control and different treatment groups revealed significant differences. The TLC in different groups are illustrated in Table 4.6 and graphically depicted in Fig.4.5.

Table 4.6. The mean hematological values in control and different treatment groups related to total leucocyte count and differential leucocyte count

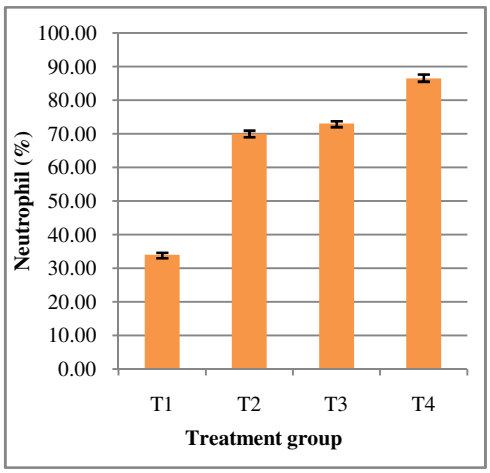
Groups	TLC(103/Cumm)	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
T1	8.53±0.58^{b}	34±0.76^{d}	64.33±0.61^{d}	1±0.26	0.83±0.48	0.33±0.21
T2	11.92±0.22^{a}	70±0.93^{c}	27±0.72^{b}	1±0.26	1±0.37	0.5±0.22
T3	5.87±0.13^{c}	73±0.77^{b}	25±0.88^{b}	0.83±0.17	0.5±0.22	0.33±0.21
T4	4.10±0.15^{d}	86.50±1.15^{a}	12±0.88^{c}	0.83±0.31	0.67±0.33	0.33±0.21
Significance/NS	**	**	**	NS	NS	NS
CD (0.05)	0.961	2.706	2.306	-	-	-

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

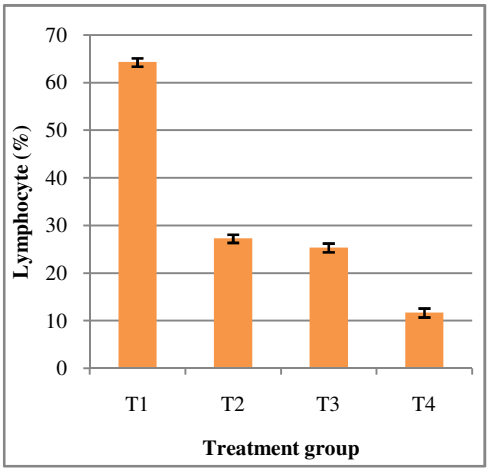
Significance levels *P≤ 0.05, NS= Non significant



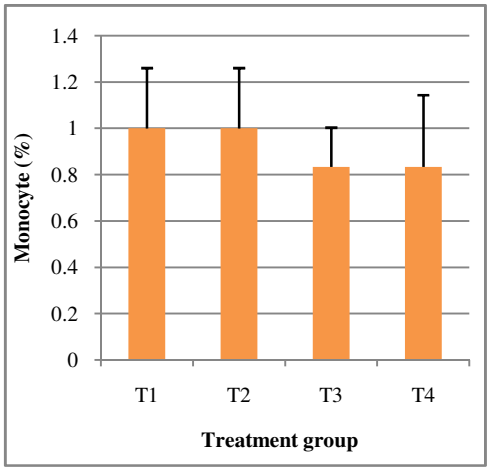
A) Mean TLC in control and different treatment groups



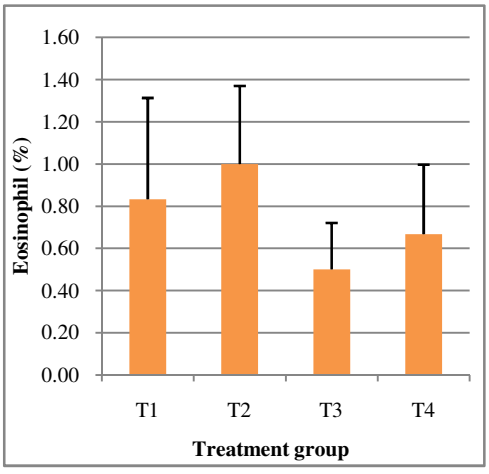
B) Mean Neutrophil (%) in control and different treatment groups



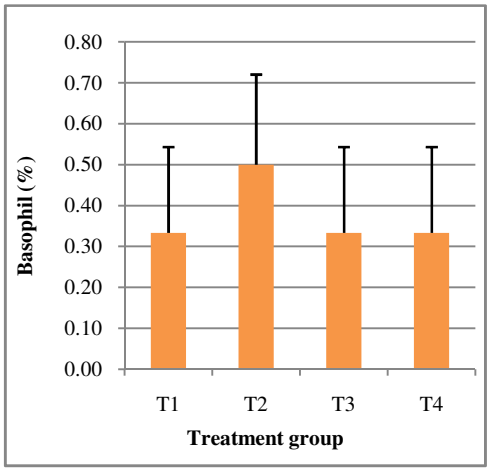
C) Mean Lymphocyte (%) in control and different treatment groups



D) Mean Monocyte (%) in control and different treatment groups



E) Mean Eosinophil (%) in control and different treatment groups



F) Mean Basophil (%) in control and different treatment groups

Figure 4.5. Haematological values related to leucocytes in control and different treatment groups (A to F)

The total leukocyte count was found to be significantly decreased in group T4 (4.10±0.15) followed by group T3 (5.87±0.13). Group T2 showed significant high count (11.92±0.22) when compared with group T1 (8.53±0.58) and group T3 and T4.

The similar finding of significant decrease in the value of total leucocyte count is in accordance with Singh *et al.* (1995) in cow calf intoxicated with ethylene glycol. Fentahun *et al*. (2020) recorded non significant decrease in total leucocyte count in mice when treated with 80% methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) at @ 100, 200 and @400 mg/kg b.wt. Significant decrease in total leucocyte count in EOC treated groups suggested a decrease in leukocyte output, known as leukopenia, which means the body is less able to combat infections (Fentahun *et al*., 2020). Contrary to present findings, Bajaj *et al*. (2011) recorded significant increase in leucocyte count in buffalo calves treated with Napier grass *(pennisetum purpureum)*. Reddy (2012) reported non significant difference in leucocyte count in rats treated with methanolic extract of *Oxalis corniculata l.* @ 100, 200 and 400 mg/kg of b.wt.

Mugisha *et al*. (2014) reported no significant difference in total leucocyte count during study of acute and sub-acute toxicity ethanolic leaf extracts *of Rumex abyssinica* Jacq. (Polygonaceae) and *Mentha spicata* L. (Lamiaceae).

4.3.6 Differential leucocyte Count (DLC)

a) Neutrophil count (%)

The average mean neutrophil counts in control and different treatment groups are illustrated in Table 4.6 and graphically represented in Fig.4.5. At the end of 4th week, the mean neutrophil count in group T1, T2, T3 and T4 were observed as 34±0.76, 70.00±0.93, 73.00±0.77 and 86.50±1.15, respectively suggested dose dependant increase in treatment groups. Significant increased neutrophil count was recorded in group T4 (86.50±1.15 %) followed by T3 (73±0.77%) and T2 (70±0.93%) group when compared with control group. The finding of increase in the value of neutrophil count

was observed by Aslani *et al*. (2011) in acute oxalate intoxication associated to ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep.

The increase in the count of neutrophils might be due to the effect of oxalate present in plant causes delayed toxicity resulting in the systemic stress which releases endogenous corticosteroids and results into the non inflammatory neutrophilia (Benjamin, 2001).

Contrary to present investigation, Bajaj *et al*. (2011) found significant decrease in neutrophil count during study of induced oxalate toxicity by feeding Napier grass to buffalo calves and Reddy (2012) reported non significant difference in neutrophil count in rats treated with methanolic extract of *Oxalis corniculata l.*

b) Lymphocyte count (%)

The mean value of lymphocyte count in control and various treatment groups at the end of experiment are illustrated in Table 4.6 and graphically represented in Fig.4.5.

The average mean lymphocyte count revealed significant differences ($P<0.05$) among control and different treatment groups and are observed as 64.33±0.61, 27±0.72, 25±0.88 and 12±0.88 in T1, T2, T3 and T4 groups, respectively. The significant decreased lymphocyte count was observed in group T4 (12±0.88) followed by T3 (25±0.88) and T2 (27±0.72) group when compared with control group (64.33±0.61).

The finding of mild decrease in the value of lymphocyte count was observed by Aslani *et al*. (2011) in acute oxalate intoxication associated to ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep.

Contrary to present investigation, Bajaj *et al*. (2011) found significant increase in lymphocyte count during study of induced oxalate toxicity by feeding Napier grass to buffalo calves and Reddy (2012) reported non significant difference in lymphocyte count in rats treated with methanolic extract of *Oxalis corniculata l.*

Histopathological examination of kidney revealed inflammatory changes which causes systemic stress which may leads to lymphopenia (Benjamin, 2001) and might be the reason for present finding in toxicated groups.

c) Monocyte count (%)

The mean monocyte count revealed non-significant differences between control and different treatment group mice and was observed as 1.00±0.26, 1.00±0.26, 0.83±0.17 and 0.83±0.3 in T1, T2, T3 and T4 groups, respectively (Table 4.6 and Fig.4.5). Similar finding of normal range of monocyte was also recorded by Bajaj *et al*. (2011) during study of induced oxalate toxicity by feeding Napier grass to buffalo calves and Reddy (2012) reported non significant difference in monocyte count in rats treated with methanolic extract of *Oxalis corniculata l.*

d) Eosinophil count (%)

The mean values of eosinophil count revealed non-significant difference among control and different treatment groups and are illustrated in Table 4.6 and graphically depicted in Fig.4.5. The mean eosinophil count in group T1, T2, T3 and T4 were observed as 0.83±0.48, 1±0.37, 0.5±0.22 and 0.67±0.33 respectively. Similar finding of normal range of eosinophil were also recorded by Bajaj *et al*. (2011) and found normal eosinophil count during study of induced oxalate toxicity by feeding Napier grass to buffalo calves. The present findings are in line with the findings of Reddy (2012) who also reported non significant difference in eosinophil count in rats treated with methanolic extract of *Oxalis corniculata l.*

e) Basophil count (%)

The mean basophil count at the end of 28^{th} day were observed as 0.33±0.21, 0.5±0.22, 0.33±0.21 and 0.33±0.21 in T1, T2, T3 and T4 groups, respectively (Table 4.6 and Fig.4.5) indicated non significant differences between control and treatment group mice. Similar finding of normal range of basophil was also recorded by Bajaj *et al*. (2011) found normal basophil count

during study of induced oxalate toxicity by feeding Napier grass to buffalo calves. The present findings are in accordance with Reddy (2012) who also reported non significant difference in basophil count in rats treated with methanolic extract of *Oxalis corniculata l.*

4.4 Biochemical parameters

The physiological functioning of mice in control group as well as in treatment groups was assessed on the basis of biochemical parameters which includes serum total protein, albumin, globulin, AST, ALT, creatinine, BUN, calcium and phosphorus. The values estimated are presented in Table 4.7 and signified graphically in Fig.4.6.

4.4.1 Serum asparate aminotransferase (AST/ SGOT) (IU/L)

Table 4.7 and Fig.4.6 showed serum aspirate aminotransferase (SGOT) in control and different treatment groups analyzed at the end of 28th day of experiment.

The mean serum AST level in different treatment groups revealed significant difference ($P<0.05$) and were recorded as137.17±4.57 IU/L, 158.42±10.02 IU/L, 165.93±1.56 IU/L and 175.52±1.40 IU/L in group T1, T2, T3 and T4, respectively. The serum asparate aminotransferase (AST)revealed significant dose dependant increase in treatment groups when compared with that of control group. The significant increase was observed in group T4 (175.52±1.40 IU/L) followed by group T3 (165.93±1.56 IU/L) and T2 (158.42±10.02 IU/L).

4.4.2 Serum alanine transaminase (ALT/ SGPT) (IU/L)

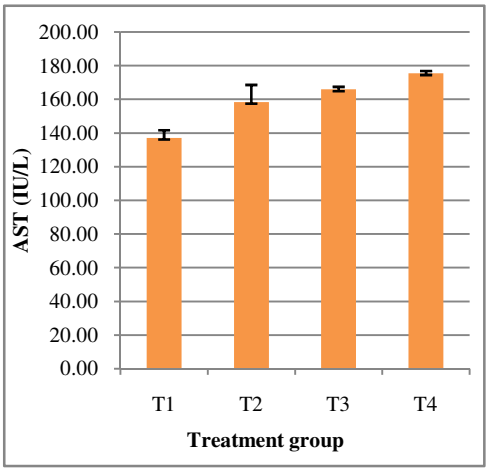
The mean value of serum alanine transaminase (ALT/ SGPT) in control and different treatment groups are illustrated in Table 4.7 and Fig.4.6. Results obtained showed dose dependant significant increased serum SGPT level in group T4 (84.67±1.11 IU/L) followed by T3 (49.817±2.5 IU/L) and T2 (41.017±2.22 IU/L) when compared with T1 (36.15±0.46 IU/L).

Table 4.7. Serum biochemical parameters in control and different treatment groups at the end of 28th day of experiment

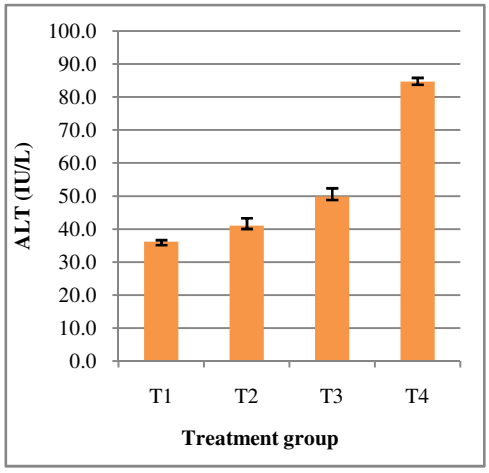
Groups	AST/SGOT (IU/L)	ALT/SGPT (IU/L)	Creatinine (mg/dL)	BUN (mg/dL)	Calcium (mg/dL)	Phosphorous (mg/dL)	Total Protein (gm/dL)	Albumin (gm/dL)	Globulin (gm/dL)
T1	137.17±4.57^{c}	36.15±0.46^{c}	0.51±0.012^{c}	26.25±0.58^{c}	9.93±0.14b	8.36±0.11^{a}	8.77±0.27^{b}	3.74±0.05^{a}	5.03±0.23 b
T2	158.42±10.02 b	41.017±2.22 c	0.53±0.018^{c}	29.97±0.65 b	10.46±0.07 a	7.32±0.08^{b}	10.10±0.08 a	3.27±0.02 b	6.82±0.08^{a}
T3	165.93±1.56^{ab}	49.817±2.5^{b}	0.64±0.030 b	33.55±0.65^{a}	8.78±0.14^{c}	6.50±0.08^{c}	7.83±0.11^{c}	2.55±0.12^{c}	6.56±0.37^{a}
T4	175.52±1.40^{a}	84.67±1.11^{a}	0.79±0.020^{a}	33.8±0.75^{a}	7.28±0.16^{d}	5.35±0.04^{d}	7.13±0.34^{d}	2.17±0.07 d	4.97±0.32 b
Significance/NS	**	**	**	**	**	**	**	**	**
CD (0.05)	16.476	5.235	0.063	1.949	0.385	0.242	0.672	0.223	0.806

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.

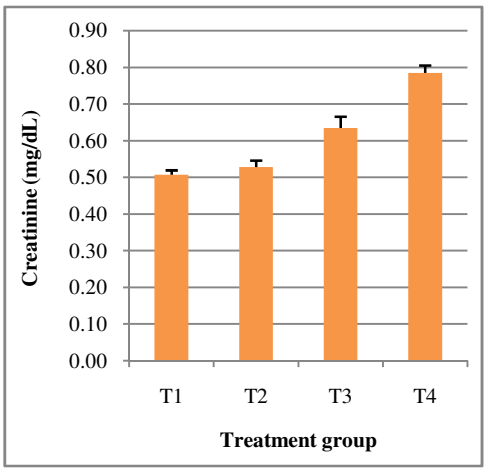
Significance levels *P≤ 0.05, NS= Non significant



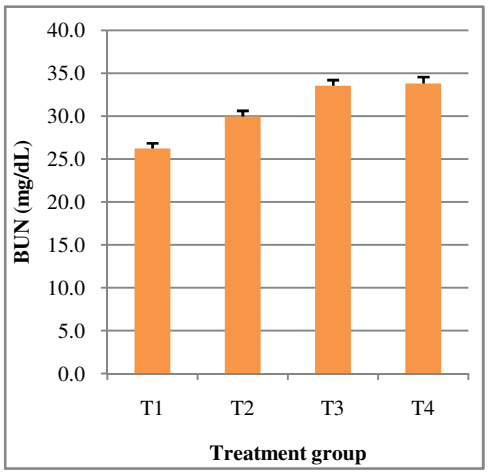
A) Mean Serum AST in control and different treatment groups



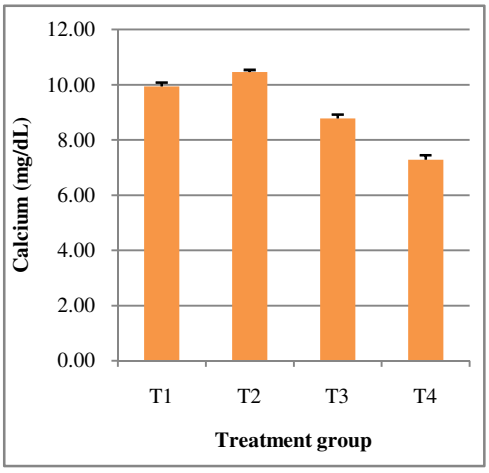
B) Mean Serum ALT in control and different treatment groups



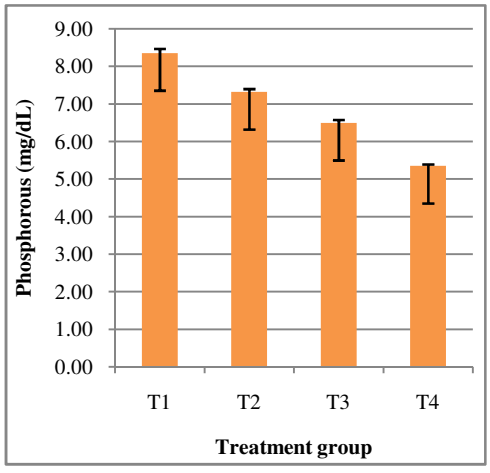
C) Mean Serum Cretinine in control and different treatment groups



D) Mean Serum BUN in control and different treatment groups

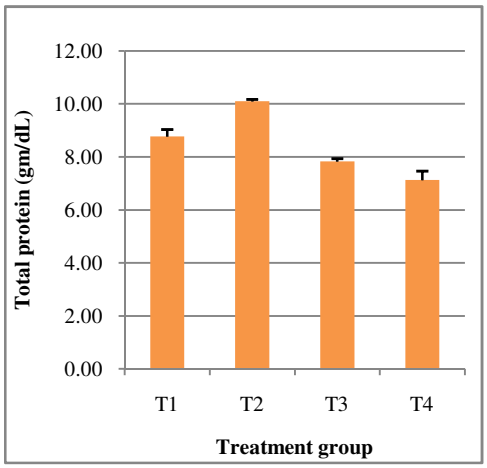


E) Mean Serum Calcium in control and different treatment groups

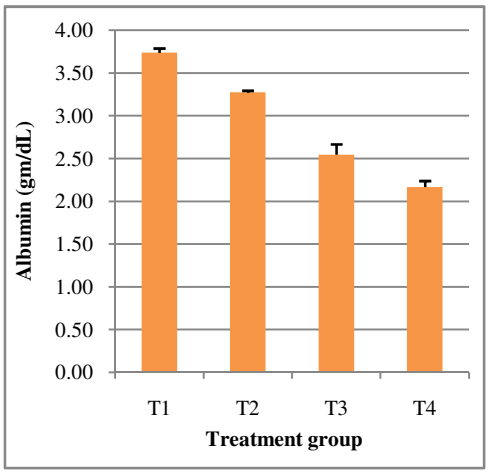


F) Mean Serum Phosphorous in control and different treatment groups

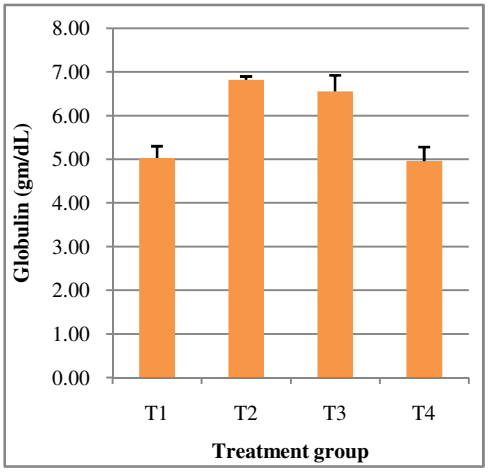
Figure 4.6. contd....



G) Mean Serum total protein in control and different treatment groups



H) Mean Serum albumin in control and different treatment groups



I) Mean Serum globulin in control and different treatment groups

Figure 4.6. Serum biochemical values in control and different treatment groups (A to I)

Significant increased serum AST and ALT values are also recorded by Singh *et al.* (1995) in ethylene glycol induced toxicity in cow calves and suggested cell necrosis of different tissue such as liver and kidney. Bajaj *et al*. (2011) also observed the slight increase in AST enzyme activity in oxalate toxicity by napier grass in buffalo calves. Reddy (2012) conducted an experiment on acute and chronic toxicity studies of methanolic extract of *Oxalis corniculata l.* in rat and recorded no significant treatment-related changes in the levels SGOT and SGPT. Alebachew *et al*. (2014) recorded significant increased in AST and ALT level in mice treated with extract of *Vernonia bipontini Vatke*. Mugisha *et al*. (2014) also observed dose dependant increased ALT level in rats treated with ethanolic leaf extracts *of Rumex abyssinica* Jacq and and *Mentha spicata* L. (Lamiaceae). Selçuk *et al*. (2015) reported increased serum AST and ALT level in *Rumex acetosa* toxicity in a boy. Contrary to present findings, Singh and Prakash (2014) recroded no significant ($p>0.05$) changes in GOT, GPT, ALP during toxicity assessment of *Oxalis corniculata.*

Increased AST/SGOT and ALT/SGPT levels in the blood are a known indicator of hepatic degeneration or injury. There will be an increase in cell membrane permeability as a result of hepatic degeneration or injury, which will eventually lead to enzyme leakage in the blood circulation (Alebachew *et al*., 2014). This can be correlated with the finding recorded in histopathological findings in liver of treatment groups and dose dependant degenerative and necrotic changes in the hepatocyte might be the reason for elevation of serum AST and ALT level.

4.4.3Serum creatinine (mg/dL)

The mean serum creatinine level in control and other treatment groups revealed significant ($P<0.05$) differences and are recorded as 0.51±0.012was recorded at the end of experiment and the values are presented in Table 4.7 and Fig.4.6.

The mean of (± SE) serum creatinine levels were recorded as 0.51±0.012 mg/dL, 0.53±0.018 mg/dL, 0.64±0.030 mg/dL, 0.79±0.02 mg/dL in T1, T2, T3 and T4 group, respectively. Results thus revealed significant increased serum creatinine level in group T4 (0.79±0.02 mg/dL) followed by group T3 (0.64±0.030 mg/dL) and T2 (0.53±0.018 mg/dL).

Similar result was also recorded by Singh *et al.* (1995) in ethylene glycol induced acute nephrotoxicity in cow calves., Fang *et al*. (2001) in oxalate nephropathy induced by star fruit in rats, Aslani *et al*. (2011) after ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep, Selçuk *et al*. (2015) in boy ingesting large amount of *Rumex acetosa*. Contrary to this finding Singh and Prakash (2014) recorded non significant differences in serum creatinine level in rats given *Oxalis corniculata.* Significantly increased in serum creatinine level indicating renal toxicity of ethanolic extract of *Oxalis corniculata* given for 28 days. Reddy (2012) during acute and chronic toxicity studies of methanolic extract of *Oxalis corniculata l.* in rat recorded no significant treatment-related changes in the levels creatinine, urea and uric acid.

Calcium bind with oxalate present in plant extract and form insoluble calcium oxalate, these calcium oxalate may precipitate to form large crystals, resulting in renal tubular damage and renal insufficiency and also hypovolaemia caused due to dehydration may impaired excreation of creatinine and results into increased in serum creatinine value (Singh *et al.,*1995) and might be the reason for elevation of serum creatinine level in treatment groups.

4.4.4 Blood urea nitrogen (BUN)

The mean value of blood urea nitrogen (nmol/L) in control and different treatment groups showed significant differences and are depicted in Table 4.7 and Fig.4.6. The blood urea nitrogen level in different groups were observed as 26.25±0.58, 29.97±0.65, 33.55±0.65 and 33.80±0.75 in T1, T2,

T3 and T4 groups, respectively indicating significant increased BUN in group T2, T3 and T4 when compared with control group. Significant increased serum blood urea nitrogen level was observed in group T4 given *Oxalis corniculata* @ 500 mg/kg.b.wt.

The similar findings in treatment groups were also reported by Singh *et al.* (1995) during ethylene glycol induced acute nephrotoxicity in cow calves, Aslani *et al*. (2011) during acute oxalate intoxication associated to ingestion of eshnan (*Seidlitzia rosmarinus*) in sheep; Bajaj *et al*. (2011) in buffalo calves during induced oxalate toxicity by ad libitum feeding of Napier grass *(pennisetum purpureum)*, Singh and Prakash (2014) during toxicity assessment of *Oxalis corniculata* and *Phyllanthus Fraternus* plants in rats and Selçuk *et al*. (2015) in a case of acute tubule interstitial nephritis due to large amount of Sorrel (*Rumex acetosa*) intake in twelve year old boy.

Significantly increased in serum BUN suggested that it might be due to cellular damage and outflow of the nitrogenous waste products such as urea and creatinine which was further confirmed by histopathological findings in kidneys.

4.4.5Serum calcium (mg/dL)

The mean serum calcium level in control and different treatment groups showed significant differences at the end of experiment and were observed as 9.93±0.14, 10.46±0.07, 8.78±0.14 and 7.28±0.16 mg/dL in T1, T2, T3 and T4 groups, respectively (Table 4.7 and Fig.4.6). Significant decreased serum calcium was observed in group T4 (7.28±0.16 mg/dL), T3 (8.78±0.14 mg/dL) and T2 (10.46±0.07 mg/dL) when compared with control group (9.93±0.14 mg/dL).

The decreased serum calcium level in group T3 and T4 are in collaboration with Singh *et al.* (1995) in ethylene glycol induced acute nephrotoxicity in cow calves. The significant ($P<0.01$) decrease in plasma calcium level from 9.13 to 6.19 mg/dl along with decrease in inorganic

phosphorus level (from 5.72 to 3.94 mg/dl) lead to hypocalcaemia was reported by Bajaj *et al*. (2011) in buffalo calves by inducing oxalate toxicity by ad libitum feeding of Napier grass.

Decreased in serum calcium in treatment groups suggested that it might be due to calcium present in blood bind with oxalate present in plant extract and form insoluble calcium oxalate and made it unavailable for body tissue and decreased absorption from intestine because of alkalinity leads to low level of serum calcium (Bajaj *et al*., 2011), however, present study did not reveal presence of oxalate crystals in the renal tubules, but the parenchyma was found to have different degrees of degenerative and necrotic changes.

4.4.6Serum phosphorus (mg/dL)

At the end of experiment, the mean serum phosphorus level revealed significant dose dependant decrease in treatment groups received ethanolic extract of *Oxalis corniculata* (Table 4.7 and Fig.4.6). Significant decreased serum phosphorus level was observed in group T4 (5.35±0.26 mg/dL) followed by T3 (6.50±0.08 mg/dL) and T2 (7.32±0.08 mg/dL).

The decreased serum phosphorous in T2, T3 and T4 group are in collaboration with Bajaj *et al*. (2011) in induced oxalate toxicity by ad libitum feeding of Napier grass *(pennisetum purpureum)* on health of buffalo calves.

In forage plants, soluble oxalate is one of major antinutrients. It works by binding calcium (Ca), magnesium (Mg), and other trace minerals including iron (Fe), preventing them from being assimilated. This causes problems with calcium (Ca) and phosphorus (P) metabolism, as well as excessive bone mineral mobilization (Rahman *et al*., 2012). According to Bajaj *et al*. (2011), decreased serum phosphorous might be due to reduced absorption of phosphorous resulted from alkalinity of intestine.

4.4.7Serum Total protein (gm/dl)

The average mean values of serum total protein in control and different treatment groups are depicted in Table 4.7 and Fig.4.6.

The mean value of serum total protein in group T1, T2, T3 and T4 are recorded as 8.77±0.27, 10.10±0.08, 7.83±0.11 and 7.13±0.34 gm/dL, respectively indicated significant ($P<0.05$) decreased level in treatment groups.

The mice from T2, T3 and T4 groups were found to be significant ($p<0.05$) decreased in total protein as compare to control and T2 groups. This was might be due to EOC treated groups causes damage to liver, increased protein loss, protein losing nephropathy and dietary protein deficiency due to decreased in feed intake by the animals. The similar findings of hypoproteinmia in treatment groups were also reported by Musale *et al*. (2017) during his experiment titled efficacy of calcium borogluconate and lime water therapy in *Anagallis arvensis* intoxicated cattle. Hypoproteinmia in treatment groups is might be due to leakage of protein through glomeruli filtration and its disintegration through degenerating tubules due to oxalate nephrosis, proteinuria and damage to hepatic tissues by calcium oxalate crystals (Radostits *et al.* (2007) were the factors responsible for hypoproteiniemia.

4.4.8 Serum Albumin (gm/dL)

The mean values of serum albumin levels (gm/dL) at the end of the experiment in control and different treatment groups are depicted in Table 4.7 and Fig.4.6.

At the end of the experiment, the mean values of serum albumin was recorded as 3.74±0.05 gm/dL, 3.27±0.02 gm/dL, 2.55±0.12 gm/dL and 2.17±0.07 gm/dL in T1, T2, T3 and T4 group, respectively. The mean values of serum albumin of T4 (2.17±0.07), T3 (2.55±0.12) and T2

(3.27±0.02) showed significant decreased value when compared to control group (3.74±0.05). The similar findings of treatment groups were also reported by Musale *et al*. (2017) during experiment titled efficacy of calcium borogluconate and lime water therapy in *anagallis arvensis* intoxicated cattle. Decreased synthesis of albumin due to hepatopathy by calcium oxalate crystals and proteinuria following to nephrosis (Radostits *et al*. 2007) caused decrease in serum albumin.

4.4.8 Serum Globulin (gm/dL)

The mean values of serum globulin levels (gm/dL) were recorded at the end of the experiment in control and different treatment groups are depicted in Table 4.7 and Fig.4.6.

The serum globulin values were found to be 5.03±0.23, 6.82±0.08, 6.56±0.37 and 4.97±0.32 in T1, T2, T3 and T4 group, respectively. The mean values of serum globulin in control and different treatment groups revealed significant differences. Significant decreased serum globulin was observed in group T4 when compared to group T3, T2 and T1 groups. The similar findings of treatment groups were also reported by Musale *et al*. (2017) during experiment titled efficacy of calcium borogluconate and lime water therapy in *Anagallis arvensis* intoxicated cattle.

4.5 Organ weights (gm)

After detailed necropsy examination of mice at 28th day of experiment, heart, kidney and liver of each group were separated out and organ weight was recorded. The organ weights of different group mice are depicted in Table 4.8.

Table 4.8. Average organ weight (gm) in different group at the end of 28th day of experiment

Group	Heart	Liver	Kidney
T1	0.26±0.01	5.47±0.10	0.78±0.03
T2	0.26±0.01	5.15±0.15	0.77±0.03
T3	0.26±0.02	5.26±0.26	0.79±0.02
T4	0.25±0.01	5.19±0.15	0.78±0.03
Level ofsignificance	NS	NS	NS
CD(0.05)	--	--	--

4.5.1Heart weight (gm)

At end of experiment mean heart weight was found to be 0.26±0.01gm, 0.26±0.01gm, 0.26±0.02gm and 0.25±0.01gm in group T1, T2, T3 and T4 group, respectively (Table 4.8). Results thus indicated non-significant effect of toxicity of ethanolic extract of *Oxalis corniculata* on absolute heart weight.

The similar findings of treatment groups were also reported by Udobang and Okokon (2017) given *Setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract to mice and they observed non significant change in heart weight in treated mice.

4.5.2Liver weight (gm)

At end of experiment, organ weight of liver was observed as 5.47±0.10gm, 5.15±0.15gm, 5.26±0.26gm, and 5.19±0.15gm in group T1, T2, T3 and T4, respectively (Table 4.8). Non significant change in liver weight was observed between treatment groups and control groups.

The similar findings of treatment groups were also reported by Fentahun *et al*. (2020) given methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) and Udobang and Okokon (2017) given *setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract to mice.

4.5.3Kidney weight (gm)

The absolute kidney weight in control and different treatment groups revealed non significant differences and are presented in Table 4.8. When compared with control group (0.78±0.03gm), group T2 (0.77±0.03gm), group T3 (0.79±0.02gm) and group T4 (0.78±0.03gm) showed non significant kidney weight given ethanolic extract of *Oxalis corniculata* for 28 days.

The similar findings of treatment groups were also reported by Fentahun *et al*. (2020) given methanol rhizome extracts of *Rumex abyssinicus jacq*. (plygonaceae) and Udobang and Okokon (2017) given *Setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract to mice.

4.6 Gross Pathological Observations

At the end of experiment six mice from each group were sacrificed by anaesthetizing in jar containing cotton wool soaked in diethyl ether inhalation and detailed necropsy examination was conducted. The gross lesions of pathological significance were recorded as under.

On macroscopic examination of visceral organs of group T1, T2 and T3 mice did not exhibit any gross pathological lesions of significance. However, liver from T4 group showed granular appearance, slightly swollen and focal pin point necrosis (Plate 4.1). Kidneys were swollen, pale color and showed fibrosis in some animals (Plate 4.2). Spleen exhibited enlargement with mild congestion (Plate 4.3). Lung showed emphysema and pale discoloration and congestion (Plate 4.4).

The gross pathological observations recorded were similar with the finding of James (1972) who observed pale and fibrous kidneys, Canfield and Dickens (1982) who recorded enlargement of both the kidneys and emphysemain lung of koala, Suckling (1886) who observed swollen and congested liver and kidney and congestion in lung of boy. Mckenzie *et al*. (1987) also observed swollen and congested liver and kidney. Gulbahar *et al*.

(2002) reported pale and soft kidney in calf while Aslani *et al*. (2011) also observed pale, swollen and enlarged kidneys in sheep.

The dose dependant lesions observed in liver in present study might be due to the presence of bioactive compounds viz. tannins, flavonoids, oxalate, and glycosides in plant and the lesions observed over kidney might be due to nephrotoxic action of plant in kidney (Gulbahar *et al*., 2002).

4.7 Histopathological observations

The tissues of different organs were fixed in 10 per cent neutral buffer saline, processed for routine histopathological process and sections of 4-6 micron were cut and stained by H & E stain (Luna, 1968). The tissue sections were further observed under microscope and observations recorded in different groups are discussed as under.

4.7.1 Liver

Sections of liver from control group showed normal cord like hepatic cell arrangement, mild blood venous congestion, and normal hepatic parenchyma with normal central vein and surrounding polyhedral hepatocytes separated by sinusoids and covered by thin capsule (Plate 4.5).

While, sections of liver from T2 group showed mild mononuclear or lymphoid cells infiltration in parenchyma (Plate 4.6). Foci of sections also showed mild granular and vacuolar degenerative changes in hepatocytes, focal necrosis, mild blood venous congestion (Plate 4.7). Most of the sections (4/6) revealed comparatively normal hepatic parenchyma (Plate 4.8) to that of control group suggesting mild hepatotoxicity only.

T3 Group liver sections showed mild to moderate granular and vaccuolar degenerative changes in the hepatocytes, centrilobular and focal areas of necrosis and mild to moderate blood venous congestion and infiltration of mononuclear cells the sinusoids (Plate 4.9, 4.10). Mild to

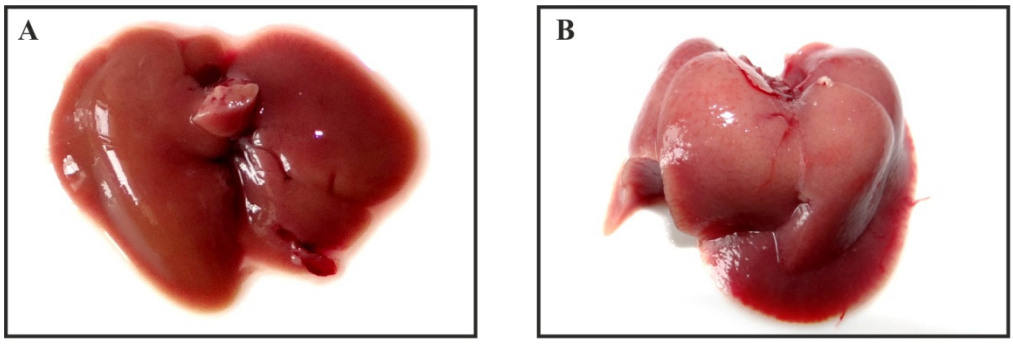


Plate 4.1. Liver: A) Group T1 showing normal morphology B) Group T4 showing granular and swollen liver

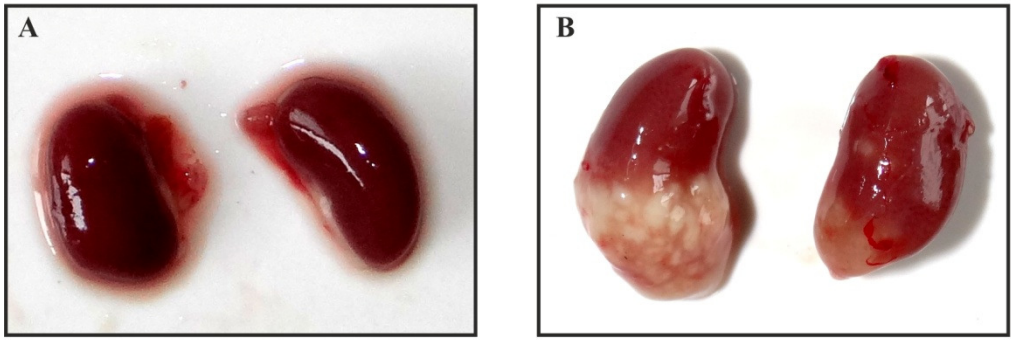


Plate 4.2. Kidney: A) Group T1 showing normal morphology B) Group T4 showing pale colour, swollen and fibrosis.

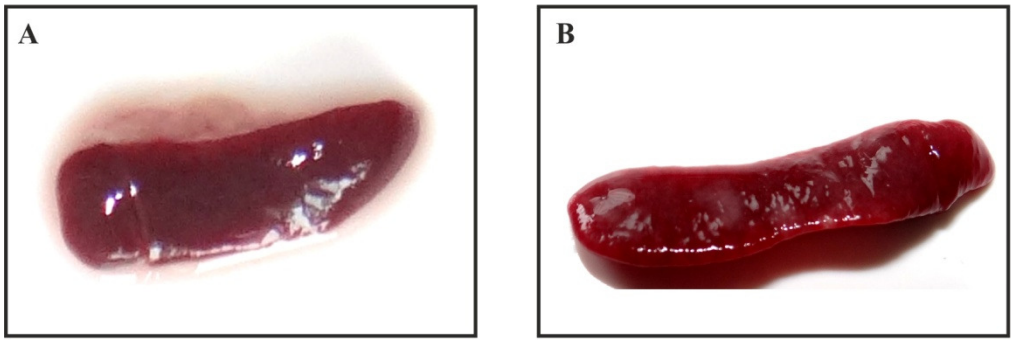


Plate 4.3. Spleen: A) Group T1 showing normal morphology B) Group T4 spleen showing enlargement and congestion

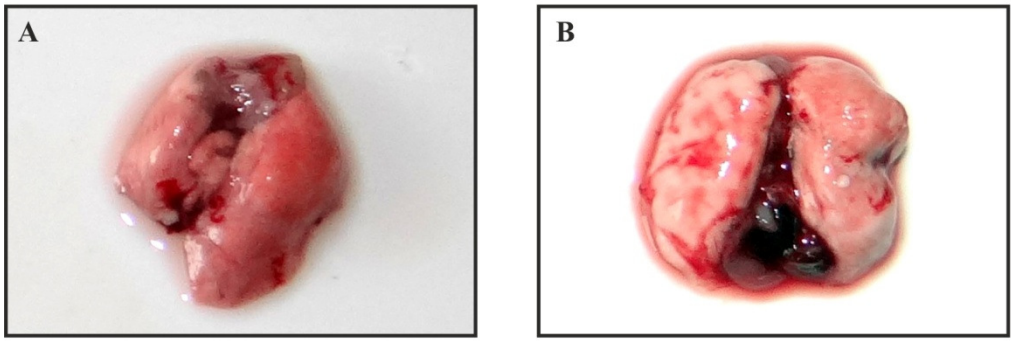


Plate 4.4. Lung: A) Group T1 showing normal morphology B) Group T4 showing pale color, emphysema and congestion

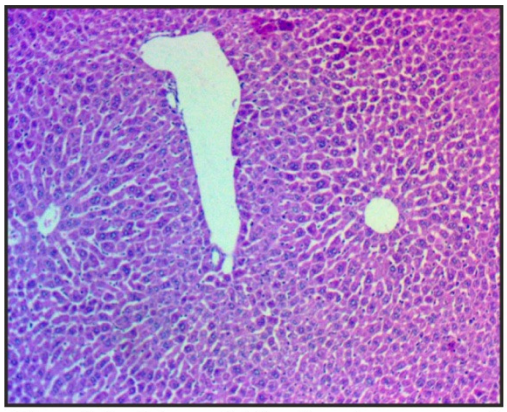


Plate 4.5. Liver (T1) showing normal hepatic parenchyma with normal central vein, polyhedral hepatocytes separated by sinusoids (H& E x100)

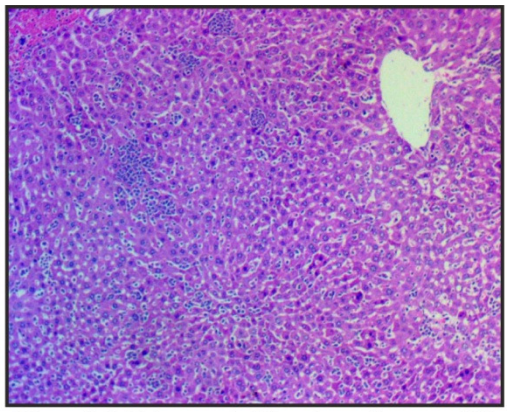


Plate 4.6. Liver (T2) showing mild mononuclear cell infiltration in the parenchyma (H & E x100)

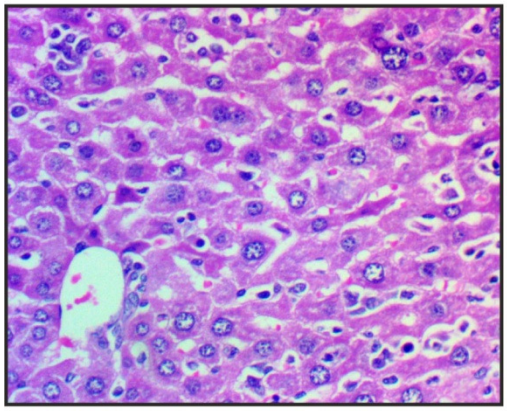


Plate 4.7. Liver (T2) showing mild granular and vacuolar changes in the hepatocytes and focal necrosis (H & E 400)

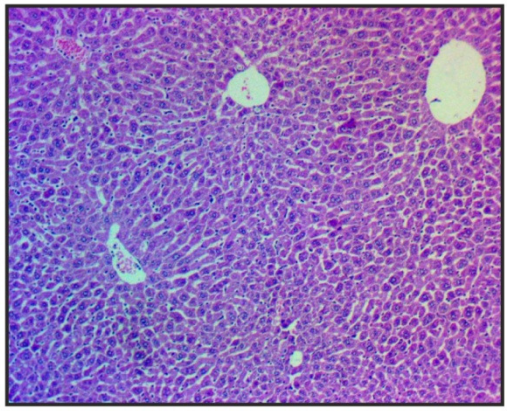


Plate 4.8. Liver (T2) showing normal hepatic cords showing comparatively normal hepatic parenchyma (H & E 100)

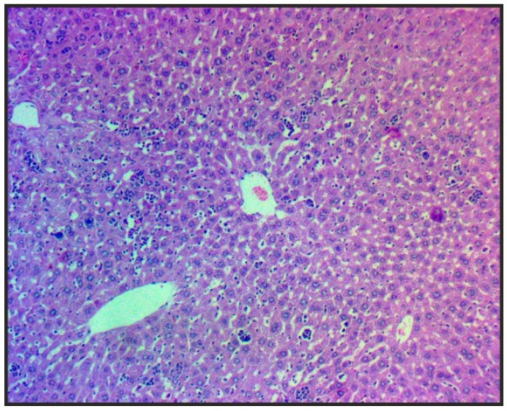


Plate 4.9. Liver (T3) showing centrilobular and focal necrosis with mononuclear cell infiltration in the sinusoides (H & E x400)

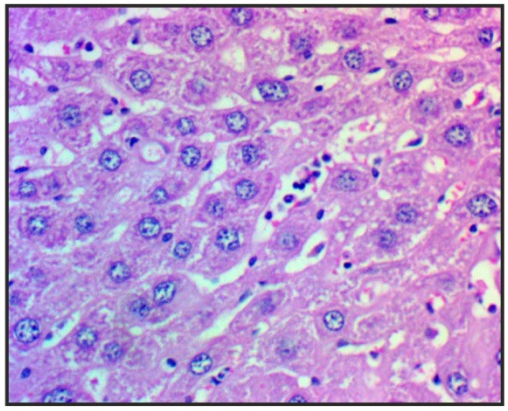


Plate 4.10. Liver (T3) showing mild to moderate granular and vacuolar changes in the hepatocytes (H & E x400)

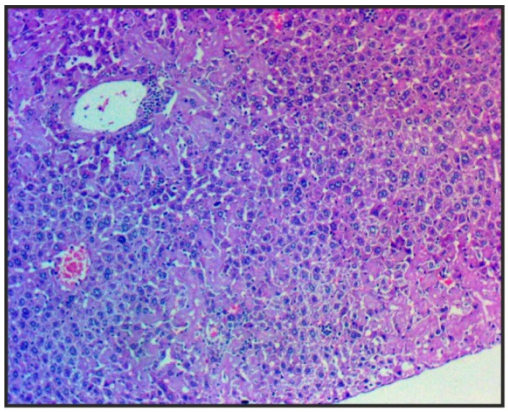


Plate 4.11. Liver (T3) showing perivascular lymphoid aggregation, haemorrhages and necrosis (H & E 100)

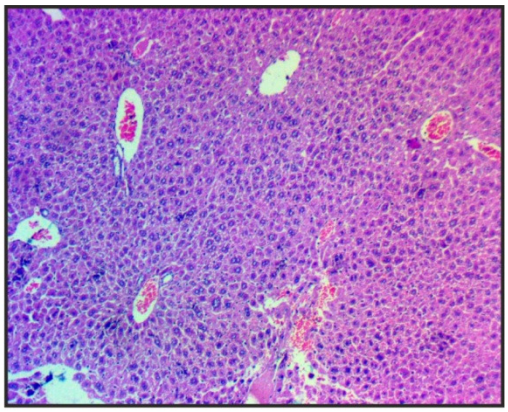


Plate 4.12. Liver (T4) showing prominent venous congestion, haemorrhages and degenerative changes (H & E x100)

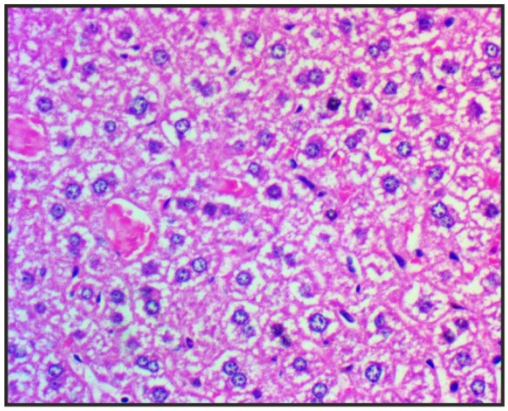


Plate 4.13. Liver (T4) showing extensive granular and vacuolar changes in hepatocytes (H & E x400)

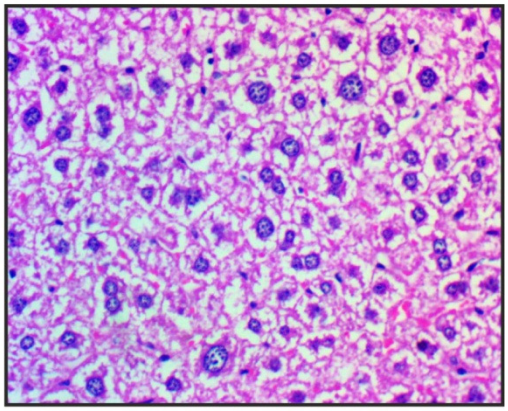


Plate 4.14. Liver (T4) showing distortion of hepatic parenchyma, pyknosis, shrinkage of hepatocyts (H & E x400)

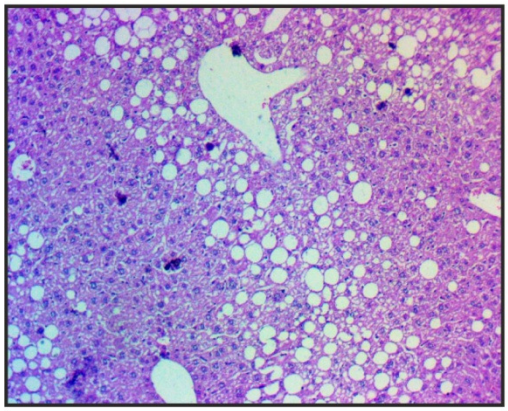


Plate 4.15. Liver (T4) showing fatty changes (H & E x100)

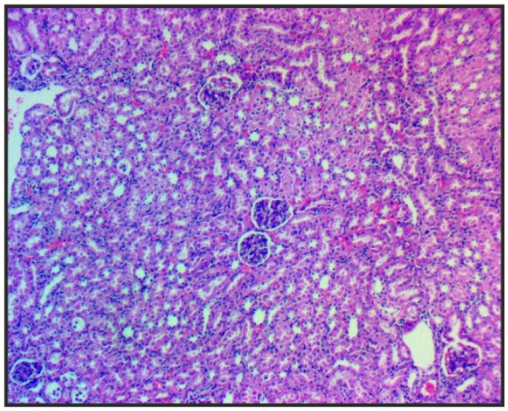


Plate 4.16. Kidney (T1) showing nomal renal parenchyma (H & E x100)

moderate perivascular lymphoid aggregation and hemorrhages was also observed in some of the sections (Plate 4.11).

However, sections of liver in T4 group showed prominent blood venous congestion, hemorrhages, centrilobular and periportal necrosis and diffuse necrosis of hepatocytes (Plate 4.12). Sections also revealed mononuclear lymphoid aggregation in the parenchyma. Few sections (3/6) showed extensive granular and vaccuolar changes in hepatocytes, distortion of hepatic parenchyma, pyknosis, shrinkage of hepatocyts along with Kupffer cell proliferation (Plate 4.13 and 4.14). Two sections showed fatty changes (Plate 4.15). Degenerative changes were more prominent towards periphery of the parenchyma.

Present findings are in accordance with Aslani *et al*. (2011) who reported acute oxalate intoxication associated to ingestion of Eshnan (*Seidlitzia rosmarinus*) in sheep and observed mild to moderate congestion with vacuolar degeneration in the liver. In addition to the present findings, Alebachew *et al*. (2014) observed dilated sinusoids, nuclear enlargement, lots of binucleated hepatocytes and also peripheral cramped chromatin, shrinkages (single cell death) of hepatocytes and fragmentation of hepatocytes given methanol leaves extract of *Vernonia bipontini Vatke* @ 800 mg/kg of b. wt in mice. Mugisha *et al*. (2014) observed focal cellular necrosis, congestion and haemorrhages. Udobang and Okokon (2017) reported mild toxic effect in liver such as presence of thrombosed to congested portal blood vessels and inflammation after feeding of *setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract @ 300 and 450 mg/kg in rats and Belsty *et al*. (2019) observed congestion and hemorrhage of portal vein and hepatic artery, cellular infiltration in the central vein, sinusoidal dilatation, Kupffer cell hyperplasia and binucleated hepatocytes.

The dose dependant lesions observed in liver in present study might be due to the presence of bioactive compounds viz. tannins, flavonoids, oxalate, and glycosides in plant that may have antiradical activities and free radicals setup a chain reaction that can cause biological damage to hepatocytes by stimulating glycation of protein, inactivation of enzymes, alteration in the structure and function of collagen basement and other membranes. Alcoholic leaf extracts were more effective stable free radical scavengers and presence of oxalate in the food is also associated with acidity and toxicity which results into retention of water inside hepatocyte resulting in cell enlargement which may be due to reduction of energy necessary for ion regulation in the cells (Alebachew *et al*., 2014).

4.7.2 Kidney

The microscopic examination of kidney from control group showed normal renal parenchyma and did not reveal any detectable significant histopathological changes in renal tubules; glomerular epithelium excepts only mild blood venous congestion in some sections (Plate 4.16).

Sections of kidney from group T2 showed mild blood venous congestion, focal areas of glomerular degeneration, mild haemorrhages in between tubules and mild granular changes in tubular epithelium (Plate 4.17). Lymphoid aggregation in was prominent at perivascular area in intratubular region in some sections (3/6) (Plate 4.18). and However, most of the section revealed comparatively normal renal parenchyma with normal renal tubules and normal glomuruli.

Sections of kidneys from T3 group showed mild to moderate blood venous congestion, tubular and intratubular haemorrhages, mild focal glomerular degeneration, mild to moderate granular and vaccular changes in tubular epithelium and tubular necrosis (Plate 4.19). Renal tubules were dilated. Only few sections (3/6) revealed loss of tubular architecture and

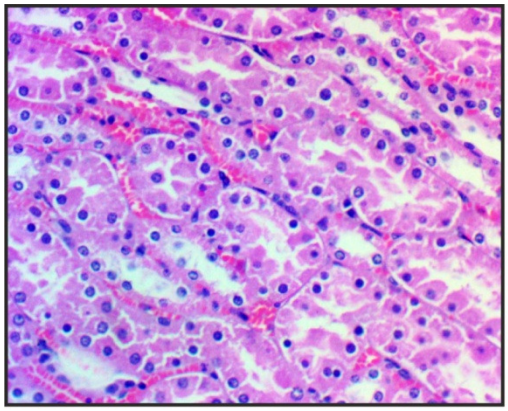


Plate 4.17. Kidney (T2) showing mild degenerative changes in the tubules and glomeruli and haemorrhages (H & E x400)

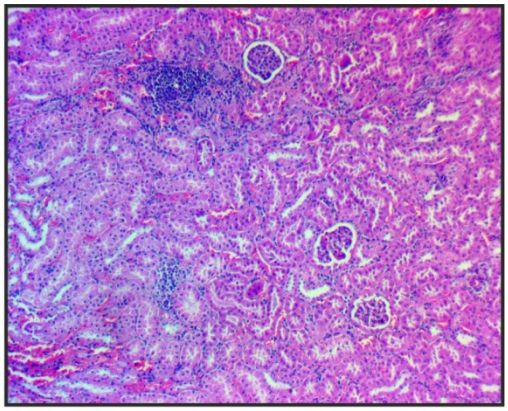


Plate 4.18. Kidney (T2) showing lymphoid aggregation and intratubular haemorrhages (H & E x100)

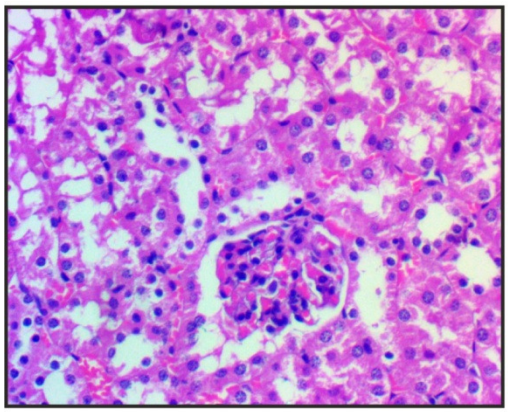


Plate 4.19. Kidney (T3) showing intertubular and glomerular haemorrhages, degenerative changes in tubules and tubular necrosis (H & E x400)

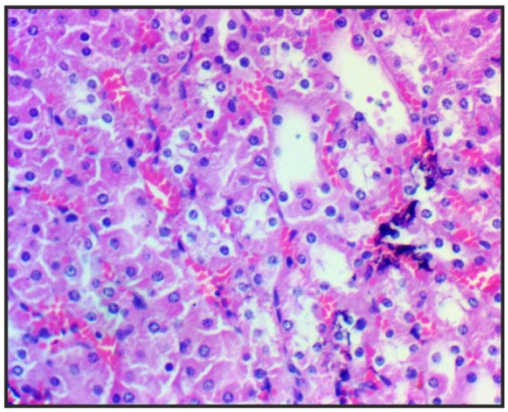


Plate 4.20. Kidney (T3) showing tubular necrosis and detachment of tubular epithelium from the basement membrane (H & E x100)

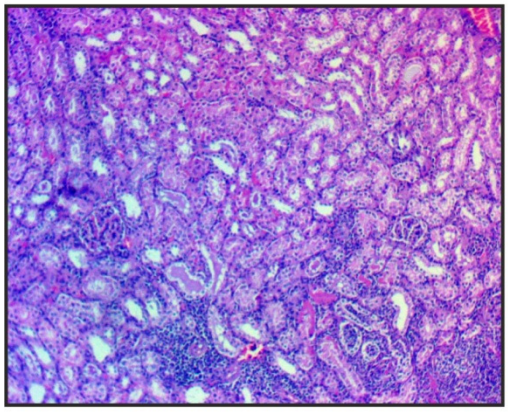


Plate 4.21. Kidney (T4) showing haemorrhages, and mononuclear cell infiltration and perivascular lymphoid aggregation (H & E x100)

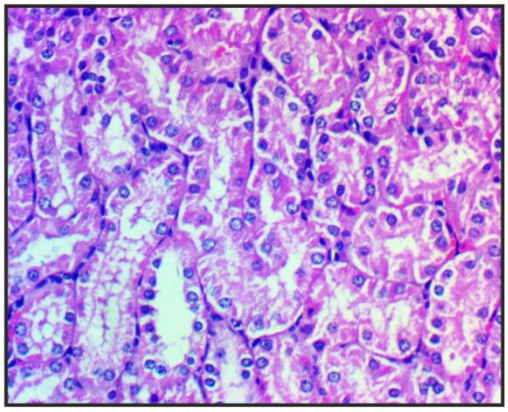


Plate 4.22. Kidney (T4) showing degenerative changes in tubular epithelium and tubular necrosis (H & E x400)

detachment of tubular epithelium from basement membrane (Plate 4.20). Lymphoid aggregation was observed in few sections (2/6) at some places.

Microscopic examination of kidney from T4 group showed mild to moderate blood venous congestion, perivascular lymphoid aggregation and haemorrhages. There were prominent lymphoid aggregations in renal parenchyma. (Plate 4.21). Sections showed moderate to extensive granular and vaccuolar changes in tubular epithelium, dilatation of tubules, detachment of tubular epithelium from basement membrane, swelling of tubular epithelium, intertubular haemorrhages and loss of tubular architecture were seen in most of the sections (Plate 4.22 and 4.23). In addition to lesions in earlier groups there were prominent and extensive accumulations of proteineous mass in the renal tubules in some sections (Plate 4.24). Glomeruli showed reduction in urinary space with degenerative changes in some of them. Interstitial nephritis was most prominent lesions in few of the sections (2/6) showing mononuclear and polymorphonuclear cell infiltration in renal parenchyma (Plate 4.25).

Present findings are in accordance earlier finding of Walker (1939) who reported contracted glomeruli, necrotic epithelium of the convoluted tubules, and in several places had desquamated apart from this he also observed collection of crystals throughout the whole of the conical zone and in close proximity to the convoluted tubules during *Oxalis corniculata* poisoning in sheep, however, visible crystal formation was not observed in any of the treatment group. The variation in species or dose might be the reason for no visible crystal formation in kidneys of mice in present investigation. Mckenzie *et al*. (1987) reported nephrotic with necrosis of some cortical tubules, dilation of most tubules, hyaline casts and many rosettes of birefringent calcium oxalate crystals in tubular lumens and oedema of the interstitium during study of acute oxalate poisoning of sheep by buffel grass. Fang *et al*. (2001) observed dilatation of renal tubules, flattening of tubular epithelium and interstitial mononuclear cell infiltration and also numerous refractile oxalate crystals were observed in some tubules. In addition to

present findings, Gulbahar *et al*. (2002) reported dilated cortical and medullary tubules with birefringent crystalline casts, focal tubular epithelial hyperplasia and diffuse interstitial fibrosis. Khan and Glenton (2010), Aslani *et al*. (2011), Alebachew *et al*. (2014) reported increased cellularity of glomerulus, urinary space obliteration and enlarged macula densa, Mugisha *et al*. (2014), Mitchell *et al*. (2017), Udobang and Okokon (2017), Belsty *et al*., (2019) reported congestion of portal and central veins, sinusoid dilatation and Kupffer cell proliferation.

The dose dependant renal toxicity in EOC treated groups might be due to the presence of oxalates in plant which involves more mechanical obstruction to renal parenchyma and may be due to in part to intracellular chelation of calcium and magnesium and hence interference with oxidative phosphorylation which results into increased oxidative stress a crucial pathogenetic factor in cellular damage to renal parenchyma (Gulbahar *et al*., 2002).

4.7.3Lung

Microscopic examination of lung from control group of mice showed normal lung alveoli, bronchi, bronchioles, blood vessels suggesting normal lung parenchyma with mild to moderate blood venous congestion (Plate 4.26).

Sections of lung from group T2 mice showed mild edematous changes and foci showed mild thickened alveolar septa. Mild perivascular and peribronchial lymphoid aggregation was also observed (Plate 4.27). Comparatively normal lung parenchyma was seen in almost all the sections.

Sections of lung from group T3 showed mild to moderate thickened alveolar septa, mild perivascular and peribronchial lymphoid aggregation, edema and blood vessel congestion (Plate 4.28).

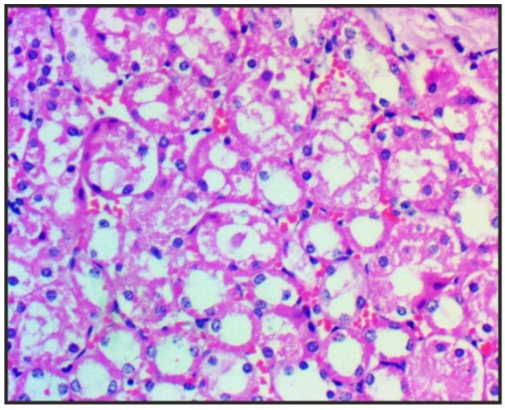


Plate 4.23. Kidney (T4) showing extensive degenerative changes in tubular epithelium and tubular necrosis (H & E x400)

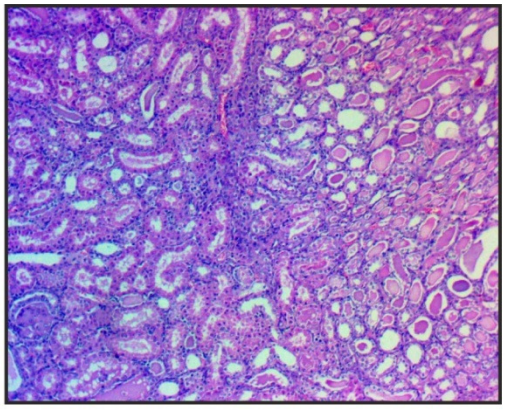


Plate 4.24. Kidney (T4) showing extensive accumulations of proteineous mass in the renal tubules (H & E x100)

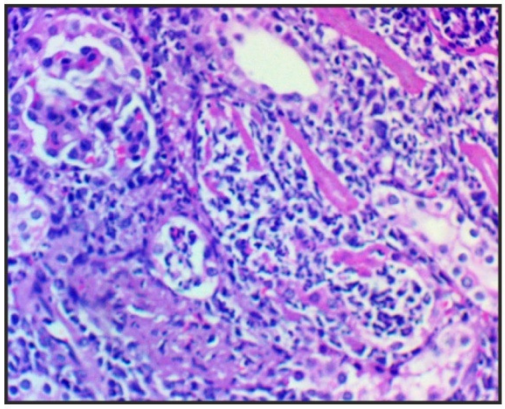


Plate 4.25. Kidney (T4) showing showing mononuclear and polymorphonuclear cell infiltration in renal parenchyma (H & E x400)

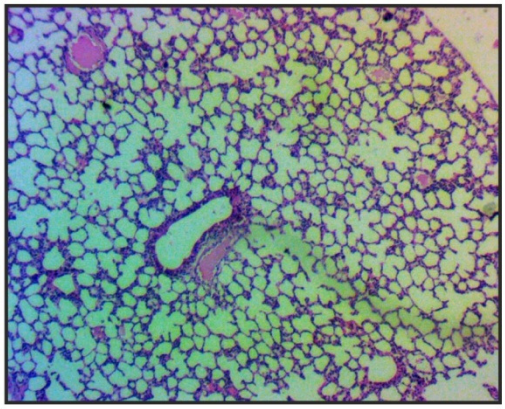


Plate 4.26. Lung (T1) showing normal lung parenchyma (H & E x100)

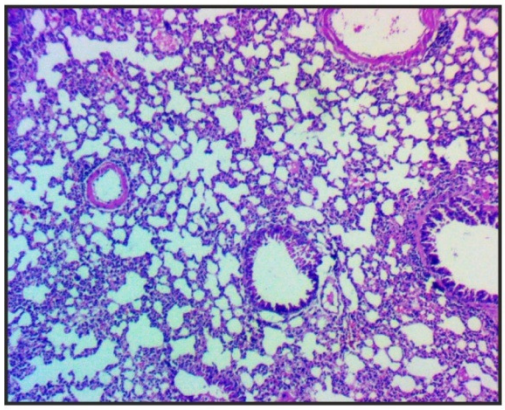


Plate 4.27. Lung (T2) showing peribrobchial lymphoid aggregation and mild thickened alveolar septa (H & E x100)

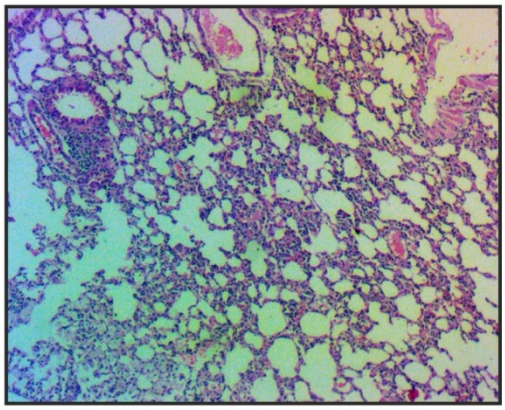


Plate 4.28. Lung (T3) showing mild to moderate thickened alveolar septa, mild perivascular and peribronchial lymphoid aggregation and edema (H & E x100)

Microscopic examination of lung sections from group T4 showed perivascular and peribronchial lymphoid aggregation and edematous changes. Some sections showed moderate thickened alveolar septa, haemorrhages and blood venous congestion. Sections also showed polymorphonuclear cell infiltration at some places (Plate 4.29 and 4.30). Lesions are indicative of mild to moderate degenerative changes in lung parenchyma.

Present findings are in accordance with Mugisha *et al*. (2014) who also observed cellular infiltration and tissue degeneration in lungs given ethanolic leaf extracts *of Rumex abyssinica* Jacq (Polygonaceae) and *Mentha spicata* L. (Lamiaceae). *M. spicata* @ 1000 and 1500 mg/kg b.wt. and Udobang and Okokon (2017) reported thrombosed to congested blood vessels within the inter-alveolar septae given *Setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract @ 300 and 450 mg/kg in rats.

The above observations found in EOC treated groups might be due to induction of inflammatory process by active chemicals such as tannins, flavonoids, oxalate, and glycosides, in the extract (Mugisha *et al*., 2014).

4.7.4 Heart

Microscopic examinations of sections of heart from control group mice showed normal arrangement of cardiac muscle fibers with prominent nucleus and cross striations suggesting normal cardiac architecture (Plate 4.31).

Histological sections of heart from group T2 mice showed normal cardiac muscle with mild separation (Plate 4.32). Mild vaccuolar degenerative changes in cardiac muscle fibers, mild blood venous congestion and mild focal infiltration of mononuclear cells in some sections of heart (2/6) (Plate 4.33). Comparatively normal heart parenchyma was observed in almost all the sections.

Sections of heart from group T3 mice showed mild haemorrhages, mild vaccuolar degenerative changes, and focal area of necrosis (Plate 4.34). Myocardial muscles showed mild to moderate separation, degenerative changes and loss of cross striations (Plate 4.35). Mild blood venous congestion was also seen in some portion of heart.

Microscopically, heart of T4 group showed mild to moderate separation of cardiac muscle fibers, myocardial degeneration and mild to moderate vaccuolar degenerative changes (Plate 4.36). Some sections showed infiltration of mononuclear cells and necrosis of muscle fibers (Plate 4.37). The lesions are more prominent in T4 group when compared with that of group T2 and group T3 suggesting dose dependant toxicity changes in mice.

Present findings are in accordance with that of Udobang and Okokon (2017) who also reported striated muscle fibers admixed with areas of scanty cytoplasm given *setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract @ 300 and 450 mg/kg in rats.

The above observations of heart found in EOC treated groups might be due to oxalic acid present in the plant which is highly corrosive and irritating and on accumulation it causes severe damages (Fong *et al*., 2000).

4.7.5Spleen

Microscopic examination of spleen from T1 group mice showed normal histological architecture with normal lymphoid population, evenly distributed normal lymphoid cells in white pulp and red cells in red pulp and normal germinal centers (Plate 4.38).

Sections of spleen from T2 group mice showed comparatively normal spleenic architecture. Some sections showed atrophy of lymphoid follicles and only mild depletion of lymphoid follicles (Plate 4.39).

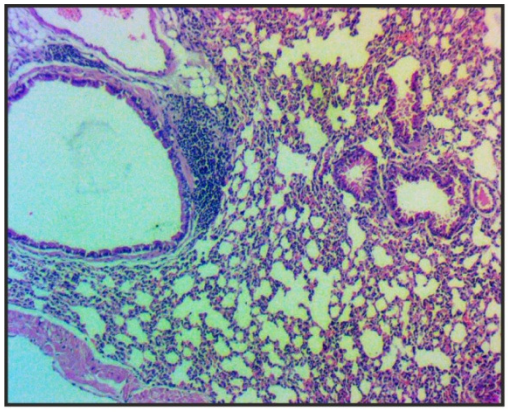


Plate 4.29. Lung (T4) showing peribrobchial lymphoid aggregation, thickened alveolar septa and edema (H & E x100)

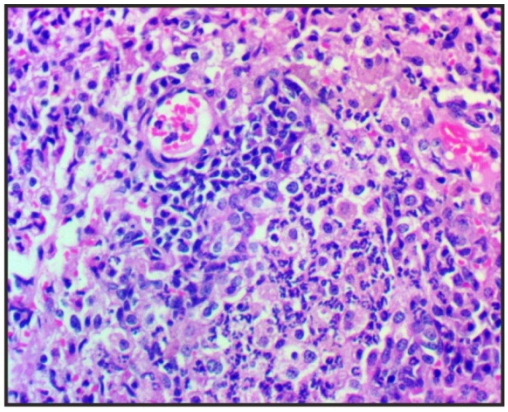


Plate 4.30. Lung (T4) showing thickened alveolar septa and polymorphonulcar cell infiltraion (H & E x400)

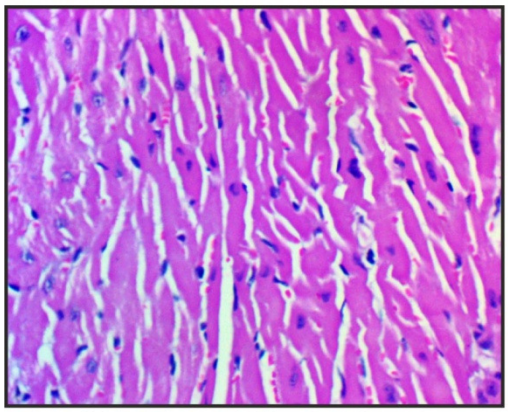


Plate 4.31. Heart (T1) showing normal cardiac architecture (H & E x100)

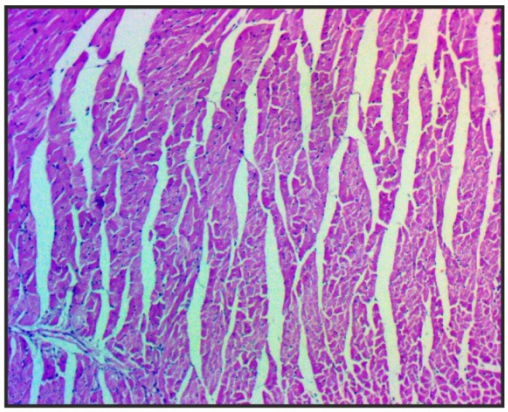


Plate 4.32. Heart (T2) showing mild separation of cardiac muscle fibers with comparatively normal heart parenchyma (H & E x 100)

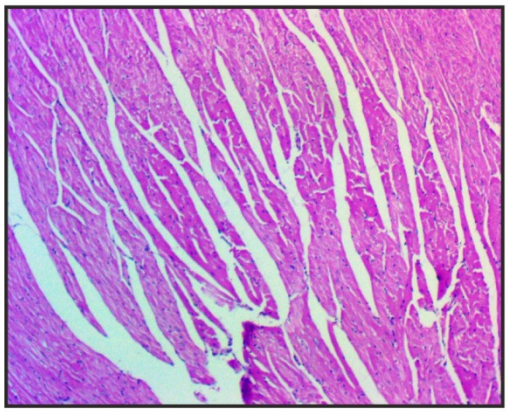


Plate 4.33. Heart (T2) showing mild focal infiltration of mononuclear cells (H & E x100)

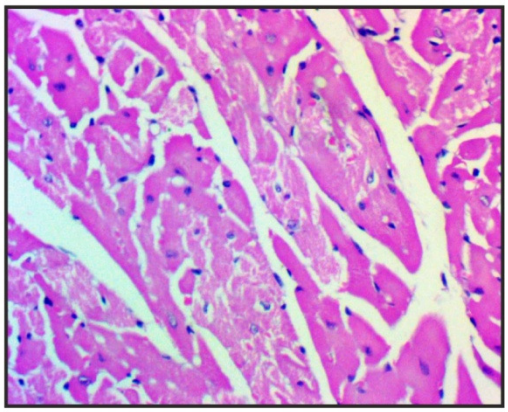


Plate 4.34. Heart (T3) showing vaccuolar degenerative changes, and focal area of necrosis (H & E x100)

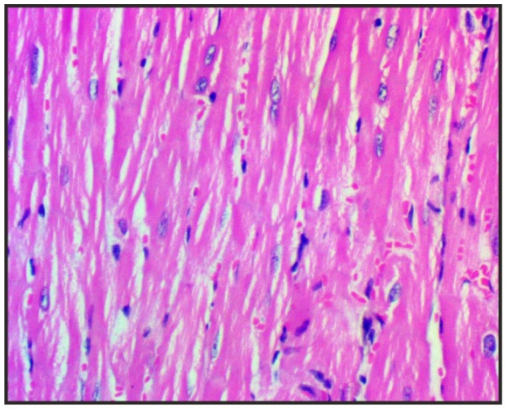


Plate 4.35. Heart (T3) showing degenerative changes and loss of cross striations (H & E x100)

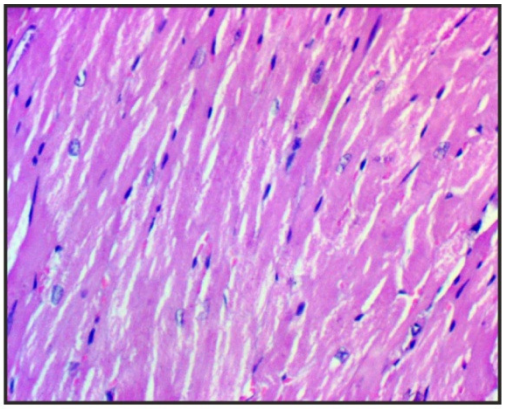


Plate 4.36. Heart (T4) showing separation of muscle fibers, myocardial degeneration and necrosis (H & E x100)

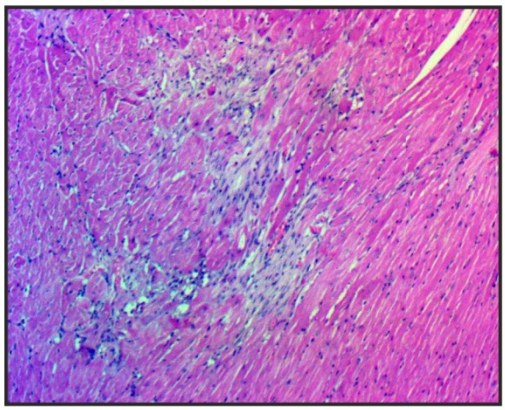


Plate 4.37. Heart (T4) showing mononuclear cell infiltration, myocardial degeneration and necrosis (H & E x100)

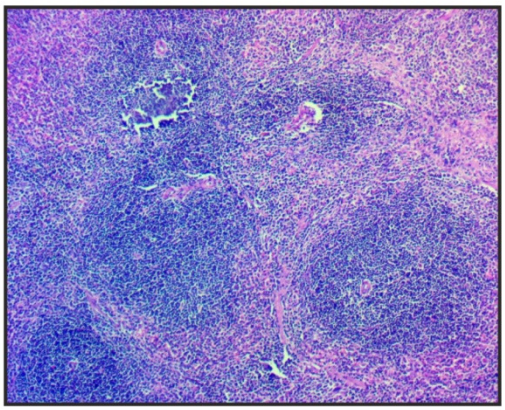


Plate 4.38. Spleen (T1) showing normal spleenic parenchyma (H & E x100)

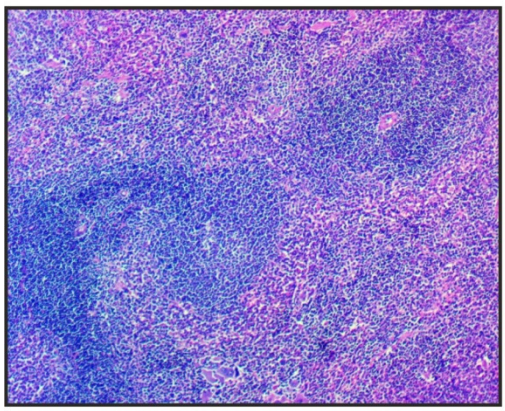


Plate 4.39. Spleen (T2) showing ncomparatively normal spleenic parenchyma with mild depletion of lymphoid follicles (H & E x100)

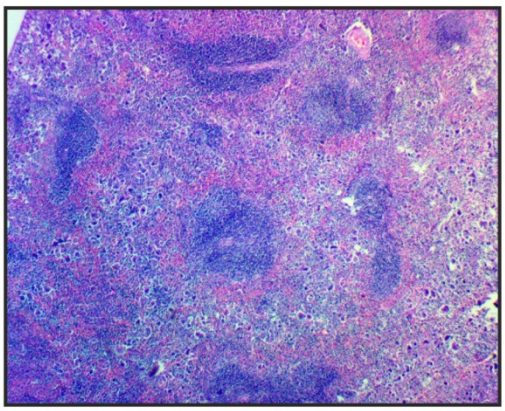


Plate 4.40. Spleen (T3) showing depletion of lymphoid population and atrophy of lymphoid follicles (H & E x40)

Histological sections of spleen from T3 group showed moderate to extensive depletion of lymphoid population and atrophy of lymphoid follicles (Plate 4.40). Some sections (4/6) showed degenerative changes, hemorrhages and necrosis of lymphoid follicles (Plate 4.41).

Sections of spleen from T4 group showed atrophy and moderate to extensive degenerative changes and severe depletion of lymphoid population in lymphoid follicles (Plate 4.42). White pulp showed necrosis of lymphoid follicles and thinning of lymphoid populations indicating immunosuppression (Plate 4.43).

Similar to present findings Udobang and Okokon (2017) also recorded variably sized lymphoid follicles and areas of hemorrhage in spleen given *Setaria megaphylla* (steud) t. dur and sphinz (poaceae) root extract @ 300 and 450 mg/kg in rats.

4.7.6 Duodenum

Microscopically, sections of duodenum from control group mice showed normal finger like tall branching villi with narrow apical end, broad basal end having normal muscular and glandular structure with normal goblet cells and brunner's gland population. Lamina propria and muscularis mucosa showed normal architecture with normal lymphoid population (Plate 4.44).

Sections of duodenum from T2 group showed comparatively normal histoarchitecture of intestinal villi with mild degenerative changes at the tip of villi and mild fusion of villi (Plate 4.45). Some sections showed thinning of lymphoid population in lamina propria and mild degenerative changes in lamina propria (Plate 4.46).

Microscopically, sections of duodenum from T3 group mice showed blunt, short and fused villi with mild to moderate degenerative changes in villi and loss of branching pattern in villi (Plate 4.47). Some

section showed mild degenerative changes in lamina propria and sloughing of epithelium in the intestinal lumen (Plate 4.48).

Group T4 showed shorter, blunt and fused villi when compared with group T1, T2 and T3. There were moderate degenerative changes in villi in lamina propria, necrosis in the villi and sloughing of villi epithelium in the lumen and mild inflammatory changes in the muscularis mucosa and lamina propria (Plate 4.49 and 4.50).

Present findings are in accordance with Mugisha *et al*. (2014) who also reported cellular infiltration in intestinal villi and sloughing of the intestinal villi given ethanolic leaf extracts *of Rumex abyssinica* Jacq @ 1000 mg/kg and @ 1500 mg/kg b.wt. in rats respectively.

The above observations such as sloughing of the intestinal villi and cellular infiltration were found in EOC treated groups might be due to induction of inflammatory process by active chemicals such as tannins, flavonoids, oxalate, and glycosides in the extract (Mugisha *et al*., 2014).

4.7.7 Stomach

Histopathological sections of stomach from control group showed normal glandular portion. Normal gastric glands along with normal gastric pits were observed. Mucosa, submucosa, mascularis mucosae were found to be normal. Normal surface mucous cells were observed with mucous (Plate 4.51 and 4.52).

Sections of stomach from T2 group mice showed comparatively normal architecture of gastric glands. Some sections showed mild degenerative changes in glandular epithelium and mild granular changes in surface mucosal epithelium in the gastric glands (Plate 4.53 and 4.54).

Sections of stomach from T3 group mice showed mild mononuclear cells infiltration, haemorrhages, blood venous congestion in submucosa and muscularis mucosa. Some sections (3/6) showed mild

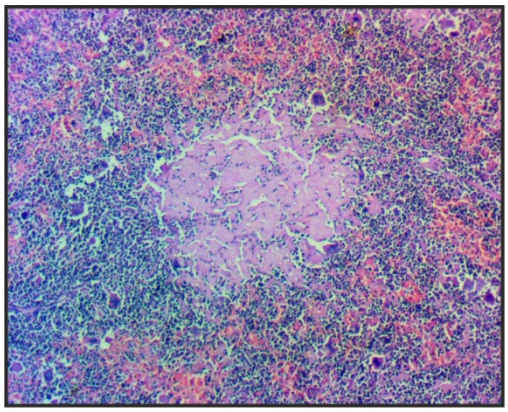


Plate 4.41. Spleen (T3) showing degenerative changes, hemorrhages and necrosis of lymphoid follicles (H & E x100)

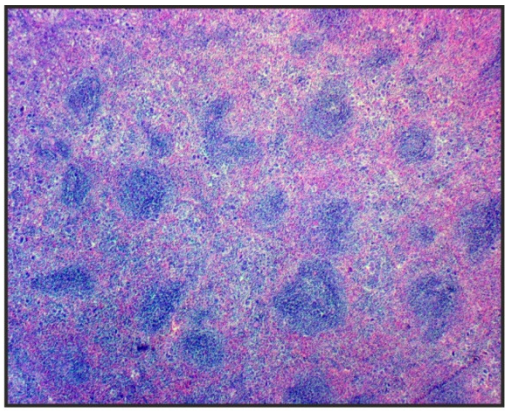


Plate 4.42. Spleen (T4) showing atrophy of lymphoid follicles and depletion of lymphoid populations (H & E x40)

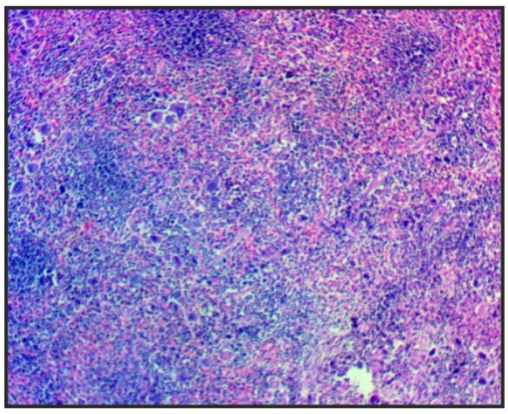


Plate 4.43. Spleen (T4) showing severe depletion of lymphoid population, degenerative changes and necrosis (H & E x100)

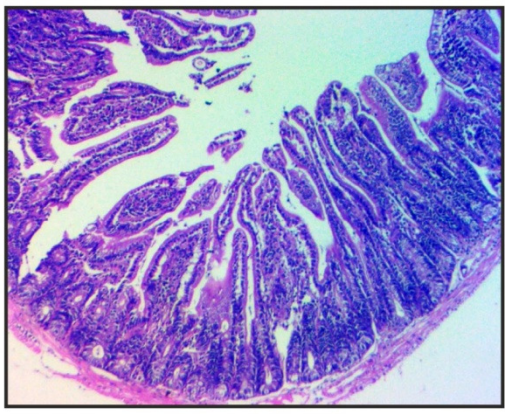


Plate 4.44. Duodenum (T1) showing normal histoarchitecture with tall finger like villi (H & E x 100)

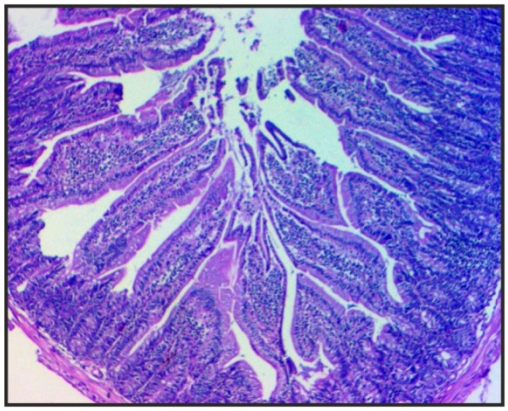


Plate 4.45. Duodenum (T2) showing mild degenerative changes at tip of villi and fusion of villi (H & E x100)

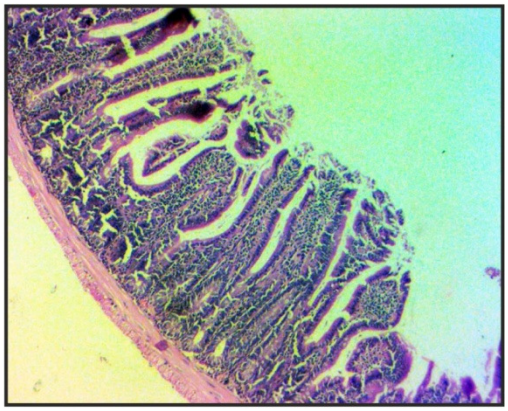


Plate 4.46. Duodenum (T2) mild degenerative changes in lamina propria and necrosis at tip of villi (H & E x100)

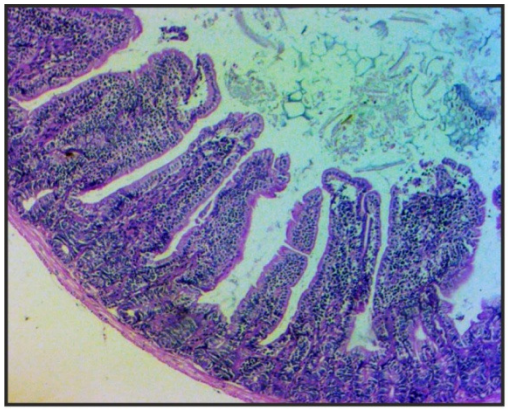


Plate 4.47. Duodenum (T3) showing fused villi with degenerative changes in lamina propria (H & E x100)

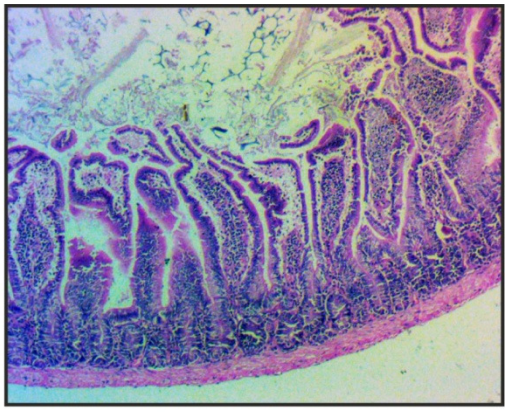


Plate 4.48. Duodenum (T3) showing degenerative changes and sloughing of villi epithelium in the lumen (H & E x100)

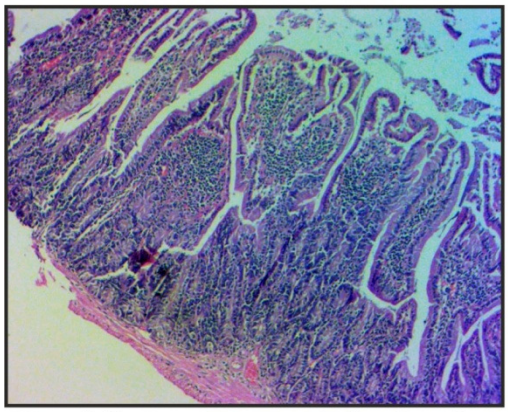


Plate 4.49. Duodenum (T4) showing fused villi with mil inflammatory and degenerative changes in lamina propria (H & E x100)

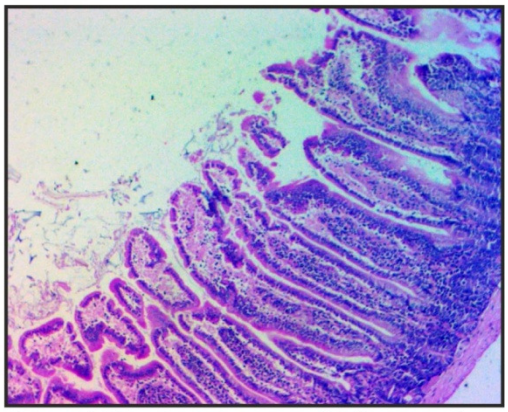


Plate 4.50. Duodenum (T4) showing necrosis in the villi (H & E x100)

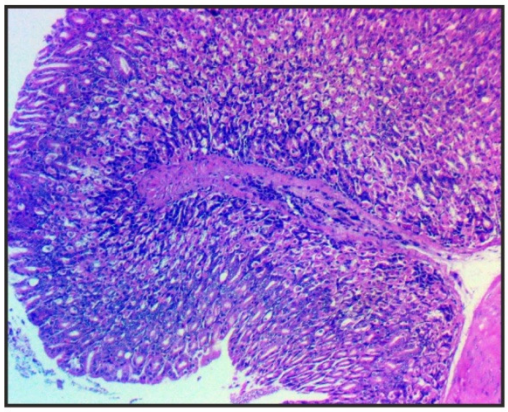


Plate 4.51. Stomach (T1) showing normal gastric pits mucosa, submucosa, mascularis mucosae and surface mucous cells (H & E x100)

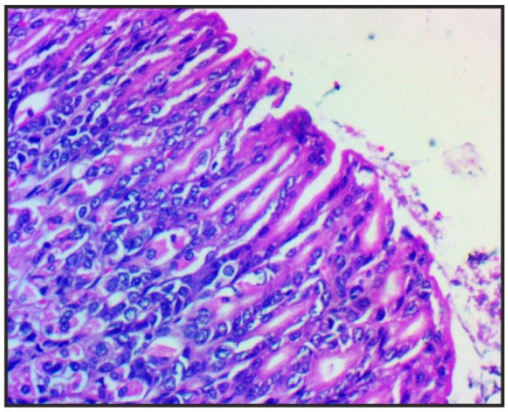


Plate 4.52. Stomach (T1) showing normal gastric pits and gastric glands(H & E x400)

degenerative changes in mucosal surface and in gastric glands and mild vacuolar and granular changes along with widening of gastric pits were also observed (Plate 4.55).

Histopathological sections of stomach from T4 group showed degeneration and necrosis of gastric gland (Plate 4.56). Moderate granular and vaccuolar changes and mild infiltration of mononuclear cells in mascularis mucosae were observed (Plate 4.57). Some sections showed polymorphonuclear cells infiltration in muscularis mucosa, necrosis and atrophy of gastric glands were also observed (Plate 4.58).

Histopathological observations thus revealed dose dependant toxic or inflammatory changes in the stomach of mice given *Oxalis corniculata* for 28 days. However, lesions observed were of no pathological significance even at the dose of 500 mg/kg body weight.

4.7.8 Brain

Microscopic examination of brain of control group of mice showed normal architecture of brain showing well distinguished grey matter and white matter, normal Virchow Robin space, normal neuronal architecture and mild blood venous congestion showing normal brain parenchyma (Plate 4.59).

Sections of brain from T2 group revealed mild neuronal degeneration, mild blood venous congestion and satelitosis. Comparatively normal histoarchitecture and normal brain parenchyma was observed as that of control group (Plate 4.60).

Histological sections of brain from T3 group showed mild blood venous congestion, mild increase in Virchow-Robin spaces along with mild vacuolar and mild degenerative changes in the cytoplasm. In some sections (2/6), mild neuronal degenerative changes were also observed (Plate 4.61).

Sections of brain from T4 group showed prominent blood venous congestion, microhemorrhages, accumulation of glial cell and mild neuronal degeneration in most of the sections (4/6). Some section showed mild increase in Virchow-Robin spaces (Plate 4.62 and 4.63).

Present findings are somewhat in accordance with James (1972) who recorded microhemorrhage in sheep at 6% soluble oxalate dose for 110 days.

The above observations of brain found in EOC treated groups might be due to oxalic acid which is highly corrosive and irritating and on accumulation it causes severe damages (Fong *et al*., 2000).

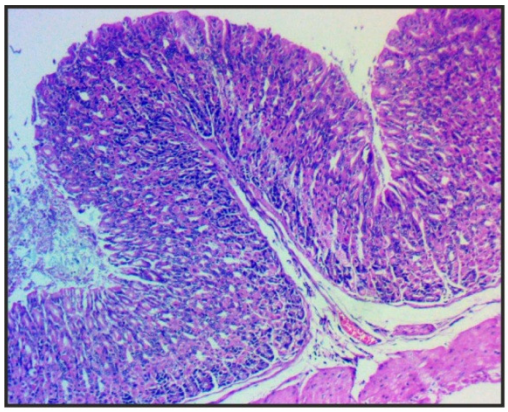


Plate 4.53. Stomach (T2) showing mild degenerative changes in surface mucosal cells(H & E x100)

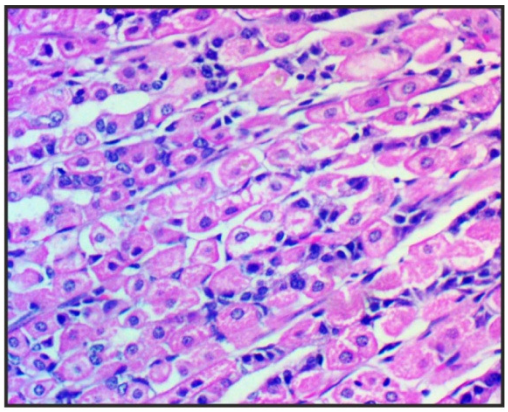


Plate 4.54. Stomach (T2) showing mild granular and vacuolar changes in the gastric mucosal cells(H & E x400)

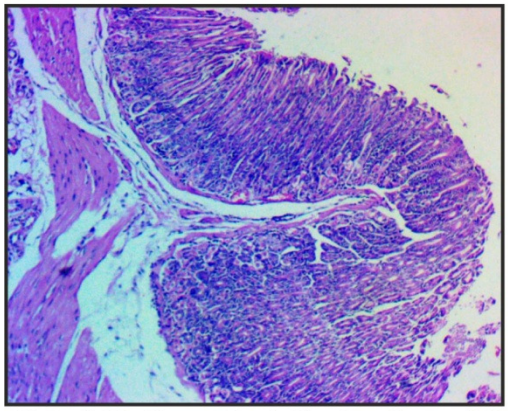


Plate 4.55. Stomach (T3) showing mild mild mononuclear cells infiltration, haemorrhagesin submucosa and muscularis mucosa (H & E x100)

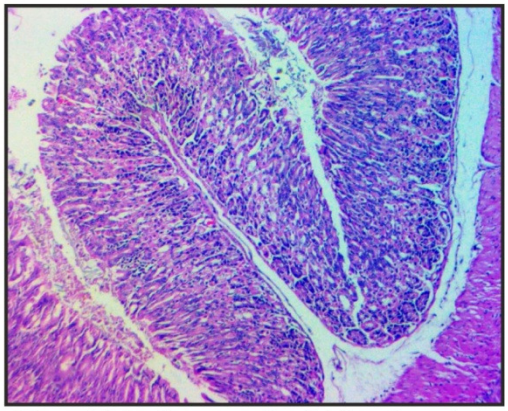


Plate 4.56. Stomach (T4) showing degeneration and necrosis of gastric glands(H & E x100)

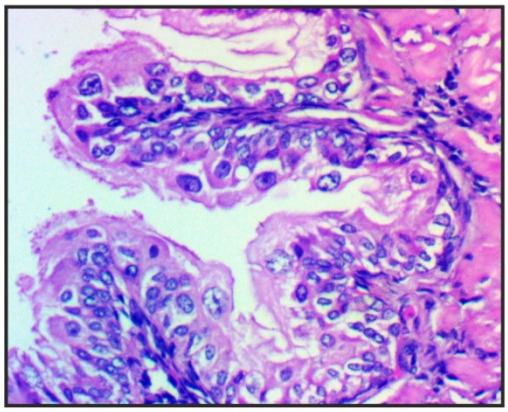


Plate 4.57. Stomach (T4) showing degeneration and necrosis of gastric glands(H & E x400)

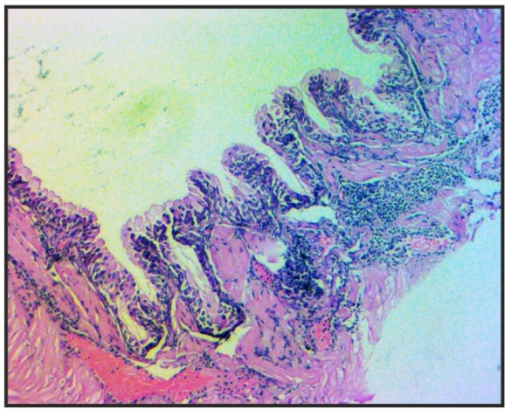


Plate 4.58. Stomach (T4) showing polymorphonuclear cells infiltration in muscularis mucosa, necrosis and atrophy of gastric glands (H & E x100)

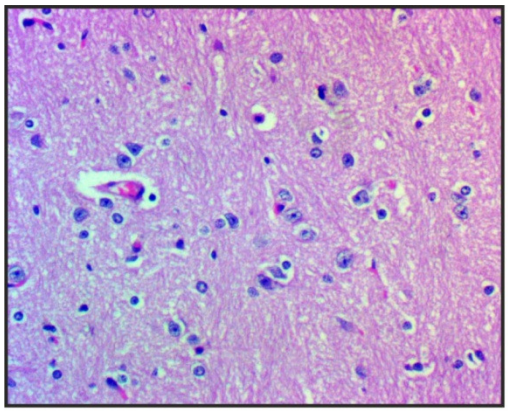


Plate 4.59. Brain (T1) showing mild venous congestion and normal brain parehcnyma (H & E x400)

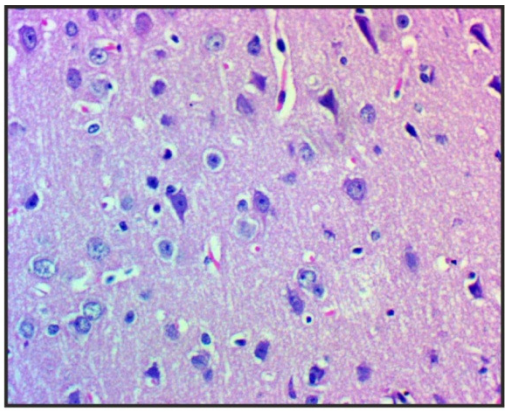


Plate 4.60. Brain (T2) showing mild neuronal degenerantion and normal brain parehcnyma (H & E x400)

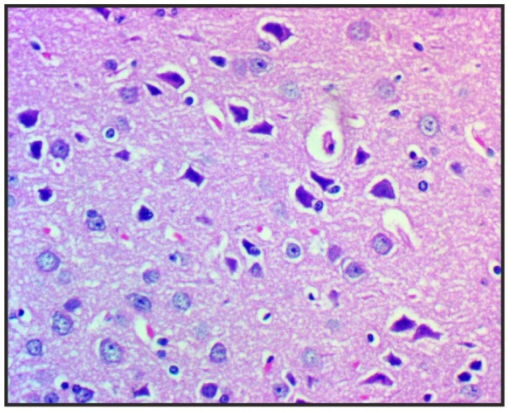


Plate 4.61. Brain (T3) showing mild neuronal degenerantion and mild increase in Virchow-Robin spaces (H & E x400)

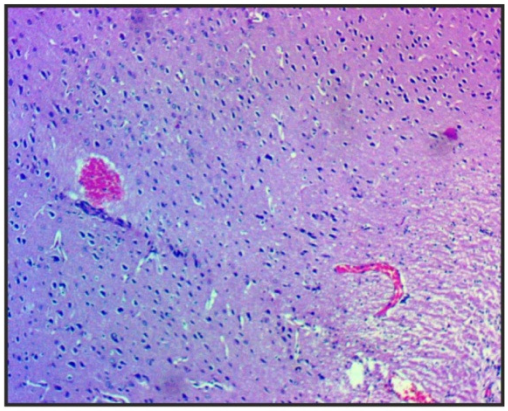


Plate 4.62. Brain (T4) showing blood venous congestion and hemorrhages (H & E x100)

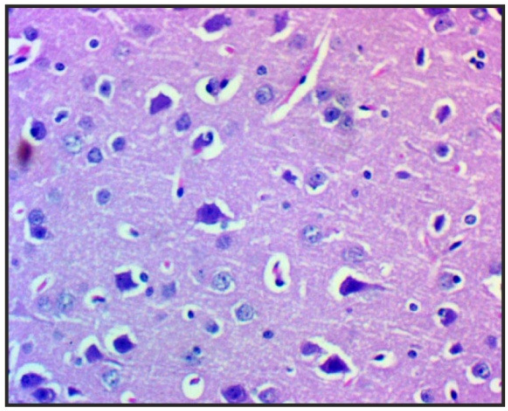


Plate 4.63. Brain (T4) showing mild neuronal degeneration (H & E x400)

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation entitled, “Toxicopathological studies of *Oxalis corniculata* in mice” was carried out to study the toxic effect of *Oxalis corniculata* givne for 28 days at different doses. The plant *Oxalis corniculata* L. (creeping wood sorrel) was collected from campus of Post Graduate Institute of Veterinary and Animal Sciences, Akola and from Nagarjun Medicinal Plants Garden of Dr. Panjabrao Deshmuck Krishi Vidydapeeth, Akola. Whole plant was air dried in a room and grinded into a fine powder and ethanolic extract was prepared. Phytochemical analysis of extract revealed presence of carbohydrates, glycosides, phytosterols, phenolilc compounds/ tannis, favonoides, proteins and amino acids.

In order to evaluate its experimental subacute toxicity in mice, the experiment was priory approved from the IAEC of institute. Thirty two male mice of 4 - 5 weeks weighing around 35-40 gm were procured form CPCSEA recognized firm. After acclimatization period of seven day, the mice were randomly divided into four equal groups comprising 8 mice in each. All the group animals were maintained in standard identical conditions with 12 hr light and 12 hr dark cycle period and kept in polypropylene cages having dimension of 47×34×18 cm layered with rice husk as bedding material. Throughout the trial period of 28 days, the mice were provided with ad libitum feed and clean drinking.

Group T1 was served as control, while group T2 was served with ethanolic extract of *Oxalis corniculata* (EOC) @ 125 mg/kg body weight orally. Group T3 was given EOC@ 250 mg/kg body weight orally and group T4 was served EOC@ 500 mg/kg body weight orally. The experiment was conducted for a period of 28 days.

During experimental period of 28 days animals from group T1, T2, T3 and T4 did not reveal any clinical sign and symptoms or any change in their behavior pattern and no mortality was recorded.

Pooled mean values for feed consumption revealed significant decreased feed consumption in group T4 (31.40±2.08) when compared to group T1 (36.73±0.07), T2 (35.35±0.13) and T3 (35.83±0.45). Average weekly body weight and body weight gain revealed non significant changes at 1^{st} and 2^{nd} week of experiment. Significant decreased body weight was recorded at 3^{rd} and 4^{th} week in all the treated groups compared to T1 group. Pooled mean values for body weight gain revealed significant decreased body weight gain in group T4.

At the end of 28^{th} day, all animals from each group were humanly sacrificed, before euthanasia, blood samples were collected in anticoagulant (EDTA) vial for hematological estimation and serum was separated from anticoagulant free blood for biochemical examination. Hematolgical observation revealed dose dependant significant decrease Hb concentration in T2 (7.5±0.2 g/dL), T3 (7.2±0.1 g/dL) and T4 (6.7±0.15) groups compared to T1 group (10.6±0.24 g/dL). Significant increased PCV was observed in group T4 (45.3±1.91%) followed by group T3 (43±1.56%) and T2 (36.33±2.99%) compared to T1 (34.83±1.22%). Group T3 (16.20±0.28) and T4 (22.30±0.56) showed significant increased TEC count when compared with T1 and T2 group. The mean values of MCV and MCHC indicated significant decrease in treatment groups. The total leukocyte count revealed Significant decreased TLC was observed in T4 (4.10±0.15) followed by T3 (5.87±0.13) while significant increase was observed in T2 group (11.92±0.22).

The mean of differential leukocyte count revealed significant increase in neutrophil (%) in group T4 followed by group T3 compared to control and group T2. The lymphocyte (%) count in T4 group showed significant decrease followed by T3 and T2 group suggested toxic effect of

ethanolic extract of *Oxalis corniculata*. The mean values of monocytes (%), eosinophil (%) and basophil (%) revealed non-significant differences.

Biochemical observations revealed significant increase in ($p<0.05$) serum AST and ALT in group T3 and T4 when compared with control group suggesting dose dependant hepatotoxicity. The serum creatinine revealed dose dependant significant increase in group T4 (0.79±0.02 mg/dL) followed by group T3 (0.64±0.030 mg/dL) and T2 (0.53±0.018 mg/dL). The blood urea nitrogen level were observed as 26.25±0.58, 29.97±0.65, 33.55±0.65 and 33.80±0.75 in T1, T2, T3 and T4 groups, respectively indicating significant increased BUN in group T2, T3 and T4. Significant dose dependant decreased serum calcium and phosphorus level was observed in treatment groups when compared with control group. Serum total protein and albumin revealed dose dependant decreased in group T2, T3 and T4. Significant decreased serum globulin was observed in group T4.

At the end of 28th day of experiment after were sacrificing of animals in each group a detailed necropsy examination was performed and gross pathological observations were recorded. Gross examination of liver from T4 group showed granular appearance, slightly swollen and focal pin point necrosis. Kidneys were swollen, slight and pale color and showed fibrosis. Spleen exhibited enlargement with mild congestion. Lung showed emphysema and pale discoloration. While group T1, T2 and T3 did not reveal any specific lesions of pathological significance. The absolute organ weight revealed non significant differences in weight of heart, liver and kidney.

Histopathological examinations of liver, kidney, lung, heart, spleen, intestine, stomach and brain from control group mice revealed normal histoarchitecture and histomorphology. Sections of these organs revealed mild degenerative changes and comparatively normal parenchyma in group T2 when compared to control group.

Sections of liver from T3 group showed mild to moderate degenerative lesions compared to group T4. Group T4 sections showed extensive granular and vacuolar degenerative changes, centrilobular and

periportal necrosis, Kupffer cell proliferation, pyknosis and shrinkage of hepatocytes and coagulative necrosis.

Kidney section of group T3 showed mild to moderate lesions of congestion, haemorrhages, mild to moderate granular and vaccular changes in tubular epithelium and tubular necrosis. Some section showed loss of tubular architecture and detachment of tubular epithelium from basement membrane. In addition to this group T4 showed accumulations of proteineous mass in the renal tubules. Glomeruli showed reduction in urinary space with degenerative changes. Interstitial nephritis was most prominent and showing mononuclear and polymorphonuclear cell infiltration in renal parenchyma.

Histopathological leions in lung were limited to mild to moderate thickened alveolar septa, mild perivascular and peribronchial lymphoid aggregation, edema and blood vessel congestion in group T3 and T4.

Heart sections revealed dose dependant haemorrhages, vaccuolar degenerative changes, necrosis, myocardial degeneration and loss of cross striations in group T3 and T4.

Sections of spleen from group T3 showed moderate depletion of lymphoid population and atrophy of lymphoid follicles while group T4 showed atrophy and moderate to extensive degenerative changes, necrosis and depletion of lymphoid follicles.

Duodenum of group T3 showed blunt and short villi with mild to moderate degenerative changes, loss of branching pattern, fusion of villi and sloughing of epithelium in the lumen. Sections from T4 showed moderate degenerative changes in lamina propria, necrosis in the villi and sloughing of villi epithelium in the lumen and mild inflammatory changes in the muscularis mucosa and lamina propria.

Sections of stomach from T4 group showed thinning of mascularis mucosae and serosa. Moderate granular and vaccuolar changes and mild infiltration of mononuclear cells and polymorphonuclear cells infiltration

in mascularis mucosae, necrosis and atrophy of gastric glands. Similar lesion of mild intensity was observed in group T3.

Sections of brain in group T3 and T4 showed mild blood venous congestion, microhemorrhages, accumulation of glial cell and mild to moderate neuronal degeneration and mild increase in Virchow-Robin space.

Following conclusions drawn from present investigation.

1) Ethanolic extract of *Oxalis corniculata* causes dose dependant reduction in feed consumption, body weight and body weight gain in mice.
2) Ethanolic extract of *Oxalis corniculata* causes dose dependant adverse effects on hematological parameters.
3) Significant dose dependant increase in serum AST, ALT, BUN and creatinine and significant dose dependant decrease in serum calcium, phosphorous, total protein and albumin suggested dose dependant hepatotoxic and nephrotoxic effect of *Oxalis corniculata* in mice.
4) Gross and histopathological examination of visceral organs revealed dose dependant hepatotoxic, nephrotoxic and cardiotoxic effect of *Oxalis corniculata*.

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THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	**:**	**TOXICO PATHOLOGICAL STUDIES OF *Oxalis corniculata* IN MICE**
b)	**Full name of student**	**:**	**Waghmare Nilesh Ashok**
c)	**Name and address of Major advisor**	**:**	**Dr. R. S. Ingole** Assistant Professor & Head Department of Veterinary Pathology, PGIVAS, Akola
d)	**Degree to be awarded**	**:**	M. V. Sc. (Veterinary Pathology)
e)	**Year of award of degree**	**:**	2021
f)	**Major subject**	**:**	Veterinary Pathology
g)	**Total number of pages In the thesis**	**:**	102
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i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

Toxicopathological studies of *Oxalis corniculata* was evaluated for 28 day in mice. Ethanolic extract of *Oxalis corniculata* @ 125, 250 and 500 mg/kg body weight was used for inducing toxicity. Phytochemical analysis of extract revealed presence of carbohydrates, glycosides, phytosterols, phenolilc compounds/ tannis, favonoides, proteins and amino acids. Total 32 male Albino mice were divided into four equal groups. Group T1 was served as control, while group T2, T3 and T4 were served with ethanolic extract of *Oxalis corniculata* @ 125, 250 and 500 mg/kg body weight, respectively for

28 days. No clinical sign, symptoms or change in behavior was observed in any of the group. Significant decreased feed consumption, weekly body weight and weight gain was observed in group T4. Haematological observations revealed significant dose dependant decrease in Hb, MCV, MCHC, TLC and lymphocyte count and significant dose dependant increase in PCV, TEC and neutrophil count. Monocyte, eosionophil and basoophil differ non significantly. Biochemical parameters revealed dose dependant increase in serum AST, ALT, BUN and creatinine and significant dose dependant decrease in serum calcium, phosphorous, total protein, albumin and globulin in treatment groups. Gross examination revealed granular surface, slightly swollen and focal pin point necrosis in liver, swollen, pale color kidneys, swollen spleen and pale color lungs. The absolute organ weight of heart, liver and kidney revealed non significant differences. Histopathological examinations of liver, kidney, lung, heart, spleen, duodenum, stomach and brain revealed dose dependant congestion, hemorrhages, degeneration and necorsis. Liver, kidney, duodenum, spleen, heart showed extensive degenerative changes in group T4 followed by group T3, however group T2 showed mild lesion with comparatively normal parenchyma to that of control animals. It is thus concluded that *Oxalis corniculata* produces dose dependant adverse effect on hematological, biochemical and histopathologial parameters suggesting hepatotoxic, nephrotoxic and cardiotoxic effect of *Oxalis corniculata*.

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	**: उंदिरांमध्ये आंबोशीच्या विषविकृतीचा अभ्यास**
२.	**विद्यार्थ्याचे पूर्ण नांव**	**: वाघमारे निलेश अशोक**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	**: डॉ. र. सु. इंगोले** सहाय्यक प्राध्यापक आणि विभाग प्रमुख पशुविकृतीशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञानसंस्था, अकोला.
४.	**प्रदान केली जाणारी पदवी**	: एम.व्ही.एस्सी.
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०२१
६.	**मुख्य विषय**	: पशुविकृतीशास्त्र विभाग
७.	**प्रबंधामधील एकुण पाने**	: १०२
८.	**प्रबंध सारांशामधील एकुण शब्द**	: २८३
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

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अकोला.

सारांश

उंदरांमध्ये 28 दिवस आंबोशीच्या विषविकृतीशास्त्राच्या अभ्यासांचे मूल्यांकन केले गेले. विषारीपणास उत्तेजन देण्यासाठी 125, 250 आणि 500 मिग्रॅ / किलोग्राम शारीरिक वजनानुसार वनस्पतीच्या इथॅनॉलिक अर्कचा वापर केला. अर्कच्या फायटोकेमिकल विश्लेषणामध्ये कार्बोहायड्रेट्स, ग्लाइकोसाइड्स, फायटोस्टेरॉल, फिनोलिलक संयुगे / टॅनिस, फॅव्होनाईड्स, प्रथिने आणि अमीनो आम्लाची उपस्थिती दिसून आली. एकूण 32 नर अल्बिनो उंदीर चार समान गटात विभागले गेले. गट टी 1 नियंत्रणासाठी देण्यात आला, तर टी 2, टी 3

आणि टी 4 मध्ये आंबोशी @ 125, 250 आणि 500 मिलीग्राम / किलोग्राम शारीरिक वजनानुसार अनुक्रमे 28 दिवसांसाठी इथेनॉलिक अर्क दिले गेले. कोणत्याही गटात कोणतेही चिकित्सालयीन लक्षणे किंवा वर्तनात बदल दिसला नाही. गट टी 4 मध्ये अन्न खाणे, आठवड्याचे शरीराचे वजन आणि वजनातील वाढ यामध्ये लक्षणीय घट दिसून आली. रक्तवाहिन्यांसंबंधी मापदंडांच्या निरीक्षणामध्ये एचबी, एमसीव्ही, एमसीएचसी, टीएलसी आणि लिम्फोसाइट गणना आणि मात्राच्या आधारावर पीसीव्ही, टीईसी आणि न्युट्रोफिल मोजणीत लक्षणीय वाढ आढळून आली. मोनोसाइट, इओसिओनोफिल आणि बेसोफील यामध्ये लक्षणीय बदल आढळले नाही. जैवरासायिनक मापदंडांमध्ये सीरम एएसटी, एएलटी, बीयुएन आणि क्रिएटिनिनमध्ये मात्रावर अवलंबून वाढ आणि सिरेम कॅल्शियम, फॉस्फरस, एकूण प्रथिने, अल्ब्युमिन आणि ग्लोब्युलिनमध्ये उपचारांच्या गटांमध्ये लक्षणीय घट दर्शविली. स्थूल निरीक्षणामध्ये यकृतामध्ये सुज, फोकल पिन पॉईंट नेक्रोसिस आणि दाणेदार पृष्ठभाग, फिकट रंगाच्या मूत्रपिंड, सूजलेल्या प्लीहा आणि फिकट रंगाचे फुफ्फुस उघडकीस आले. हृदय, यकृत आणि मूत्रपिंडाच्या वजनांमध्ये महत्त्वपूर्ण फरक आढळले नाहीत. सूक्ष्म निरीक्षणामध्ये यकृत, मूत्रपिंड, फुफ्फुस, हृदय, प्लीहा, ग्रहणी, पोट आणि मेंदू यांच्यामध्ये मात्रा वर आधारित रक्तसंचय, रक्तस्राव, झीज आणि नेक्रोसिस लक्षात आले. यकृत, मूत्रपिंड, ग्रहणी, प्लीहा, हृदयात गट टी 4 मध्ये मोठ्या प्रमाणात डीजेनेरेटिव बदल दिसून आला, त्यानंतर गट टी 3, तथापि गट टी 2 मध्ये नियंत्रित प्राण्यांच्या तुलनेने सामान्य पॅरेन्कायमासह सौम्य घाव दिसून आले.अशा प्रकारे आंबोशीचा रक्तवाहिन्यांसंबंधी मापदंडांवर, जैवरासायिनक आणि सूक्ष्मनिरीक्षण मापदंडांवर मात्राच्या आधारित प्रतिकूल प्रभाव निर्माण करतो ज्याने आंबोशीची यकृत, मूत्रपिंड आणि हृदय यांवर विषबाधा होते असा निष्कर्ष काढला जातो.