



Subacute Oral Toxicity Assessment of *Oxalis corniculata* in Mice

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ABSTRACT

Background: *Oxalis corniculata* L. plant found to be toxic to livestock once they eaten a huge quantity of plant, however very scanty information is available on toxicities of *O. corniculata* in livestock as well as in experimental animals.

Methods: Subacute oral toxicity study of *O. corniculata* was evaluated @ 125, 250 and 500 mg/kg b.wt. by oral gavage for 28 days. General performance, hematobiochemical profile and histopathological alterations were evaluated.

Result: No abnormal behavior was observed in any of the groups. Significant decreased feed consumption and body weight was observed in high dose treated group. There was significant dose dependent decrease in Hb, MCV, MCHC, TLC and lymphocyte count and increase in PCV, TEC and neutrophil. Serum AST, ALT, creatinine and BUN increased significantly and serum calcium, phosphorous, total protein, albumin and globulin decreased significantly in high dose group. Liver and kidney showed dose dependent alterations, degeneration suggested dose depending hepatotoxicity and nephrotoxicity.

Key words: Hepatotoxicity, Nephrotoxicity, *Oxalis corniculata*, Toxicity.

INTRODUCTION

Oxalis corniculata L. plant belongs to Oxalidaceae Family of Genus Oxalis. Oxalidaceae comprising about 8 genera and 900 species and are distributed worldwide and amongst it 2 genera and dozens of species have been reported in all over the India (Mushir *et al.*, 2015). Many of the species are referred to as wood sorrels as these plant have an acidic taste like Sorrel proper (*Rumex acetosa*), which is simply distantly related. Species of Oxalis are notorious for oxalate accumulation, hence the name given to the genus as Oxalis. The amount of oxalate in *O. per-caprae* are in the range of 3.7-14.9% soluble and 5.9- 16.6 % total and in *O. corniculata* as 4.1% soluble and 7% as a total (Libert and Franceschi, 1987). Within plants, the sap pH is low and therefore the oxalate may occur as a free acid within the vacuoles and also may be because of acid/potassium salt (Oke, 1969).

The leaves of *Oxalis corniculata* are quite edible with a tangy taste and the whole plant is rich in Vit-C. The plant leaves are having three major C-glycosyl flavans namely isoorientin, isovitexin and swertisin of creeping oxalis (Mizokami *et al.*, 2008). Traditionally it is utilized in anaemia, dyspepsia, cancer, piles, diuretic and convulsions (Chetty *et al.*, 2008, Srikanth *et al.*, 2017). 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" as an appetizing drug and also used to treat fever biliousness and dysentery (Sreejith *et al.*, 2014).

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. In acute cases, main clinical symptoms observed are muscle tremors, staggering gait, collapse and sudden death (Simmonds *et al.*, 2000). In spite of toxic properties of *Oxalis corniculata* and a common weed in most part of the India, literature searched revealed very scanty information on toxicities of

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Oxalis corniculata in animals. Considering the abundant 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 subacute oral toxicity of ethanolic extract of *Oxalis corniculata* L. plant in mice.

MATERIALS AND METHODS

Collection of plant materials and preparation of ethanolic extract of *Oxalis corniculata*

The plant *Oxalis corniculata* was obtained (Fig 1) from the campus of Post Graduate Institute of Veterinary and Animal Sciences, Akola (Maharashtra), India and the whole plant was identified and authenticated from an expert taxonomist. The shade dried plants were grinded and obtained fine powder. The powder filtered by sieving was subjected to

ethanolic extraction. Briefly, 50 g powder soaked in 600mL of 95% ethanol at room temperature for 48 hrs with occasional shaking and was filtered by Whatman's filter paper (No.1). The filtrates were evaporated on rotary evaporator to concentrate in crude extract form at 40°C and obtained light green residue of 6.56 g (13.12% w/w). The aqueous suspension of extract was made by dissolving into distilled water according to dose values (Singh and Prakash, 2014). The ethanolic extract of *Oxalis corniculata* was analyzed qualitatively for the presence of phytochemicals viz. alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates and glycosides as per standard methodology (Harborne, 1998).

Experimental design

The experimental protocol was approved from the Institutional Animal Ethical Committee (IAEC). Total 32 Swiss albino male mice weighing around 35 to 40 gm were procured from CCSEA approved firm and acclimatized for a period of 7 days. The present experiment was carried out in the small animal house of Department of Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences, Akola during March to April 2021. The experimental design was as under.

Group I: Normal healthy control group and administered with normal saline for 28 days.

Group II, III and IV: Received ethanolic extract of *Oxalis corniculata* @ 125, 250 and 500 mg/kg b.wt., respectively, orally once daily for 28 days and were labeled as low, medium and high dose group.

All groups were maintained at 22±2°C and 40-60%, temperature and humidity, respectively provided with 12 hr light and 12 hr dark period in polypropylene cages layered with rice husk and were provided with ad-libitum standard commercial feed and clean drinking water.

General performance

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

Hematobiochemical observations

At the termination of experiment, blood was collected through retro orbital venous plexus under appropriate dose of Thiopentone sodium intraperitoneally in EDTA for hematological parameters while blood collected in plane vial without anticoagulant was used for separation of serum which was further used for estimations of biochemical parameters. Blood smears were prepared directly from fresh blood for differential leukocyte count. Hematological parameter viz. Hb, PCV, TEC, MCV, MCH, MCHC, TLC and DLC were estimated as per the standard methods (Benjamin, 2001). Biochemical parameters viz. serum AST, ALT, creatinine, BUN, calcium, phosphorus, total protein, albumin and globulin were estimated by using AGD Diagnostic kits supplied by M/s. AGD Biomedicals Pvt. Ltd., Mumbai using Autoanalyzer (AGD Biomedical, Model No. AGD 2020).

Histopathological observations

At the end of experiment all the animals were sacrificed by anesthetizing in a jar containing cotton wool soaked in diethyl ether inhalation. Tissues of liver and kidneys were collected in 10% neutral formal saline solution. After fixation, the tissues were processed for routine histopathological techniques and sections of 4-6 µm were cut on rotary microtome and stained by H and E stain (Luna, 1968).

RESULTS AND DISCUSSION

Phytochemical analysis revealed presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannis, flavonoides, proteins, amino acids, however found negative for alkaloids and saponins.

No clinical signs or behavioral changes were observed in any of the group and are in collaboration with acute oxalate nephropathy in rat induced by star fruit (Fang *et al.*, 2001). The findings are in agreement with Reddy (2012) who evaluated safety and potential toxicities of methanolic extract of *Oxalis corniculata* I. @ 100, 200, 400 mg/kg of



Fig 1: *Oxalis corniculata* plant.

body weight once in week. The 50% lethal dose (LD50) of *O. corniculata* (1300 mg/kg b.wt.) also revealed no positive signs of toxicity when given @ 200 and 400 mg/kg b. wt. (Singh and Prakash, 2014).

Feed consumption was significantly decreased in high dose group (31.40±2.08) when compared with control (36.73±0.07), low dose (35.35±0.13) and medium dose (35.83±0.45) (Table 1). The considerable decreased feed consumption was recorded after 2nd week. Similarly, decreased feed consumption in broiler chicks was observed by Subhani *et al.* (2018) given extract of *O. corniculata*. Plants containing oxalate damages the kidney and ultimately affects the metabolism and thereby declined feed consumption.

Average weekly body weight revealed non significant changes at 1st and 2nd weeks followed by significantly

decreased at 3rd and 4th week in all dose groups compared to control group (Table 2). Anti nutritional factors such as phytosterols, tannins and oxalates present in *O. corniculata* may form complexes with metals (Ca⁺⁺, Zn, Mg and Fe) and proteins and reduce mineral's and protein's bioavailability and might be the reason for dose dependant decrease in body weight (Alebachew *et al.* (2014).

Significant dose dependant decreased Hb was observed in low (7.5±0.2 g/dL), medium (7.2±0.1 g/dL) and high (6.7±0.15 g/dL) dose groups compared to control group (10.6±0.24 g/dL) (Table 3). Significant decreased mean corpuscular hemoglobin was observed in mice given methanol leaves extract of *Vernonia bipontini* Vatke @ 800 mg/kg of b.wt. and suggested inhibition of RBC formation, which reduced hemoglobin content (Alebachew *et al.*, 2014).

Table 1: Average weekly feed consumption (g).

Groups	1 st Week	2 nd Week	3 rd Week	4 th Week	Total feed consumption	Pooled mean
Group I	36.92	36.65	36.76	36.58	146.91	36.73±0.07 ^a
Group II	35.11	35.25	35.72	35.30	141.38	35.35±0.13 ^a
Group III	36.76	36.43	35.00	35.13	143.32	35.83±0.45 ^a
Group IV	37.6	29.8	28.8	29.4	125.53	31.40±2.08 ^b
Significance	-	-	-	-	-	*
CD(0.05)	-	-	-	-	-	3.282

Significance levels *P≤0.05, NS= Non significant.

Table 2: Average weekly body weight (g) per mice in different groups.

Group	0 Day	1 st Week	2 nd Week	3 rd Week	4 th Week	Pooled mean
Group I	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
Group II	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
Group III	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
Group IV	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
Significance	-	-	-	**	**	**
CD(0.05)	NS	NS	NS	2.552	3.086	1.292

Significance levels *P≤0.05, NS= Non significant.

Table 3: Mean hematological values related to erythrocytes.

Group	Hb (g/dL)	PCV (%)	TEC (10 ⁶ /cumm)	MCV (fL)	MCH (Pg)	MCHC (%)
Group I	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
Group II	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
Group III	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
Group IV	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	**	**	**	**	**	**
CD (0.05)	0.535	5.991	1.014	7.647	0.955	4.007

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

Table 4: The hematological values related to total leucocyte count and differential leucocyte count.

Groups	TLC(10 ³ /Cumm)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Group I	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
Group II	11.92±0.22 ^a	70±0.93 ^c	27±0.72 ^b	1±0.26	1±0.37	0.5±0.22
Group III	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
Group IV	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	-	-	-

Significance levels *P≤0.05, NS= Non significant.

Table 5: Serum biochemical parameters at the end of experiment.

Groups	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dL)	BUN (mg/dL)	Calcium (mg/dL)	Phosphorous (mg/dL)	Total Protein (gm/dL)	Albumin (gm/dL)	Globulin (gm/dL)
Group I	137.17±4.57 ^c	36.15±0.46 ^c	0.51±0.012 ^c	26.25±0.58 ^c	9.93±0.14 ^b	8.36±0.11 ^a	8.77±0.27 ^b	3.74±0.05 ^a	5.03±0.23 ^b
Group II	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
Group III	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
Group IV	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	**	**			**	**	**	**	**
CD (0.05)	16.476	5.235	0.063	1.949	0.385	0.242	0.672	0.223	0.806
Significance levels *P≤0.05, NS= Non significant									

Decrease in feed intake might be the reason for dose dependent decreased hemoglobin levels (Benjamin, 2001). Significant increased PCV was observed in high dose group (45.3±1.91%) followed by medium dose (43±1.56%) and low dose group (36.33±2.99%) compared to control group (34.83±1.22%). Imbalance in the rate of hematological parameters synthesis and catabolism was suggested during acute and sub-acute toxicity of ethanolic leaf extracts of *Rumex abyssinica* Jacq. and *Mentha spicata* L. in rats (Mugisha *et al.*, 2014). Medium dose (16.20±0.28) and high dose group (22.30±0.56) revealed significant increased TEC count. Similar findings of significant increased TEC was also recorded during case study of erythrocytosis associated with 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 (Basu *et al.*, 1974) which was further confirmed in histopathological lesions of kidney in treatment groups. Significant increase in TEC was recorded previously in methanol rhizome extracts of *Rumex abyssinicus jacq* (@ 400 mg/kg of b.wt) treated mice (Fentahun *et al.*, 2020).

The mean values of MCV and MCHC indicated significant decrease in treatment groups. indicated microcytic hypochromic anaemia which might be associated with renal disease due to oxalate poisoning of the *O. corniculata* leading to renal vascular disease or glomerular damage which provoke different kinds of anemia (Basu *et al.*, 1974).

The total leukocyte count revealed significant decrease in high dose group (4.10±0.15) followed by medium dose (5.87±0.13) while significant increase was observed in low dose group (11.92±0.22) (Table 4). Non significant decrease in total leukocyte count was observed in mice when treated with 80% methanol rhizome extracts of *Rumex abyssinicus jacq.* (polygonaceae) @ 100, 200 and @ 400 mg/kg b.wt (Fentahun *et al.*, 2020). Contrary to present findings, significant increased leucocyte count was observed in buffalo calves treated with Napier grass (*Pennisetum purpureum*) (Bajaj *et al.*, 2011). Contrary to present findings nonsignificant difference in leucocyte count in rats was recorded when treated with methanolic extract of *O. corniculata* l. @ 100, 200 and 400 mg/kg of b.wt (Reddy, 2012).

Differential leukocyte count revealed significant increase in neutrophil in high dose group followed by medium dose group as compared to control and low dose group. The increase in 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 in non-inflammatory neutrophilia (Benjamin, 2001). The significant dose dependent decrease in lymphocyte (%) count suggested toxic effect of ethanolic extract of *O. corniculata*. Contrary to present investigation, significant increase in lymphocyte count was recorded during induced oxalate toxicity by feeding Napier grass to buffalo calves (Bajaj *et al.*, 2011) and in rats treated with methanolic extract of *O. corniculata* l.

(Reddy, 2012). Monocytes (%), eosinophil (%) and basophil (%) revealed non-significant differences.

Biochemical observations revealed significant increase in ($p < 0.05$) serum AST and ALT in medium and high dose group suggested dose dependent hepatotoxicity (Table 5). Increased AST and ALT levels in the blood are known indicators 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). The dose dependent histopathological

changes are further confirmed in the liver of treatment groups. Contrary to this, previous experiment on acute and chronic toxicity studies of methanolic extract of *O. corniculata* L. in rat recorded no significant treatment related changes in the levels SGOT and SGPT (Reddy, 2012; Singh and Prakash, 2014). However, the findings of significant increase in AST and ALT level in mice treated with extract of *Vernonia bipontini* Vatke are in collaboration (Alebachew *et al.*, 2014). Dose dependant increased ALT level in rats treated with ethanolic leaf extracts of *Rumex abyssinica* Jacq and *Mentha spicata* L. (Lamiaceae) was recorded

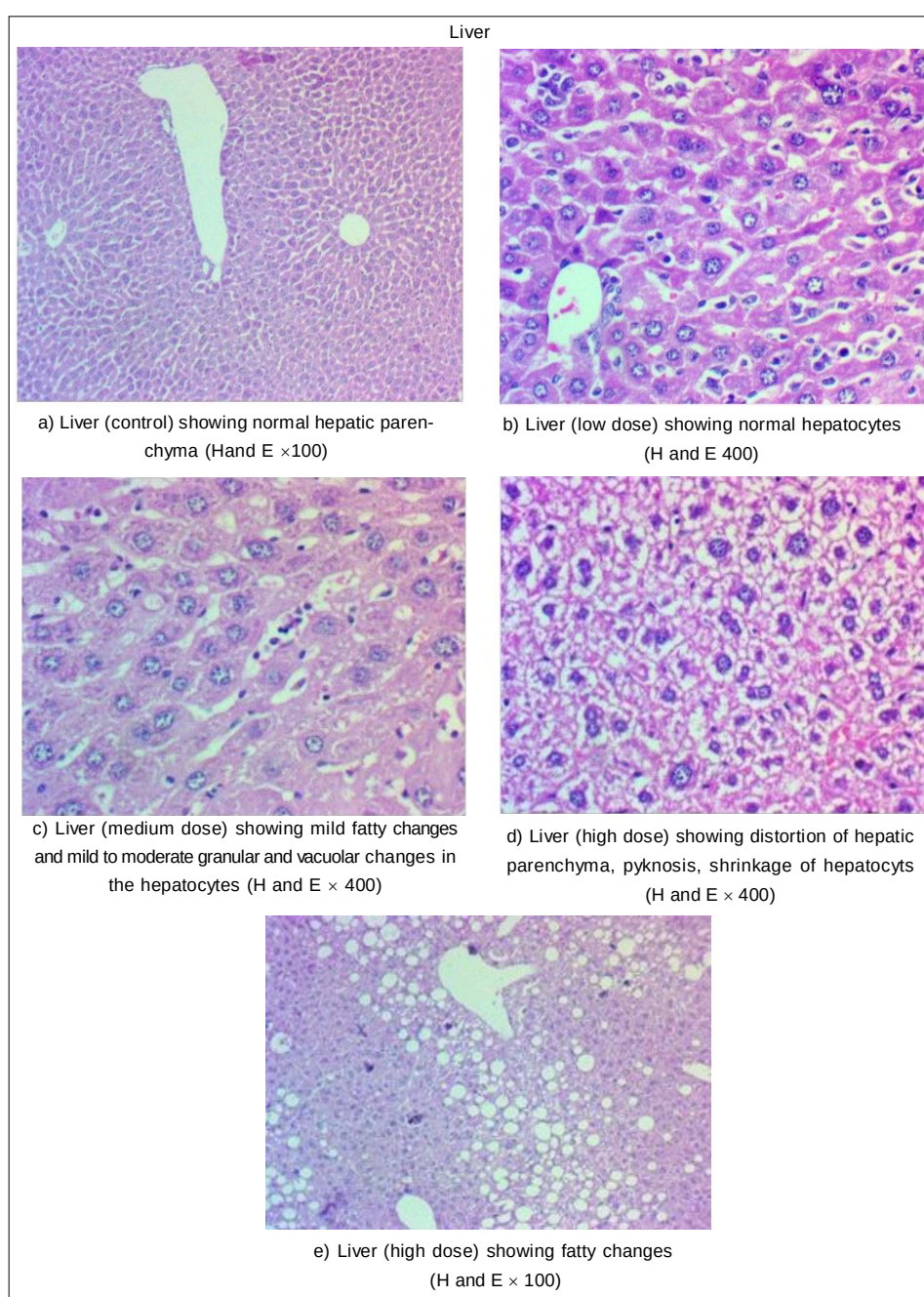


Fig 2: Histopathological observations in liver (a-e).

previously (Mugisha *et al.*, 2014). The findings of increased serum AST and ALT level in *Rumex acetosa* toxicity in a boy corroborated with present findings (Selcuk *et al.*, 2015).

Serum creatinine and BUN revealed dose dependent significant increase high dose group. Calcium bind with oxalate present in extract forming insoluble calcium oxalate which get precipitated in renal tubules and cause damage and renal insufficiency. Also hypovolaemia due to dehydration may impaired excretion of creatinine resulting in increase in creatinine and BUN (Singh *et al.*, 1995).

Significant dose dependent decreased serum calcium and phosphorus suggested binding of calcium of blood with oxalate of plant and form insoluble calcium oxalate making unavailable for body tissue and decreased absorption from intestine because of alkalinity leads to low level of calcium (Bajaj *et al.*, 2011). Leakage of protein through glomeruli filtration and its disintegration through degenerating tubules due to oxalate nephrosis, proteinuria and damage to hepatic tissue by calcium oxalate crystals lead to hypoproteinemia (Radostits *et al.*, 2007). Hepatopathy by calcium oxalate

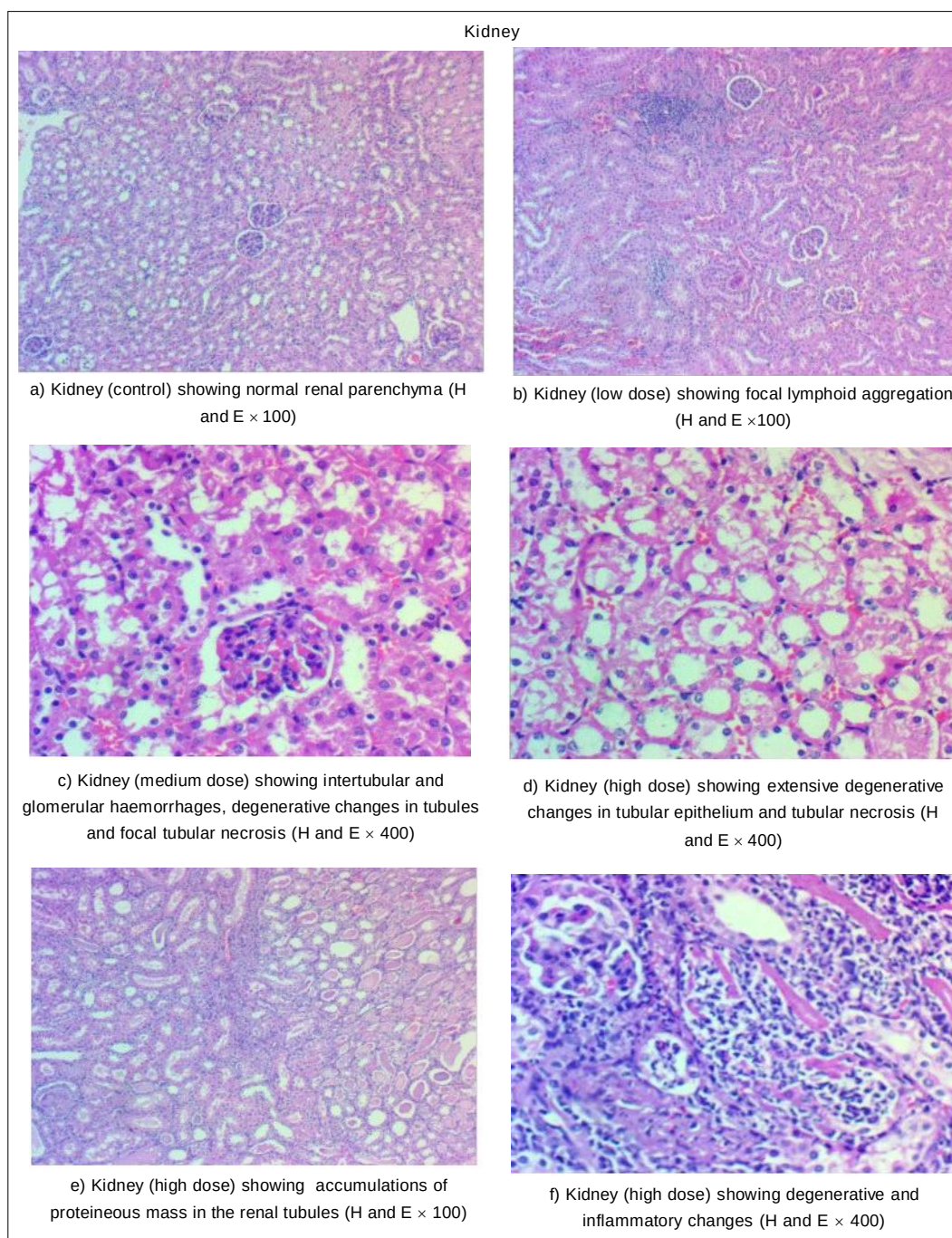


Fig 3: Histopathological observations in kidney (a-f).

crystals and proteinuria following nephrosis might be the reason for decrease in serum albumin and total protein in treatment groups (Radostits *et al.* 2007).

Gross observations of liver revealed granular appearance, slightly swollen hepatocytes and focal pin point necrosis in high dose group. Kidney of high dose group revealed pale color, swollen and fibrosis in some cases. Histopathological section of liver and kidney from control group and low dose group revealed normal renal histoarchitecture. Microsections of liver showed dose dependent degenerative changes in low to high dose group in the form of mononuclear cell degeneration, granular and vacuolar degenerative changes in the hepatocytes, venous congestion, centrilobular and periportal necrosis and distortion of hepatic parenchyma (Fig. 2). The dose dependant lesions observed in liver in present study might be due to the presence of bioactive compounds like 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. Presence of oxalate in the plant may 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) that may ultimately causes degenerative changes in the liver.

Sections of kidney also showed dose dependent venous congestion, glomerular degeneration, intratubular hemaorrhages, lymphoid aggregation, granular and vacuolar changes in the tubular epithelium, dilatation of tubules, detachment of tubular epithelium from the basement membrane and loss of tubular architecture. In addition high dose group showed prominent and extensive accumulation of proteinaceous mass in the tubules, reduction in the urinary space and interstitial nephritis in few sections (Fig 3). Present findings are in accordance with earlier finding (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 *O. corniculata* poisoning in sheep. Dose dependant renal toxicity in treatment groups might be due to presence of oxalates in plant causing mechanical obstruction in tubules lead to intracellular chelation of calcium and magnesium interfering with oxidative phosphorylation which results into increased oxidative stress a crucial pathogenetic factor in cellular damage to kidney (Gulbahar *et al.*, 2002).

CONCLUSION

Oxalis corniculata causes dose dependent adverse effects on hematobiochemical and histopathological parameters suggesting hepatotoxic and nephrotoxic effect.

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Informed consent

The experiment was approved by Institutional Animal Ethics Committee (IAEC) of Post Graduate Institute of Veterinary and Animal Sciences, Akola.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this article.

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