Biological Forum – An International Journal **15(8a): 317-325(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Toxicological Evaluation of *Trema orientalis* in Rats with Reference to Histopathology of certain Tissues

Niranjan D.[1]*, Shridhar N.B.[2], Vinuta M.H.[3], Manjunatha S.S.[4], Sunilchandra U.[5], Pradeep B.S.[6], Manju G.U.[7] and Patel Suresh Revanna[8]

[1]Assistant Professor (Contractual), Animal Husbandry Polytechnic, Konehalli, KVAFSU (Karnataka), India.
[2]Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, KVAFSU (Karnataka), India.
[3] Senior MVSc. Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, KVAFSU (Karnataka), India.
[4]Assistant Professor, Department of Veterinary Pathology, Veterinary College Shivamogga, KVAFSU (Karnataka), India.
[5]Associate Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga, KVAFSU(Karnataka), India.
[6]Assistant Professor and Head, Department of Veterinary Parasitology, Veterinary College, Shivamogga, KVAFSU (Karnataka), India.
[7]Assistant Professor and Head, Department of Animal Nutrition, Veterinary College, Gadag, KVAFSU (Karnataka), India.
[8]Associate Professor (I/C), Department of Veterinary Medicine, Veterinary College, Shivamogga, KVAFSU (Karnataka), India.

(Corresponding author: Niranjan D.)*
(Received: 19 June 2023; Revised: 24 July 2023; Accepted: 28 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: An experimental study was carried out to conduct the safety and toxicological evaluation of the methanolic extract of *T. orientalis* in Wistar rats through analyzing the histopathological changes observed in vital organs. Repeated dose 28-day oral toxicity study of Methanolic extract of *Trema orientalis* (METO) was performed in male Wistar albino rats as per the OECD guidelines 407. Twenty-four rats used in the study, were divided into 4 groups, with six in each. Group I served as control, with no medication. METO was administered daily through oral gavaging at dose levels of 250, 500 and 1000 mg/kg for 28 days in group II, III and IV respectively. The experimental rats were apparently healthy throughout the duration of the experiment, without significant changes in rate of body weight gain. After the completion of study all rats were euthanized humanely and during necropsy the external and internal examination of the carcass was done and lesions were recorded. The histopathological examination of the liver, kidneys and heart in the METO treated rats revealed dose-dependent and organ-specific circulatory, degenerative and inflammatory changes. The study concluded that the trematoxin, a glycosidictoxic principle of *T. orientalis* could be the possible reason for the pulmonary, hepatic and renal damage occurred in the experimental rats and the possibility of toxic potentiality in herbivores might need to be investigated in future.

Keywords: *T. orientalis*, Wistar albino rats, Necropsy, Histopathology and Trematoxin.

INTRODUCTION

Historically, a considerable portion of the population in developing nations have relied upon the produce obtained from forests to treat ailments in humans and animals. Several plants which are collectively referred as medicinal and aromatic plants are often used for medicinal therapeutic utility. Relatively 12.5 *per cent* of 4,22,000 plant species known globally are said to have medicinal properties, but only few plants among them are under cultivation (Rao *et al*., 2004). Due to the safer pharmacological potentiality and significant therapeutic benefits, herbal medicines are in greater demand than ever before. However, in order to put the herbs-based medications on the market as main line therapies, efforts have to be made to investigate, standardize and validate them for their potency, safety and efficacy (Dubey *et al*., 2013).

The importance of a systematic scientific approach was emphasised as being essential for future success. For instance, research on the poisons used in dart and arrows in prehistoric cultures resulted in the identification of a wide range of different compounds that are beneficial in the treatment of malaria, chronic pain, cardiac problems and also some other diseases (Philippe and Angenot 2005). *Trema orientalis* (L) Blume is a small to medium sized tree belonging to Cannabaceae family. It is a shade tree with soft leaves that grows quickly, making it best suited for gardens

and avenues. It has been used to make paper and poles. It has therapeutic qualities, which have been used medicinally to treat helminthic, inflammatory and respiratory diseases (Saleh *et al*., 2020a). The toxic principle of *T. orientalis*is trematoxin, a glycoside which contains steroidal saponins. Additional constituents in the plant include tannins, flavonoids, phytosterols, triterpenes and various xanthone components, which are responsible for the pathophysiologic qualities of the plant (Ibrahim *et al*., 2020).

Suspected toxicity conditions in goats after ingestion of *T. orientalis* leaves have been noticed by the veterinarians across Karnataka state particularly in and around Shivamogga and Dharwad district. As per the information received regarding the incidences of the plant toxicity, the ailing goats had exhibited abnormal clinical signs such as incoordination, apathy, tenesmus, paddling movements and coma before death on fifth day of ingestion of the plant. Thus, in addition to the numerous traditional claims of the medicinal properties of the aerial parts of the plant and the incidences of suspected toxicity in goats necessitated the studies on the toxicological aspects of *T. orientalis* in animals. So, this study is conducted for the safety evaluation of *Trema orientalis* in rats with reference to histopathology.

MATERIALS AND METHODS

The present experimental study on rats was conducted in small animal house of Veterinary College Shivamogga. The current study was undertaken to determine the toxicity assessment of aerial parts of *T. orientalis*. The experimental protocol was initiated following the approval of Institutional Animal Ethics Committee of Veterinary College Shivamogga vide: No.VCS/IAEC/SA-71/2022-23 and dated: 06.08.2022 as per CCSEA (formerly CPCSEA) guidelines. The fresh aerial parts of *T. orientalis* plant were gathered from different regions of Soraba taluk, Shivamogga district, Karnataka in the months of April and May 2022. Dr. Rajeshwari, N. Professor, Department of Botany and Seed Technology, Sahyadri Science College, Shivamogga, verified the plant's taxonomic identification. The plant material was collected, cleansed under running tap water and then dried in the indoors for 20 days. The plant material was first chopped in to small pieces by multipurpose shredder, then mechanically grounded in to a coarse powder using heavy duty jumbo flour and spice pulverizer (5HP AC motor) and then sieved in to a fine powder. Later both coarse and fine powder were stored in airtight containers for further use.

For histopathology, commercially available products such as Haematoxylin stain, Mayer's glycerol albumin mixture (50% glycerol and 10% egg albumin), Eosin stain, ethanol - 90%, 80%, 70%, xylene (clearing agent) and D.P.X. (Disteryne, plasticizer and xylene mixture as a permanent mountant) were the reagents and chemicals used.

Toxicity studies of methanolic extract of aerial parts of *T. orientalis.* The repeated dose sub-acute toxicity study was conducted for 28 days as per broader outlines of OECD guideline 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents).

Experimental animals. Four to five weeks old Wistar albino male rats weighing around 150±10 g were procured from Adita biosys private limited, Tumakuru (Reg No: -1868/PO/RcBt/S/16/CPCSEA. Date of Registration: - 23.02.2016 valid up to 08.04.2026). All the experimental rats were housed in polypropylene cages and acclimatized to laboratory conditions of 12 h light/dark cycle, 22 ± 3°C housing temperature, relative humidity of 50-60 % and ventilation of 12-15 air cycles per hour for at least one week before the experiment. The rats were provided with standard pellet feed and *ad libitum* water throughout the experiment. The guidelines prescribed by CCSEA were followed during the course of experimental study, with duly approval from the Institutional Animal Ethics Committee of Veterinary College, Shivamogga. Prior to the commencement of the experimental study, the experimental rats were acclimatized to the laboratory environment for seven days.

Selection and preparation of doses. Three doses 250, 500 and 1000 mg/kg were chosen as low, medium and high doses, respectively in accordance with OECD guidelines 407 based on earlier pharmacological research on methanolic extract of aerial parts of *T. orientalis* (METO) plant leaves conducted by Hemalatha *et al*. (2019); Kasim *et al*. (2015); Kouakou *et al*. (2014). Previous studies revealed that the METO had little toxic effects on liver and kidneys. The LD_{50}**.** The rats were administered with the METO for a period of 28 days by oral gavaging technique as a single dose separately for each rat every day at the scheduled time in early morning, before providing feed and water. The experimental design for repeated dose 28-day oral toxicity study of METO in ratsis depicted in Table 1.

Table 1: Experimental design for repeated dose 28-day oral toxicity study of METO in rats.

Sr. No.	Group	No. of rats	Treatment	Dosing
1.	Group I	6	Control	Administered (*per os*) with single dose of normal saline (1.5 ml)
2.	Group II	6	Low dose	Administered (*per os*) daily with single low dose (250 mg/kg) of *T. orientalis* extract.
3.	Group III	6	Medium dose	Administered (*per os*) daily with single medium dose (500 mg/kg) of *T. Orientalis* extract.
4.	Group IV	6	High dose	Administered (*per os*) daily with single high dose (1000 mg/kg) of *T. orientalis* extract.

Administration of doses. The rats were administered with the METO for a period of 28 days by oral gavaging technique as a single dose separately for each rat every day at the scheduled time in early morning, before providing feed and water. Utmost care was taken regarding the personnel and animal safety during administration of test doses.

Body weight. The body weight of each animal was taken once a week with the help of weighing balance with minimal stress to the rats, until the end on day 28 of repeated dose oral toxicity test.

Pathology. All the experimental rats in the control and treated groups were humanely sacrificed by Carbon dioxide gas (70%) in closed chamber at the end of the study period of 28 days (29th day) for the repeated dose oral toxicity studies. The necropsy was carried out and gross changes were recorded. The external and internal examination of the carcass was done and lesions were recorded.

Collection of organs for histopathological study. The necropsy was carried out in each case in order to examine for any noticeable pathological alterations. Organs such as the liver, kidneys, spleen, duodenum, lungs and heart were collected and fixed in 10% neutral buffered formalin (NBF) for 72 hours. The tissues were then processed for histopathology using an automated tissue processor before being routinely embedded in paraffin. 5-micron thick sections were taken and stained according to protocol with Haematoxylin and Eosin (Luna, 1968).

Statistical analysis. Data was expressed as mean ± SEM. Differences were considered significant at *p <0.05 when compared test groups v/s control group. For statistical analysis, One-way Analysis of Variance (ANOVA) with Dunnet's multiple comparisons test was performed using Graph Pad Prism 7.2.0 (435) software.

RESULTS

Body weight. The effect of methanolic extract of aerial parts of *T. orientalis* on body weight of male Wistar rats is illustrated in Fig. 1. The mean ± SEM values of body weights of male Wistar rats in different groups are presented in table 2. Both the control and treatment groups revealed a gradual increased trend in body weight in all the rats. The percentage changes in body weight of the METO treated groups were not significantly different from that of control rats.

Table 2: Body weights (g; Mean ± S.E.M) of experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis.*

Day	Group			
	Group I	Group II	Group III	Group IV
0	178 ± 7.52	187 ± 3.39	195 ± 5.70	207 ± 7.68
7	194 ± 9.14	203 ± 4.64	217 ± 8.15	234 ± 12.08
14	209 ± 9.67	222 ± 8.00	244 ± 9.14	260 ± 12.75
21	233 ± 9.43	259 ± 10.65	267 ± 10.20	290 ± 14.83
28	255 ± 5.92	278 ± 10.20	292 ± 11.58	316 ± 16.61

Note: Data were analysed by one-way ANOVA followed by Dunnett's multiple comparisons test and compared with control group

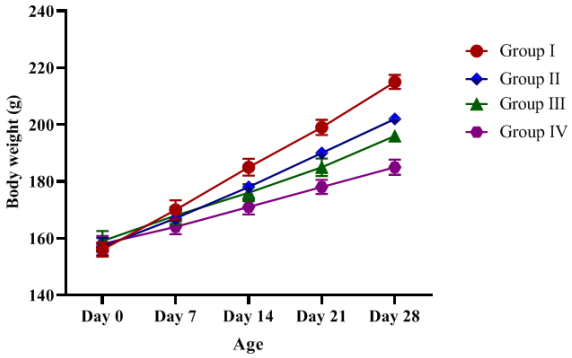


Fig. 1. Body weights (g; Mean ± S.E.M) of experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis.*

Absolute organ weight. At term the absolute organ weight of vital organs such as heart, liver, lungs, spleen and kidneys were recorded after necropsy and are presented in Table 3. The absolute organ weight of (mean ± S.E.M) heart in group I, II, III and IV are 0.743 ± 0.02, 0.807 ± 0.03, 0.845 ± 0.05 and 0.903 ± 0.06 respectively. The absolute organ weight of (mean ± S.E.M) lungs in group I, II, III and IV are 2.463 ± 0.17, 2.512 ± 0.18, 2.636 ± 0.31 and 2.686 ± 0.29 respectively. The absolute organ weight of (mean ± S.E.M) liver in group I, II, III and IV are 9.727 ± 0.86, 9.830 ± 0.18, 10.563 ± 0.55 and 11.343 ± 0.43 respectively. The absolute organ weight of (mean ± S.E.M) spleen in group I, II, III and IV are 0.952 ± 0.05, 0.973 ± 0.03, 1.076 ± 0.04 and 1.256 ± 0.02 respectively. The absolute organ weight of (mean ± S.E.M) kidneys in group I, II, III and IV are 1.765 ± 0.13, 1.825 ± 0.09, 2.025 ± 0.05 and 2.236 ± 0.13 respectively.

Table 3: Absolute organ weight (g; Mean ± S.E.M) values in various experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis.*

Organ	Group			
	Group I	Group II	Group III	Group IV
Heart	0.743 ± 0.02	0.807 ± 0.03	0.845 ± 0.05	0.903 ± 0.06
Lungs	2.463 ± 0.17	2.512 ± 0.18	2.636 ± 0.31	2.686 ± 0.29
Liver	9.727 ± 0.86	9.830 ± 0.18	10.563 ± 0.55	11.343 ± 0.43
Spleen	0.952 ± 0.05	0.973 ± 0.03	1.076 ± 0.04	1.256 ± 0.02
Kidneys	1.765 ± 0.13	1.825 ± 0.09	2.025 ± 0.05	2.236 ± 0.13

Note: Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test and compared with control group.

Relative Organ weight. The relative organ weight of vital organs such as heart, liver, lungs, spleen and kidneys were recorded after necropsy and are presented in Table 4, Fig. 2 and 3. The relative organ weight of (mean ± S.E.M) heart in group I, II, III and IV are 0.292 ± 0.05, 0.344 ± 0.06, 0.432 ± 0.03 and 0.491 ± 0.04 respectively. The relative organ weight of (mean ± S.E.M) values of lungs in group I, II, III and IV are 1.147 ± 0.08, 1.242 ± 0.08, 1.345 ± 0.15 and 1.461 ± 0.17 respectively. The relative organ weight of (mean ± S.E.M) values of liver in group I, II, III and IV are 4.516 ± 0.37, 4.866 ± 0.09, 5.385 ± 0.26 and 6.141 ± 0.27 respectively. The relative organ weight of (mean ± S.E.M) values of spleen in group I, II, III and IV are 0.443 ± 0.02, 0.482 ± 0.01, 0.549 ± 0.02 and 0.679 ± 0.01 respectively. The relative organ weight of (mean ± S.E.M) values of kidneys in group I, II, III and IV are 0.822 ± 0.06, 0.902 ± 0.04, 1.033 ± 0.02 and 1.214 ± 0.09 respectively.

Table 4: Relative organ weight (g; Mean ± S.E.M) values in various experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*

Organ	Group			
	Group I	Group II	Group III	Group IV
Heart	0.292 ± 0.05	0.344 ± 0.06	0.432 ± 0.03	0.491 ± 0.04
Lungs	1.147 ± 0.08	1.242 ± 0.08	1.345 ± 0.15	1.461 ± 0.17
Liver	4.516 ± 0.37	4.866 ± 0.09	5.385 ± 0.26	6.141 ± 0.27
Spleen	0.443 ± 0.02	0.482 ± 0.01	0.549 ± 0.02	0.679 ± 0.01
Kidneys	0.822 ± 0.06	0.902 ± 0.04	1.033 ± 0.02	1.214 ± 0.09

Note: Data were analysed by one-way ANOVA followed by Dunnett's multiple comparisons test and compared with control group

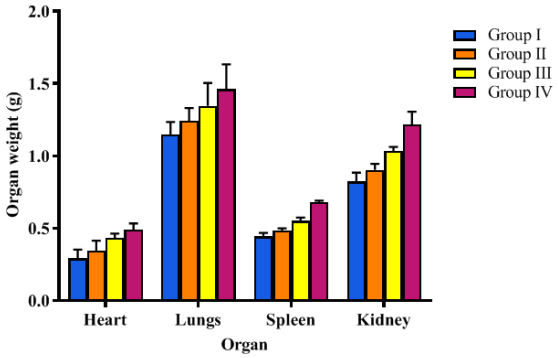


Fig. 2. Relative organ weight (g; Mean ± S.E.M) values in various experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis.*

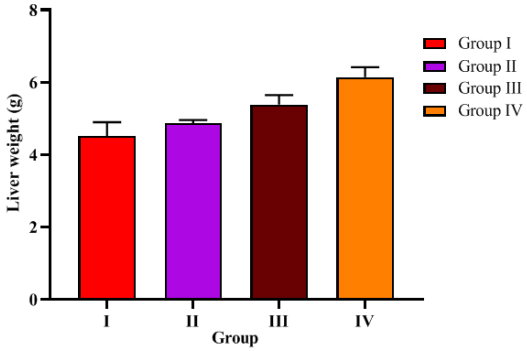


Fig. 3. Relative liver weight (g; Mean ± S.E.M) values in various experimental groups (n=6) of rats during repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis.*

Pathology of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*

Gross pathology. The rats in the control group did not reveal any abnormalities in heart, liver, lungs, kidneys, spleen and duodenum at post-mortem examination. In Group II rats, right ventricular hypertrophy and dilatation and mild coronary vessel congestion were observed in heart. In lungs there was congestion, consolidation, fibrous tissue proliferation and patchy pneumonia. There was moderate congestion and mild hepatomegaly in liver. In spleen mild to moderate congestive changes were recorded. Mild to moderate medullary and cortical congestion were identified in kidneys. In case of duodenum, excess catarrh in intestinal lumen and mild serosal congestion were observed. In case of Group III rats, heart exhibited mild coronary vessel congestion and cardiomegaly. The pathological features like congestion, consolidation, fibrous tissue proliferation and patchy pneumonia were observed in lungs. In liver congestive changes, hepatomegaly and chronic venous congestion (nut-meg liver) were noticed. Spleen exhibited only mild congestive changes. Mild cortical congestion was detected in kidneys. In duodenum excess of catarrh in intestinal lumen and mild serosal congestion were recorded. In Group IV rats, moderate coronary vessel congestion and mild right ventricular dilatation were observed in case of heart. The lungs exhibited congestion, consolidation, fibrous tissue proliferation, oedematous changes and patchy pneumonia. The congestive and haemorrhagic changes along with hepatomegaly was noticed in liver. In spleen moderate congestive changes were observed. Cortical congestion and congestive changes in cortical medullary junction were detected in kidneys. The duodenum exhibited mild serosal congestion with excess catarrh.

Histopathology

Histopathology of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*: Group I (Control)

The histological observation of liver, lungs, kidneys, heart, spleen and duodenum in the control group showed normal morphology. The histological observations of the group are presented in Plate 1.

Histopathology of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*: Group II (250mg/kg)

The section of heart exhibited mild epicardial capillary congestion and cardio-myofibers were normal. The histological section of the liver revealed vascular changes such as sinusoidal, central venular and portal vein congestion along with perivascular oedema. Inflammatory cells infiltration around the portal vein and bile duct was also recorded. The section of lungs showed mild inter-alveolar septal thickening along with congestion of alveolar capillaries and emphysematous changes. The section of kidneys under microscopy didn't show any degenerative changes. Glomeruli, proximal convoluted tubule and distal convoluted tubules appeared to be normal, compact and well defined. No atrophic changes were ascertained in interstitium or tubules. Nephron cells were normal without any haemorrhages, degeneration, necrosis or infiltration. The histopathological examination of spleen demonstrated normal morphology of white pulp and red pulp with uniformly distributed lymphoid cells. The periarteriolar lymphoid sheath surrounding the central artery found to be normal. The microscopic findings of the duodenum included desquamative changes in few columnar epithelial cells of villi. Goblet cell hyperplasia secreting mucin in few areas were observed. The histopathological observations of the group are presented in Plate 2.

Histopathology of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*: Group III (500mg/kg)

The section of heart exhibited epicardial and myocardial capillary congestion. The histological section of the liver revealed moderate form of sinusoidal, central venular and portal vein congestion. Perivascular inflammatory cells infiltration around the portal triad and over dilated sinusoids were observed. Fatty degenerative changes in hepatic parenchyma were supported by appearance of lipid vacuoles in the cytoplasm. The section of lungs exhibited moderate form of inter-alveolar septal thickening, congestion and emphysematous changes. Distended alveolar capillaries and irregular alveolar cavities were also found. The section of kidneys under microscopy showed interstitial vascular congestion and glomerular capillary congestion in renal tubular epithelial cells with focal degenerative changes. The histopathological examination of spleen revealed the congestive changes in white pulp area along with lymphoid cell depletion in germinal centres. The microscopic findings of the duodenum demonstrated desquamative changes in columnar epithelial cells of villi. Goblet cell hyperplasia with mucin secretion and inflammatory cell infiltration in between villi were observed. Sub mucosal vascular congestion was also seen. The histopathological changes of the group are presented in Plate 3.

Histopathology of rats in repeated dose 28-day oral toxicity study of methanolic extract of aerial parts of *T. orientalis*: Group IV (1000mg/kg)

The section of heart exhibited mild epicardial capillary congestion, intensive myocardial capillary congestion with vascular sclerosis and mild haemorrhagic changes. The histological section of the liver revealed severe sinusoidal vascular congestion. Perivasculitis in portal triad, fatty degenerative changes in hepatic parenchyma supported by appearance of lipid vacuoles in the cytoplasm. Multifocal hepatitis was also noticed. The section of lungs demonstrated severe form of inter-alveolar septal thickening, congestion and emphysema of alveoli. Pulmonary oedema was confirmed with the presence of fluid accumulation in interstitial spaces of alveolar septa, interlobular septa and surrounding bronchioles giving gelatinous, homogenous, eosinophilic and thickened appearance to these structures. The section of kidneys under microscopy

showed interstitial vascular congestion and glomerular capillary congestion in renal tubular epithelial cells. Focal interstitial nephritis, oedema of interstitial tissue with infiltration of lymphocytes and periglomerular inflammatory cells infiltration were seen. Degeneration and coagulative necrosis of renal tubules were also observed. The histopathological examination of spleen demonstrated severe splenic vascular congestion and severe congestive changes in white pulp area along with lymphoid cell depletion in germinal centres. The splenic sinuses were dilated and perifollicular congestion was noticed. The microscopic findings of the duodenum revealed extreme desquamative changes in columnar epithelial cells of villi. Goblet cell hyperplasia was widespread in crypts and villi along with mucin secretion. Thickening of mucosal layer and destruction of villi were also ascertained. The histopathological changes of the group are presented in Plate 4.

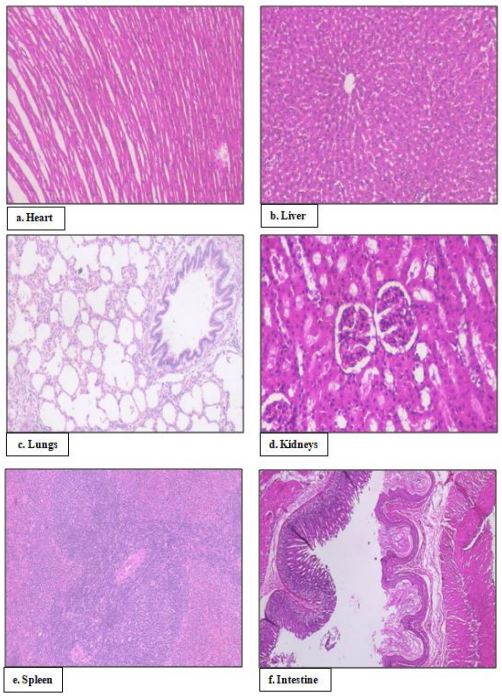
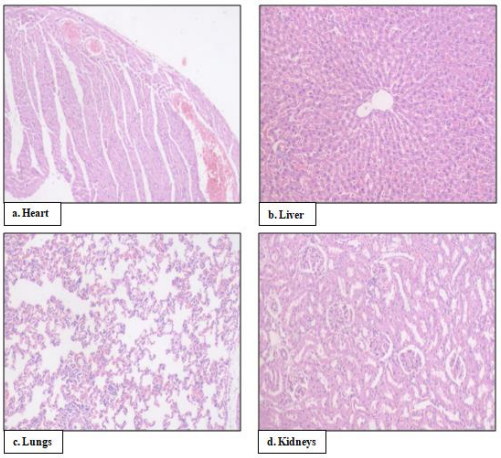


Plate 1. Histopathology: Repeated dose oral toxicity study - Group I (Control)



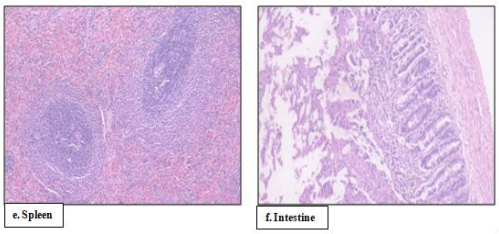


Plate 2. Histopathology: Repeated dose oral toxicity study - Group II (250 mg/kg).

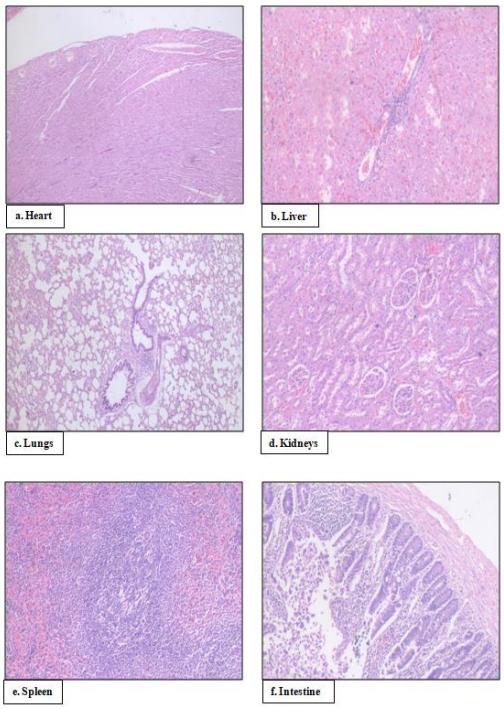


Plate 3. Histopathology: Repeated dose oral toxicity study - Group III (500 mg/kg).

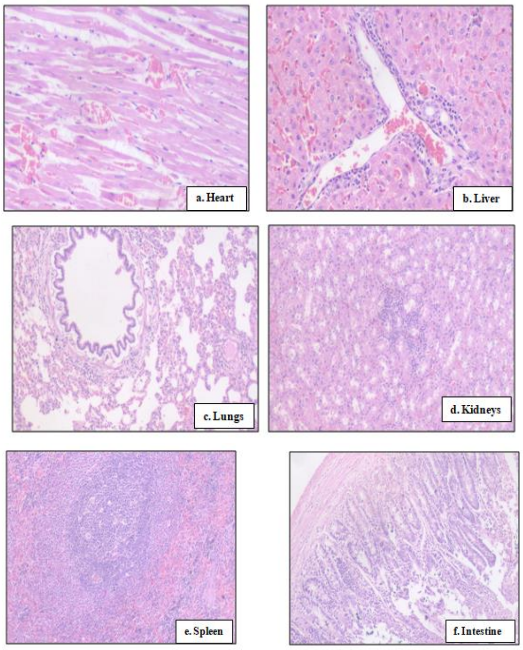


Plate 4. Histopathology: Repeated dose oral toxicity study - Group IV (1000 mg/kg).

DISCUSSION

The outcome of the current study on body weights of the rats were in agreement with Hemalatha *et al*. (2019) who claimed that there was gradual increase in the body weight values from the day 1 to 28 in all experimental rats treated with methanolic extract of *T. orientalis*. Kouakou *et al*. (2014), with ethanolic extract of *T. guineensis* in Wistar albino female rats have also reported a non-significant weight gain in all the test groups in acute toxicity study. The present study revealed that METO had no considerable impacts on their body weight or rate of growth. Similar outcomes were supposed to happen in domestic animals fed on *T. orientalis*. On the contrary, as in the case of certain goats which had clinically suffered from ill health and reduced body weight after consuming aerial parts of *T. orientalis*, as observed by farmers, there was reduction in body weights of animals. Further studies in goats might help to determine the phytoconstituent that may be the causative factor for the effect on growth rate and body weight of the animal.

As per Saleh *et al.* (2020b), at repeated oral dosages of 2.0 mg/kg between ethanolic extract of *T. orientalis* was hepatotoxic and nephrotoxic in goats, but there was no detectable toxicity at lower doses. Further, they had also observed focal haemorrhages, hepatomegaly, congestion and deep green bile in liver and congestion in kidneys in the post mortem of goats in 14-day acute toxicity study. Therefore, precautions need to be taken by the animal owners when rearing goats on grazing lands inhabited with *T. orientalis*.

Bandarra *et al*. (2011), observed that the ponies which were fed with *Trema micrantha* leaves exhibited toxicity and the absolute organ weights of the heart, lungs, liver, spleen and kidneys of all the test groups in the present study were increased without any significant difference between groups on day 28.The findings with absolute organ weight of current study are in contrast with findings of Saleh *et al.* (2020b); Andersen *et al*. (1999), who observed decrease in organ weight of goats fed with ethanolic extract of *T. orientalis* in goats for 14 days acute toxicity study. Here in the present study absolute organ weights reduced slightly and non-significantly. The interspecies variation and genetic differences among domestic and laboratory animals might be the possible reasons for the different findings. Also, they reported hepatomegaly, congestion and deep green bile in liver which could be compared with increased organ weight of liver. The increase in weight of liver could be correlated with the findings of histopathology of current study which signifies hepatic damage due to strong phytochemical compound trematoxin glycoside-containing steroidal saponins present in the *T. orientalis* as per the study of Ibrahim *et al*. (2020).

As per the findings of Whitley, (2022) *T. micrantha* and *T. aspera* had similar phytochemical properties and plant morphology with respect to *T. orientalis*. The gross pathology of organs observed in the present study were similar to the report of Quevedo *et al*. (2022), who reported necropsy findings of three sheep which died after consuming leaves of *T. micrantha*. As per their study, there were moderately apparent diffused lobular pattern in kidneys with pale appearance and multiple reddish spots in the cortex. Very less quantity of serosanguineous fluid oozing out with subpleural petechial haemorrhages and emphysema were noticed in lungs. Wouters *et al.* (2013) observed that the sheep, which died after feeding on *T. micrantha* leaves had revealed pale yellowish with enhanced lobular pattern of liver and lungs were dark red with rib markings, scanty tracheal and bronchial foam. The congestive and haemorrhagic changes in liver were noticed in the present study. Cortical congestion and congestive changes in cortical medullary junction were detected in kidneys. In lungs congestion, consolidation, fibrous tissue proliferation, oedematous changes and patchy pneumonia were found. The findings with respect to the gross pathological changes observed were similar to the previously reported studies as mentioned above.

The hepatic damage and minor renal damage resulting from *T. orientalis* were evident through histopathological study of vital organs and it was associated and consistent with earlier reports and investigations on laboratory and domestic animals. In the current study the liver exhibited severe sinusoidal vascular congestive changes, fatty degenerative changes and multifocal hepatitis. This could be compared with the study of Saleh *et al*. (2020b), in which histopathology revealed mild and moderate hepatocellular degeneration, vacuolation, necrosis and biliary duct hyperplasia in goats fed with ethanolic extract of *T. orientalis*. As per the reports of Quevedo *et al*. (2022), sheep died of *T. micrantha* poisoning exhibited mild multifocal centrilobular necrosis and moderate diffused hepatocellular degeneration in the midzonal and portal regions in liver. The severe diffused hepatocellular fatty degeneration, individual hepatocyte necrosis and multifocal ductal proliferation were the histopathological lesions found out by Bandarra *et al*. (2010), in his case report of toxicity of *T. micrantha* in horse liver.

Quevedo *et al.* (2022), revealed the presence of moderate number of hyaline casts at multifocal tubular lumen in histopathological study of kidneys of sheep after *T. micrantha* toxicity. Bandarra *et al*. (2011), had observed that tumefaction and tubular necrosis were evident in kidneys of horse after poisoning with *T. micrantha.* Saleh *et al.* (2020b), recorded congestive changes in histopathology of kidneys of goat administered with *T. orientalis* extract.

The histopathological findings of kidneys in the present study exhibiting focal interstitial nephritis, degenerative changes, coagulative necrosis of renal tubules, increased levels of creatinine and BUN on day 28 were in agreement with the previous author reports. As per the study of Wouters *et al.* (2013), significant pulmonary oedema, congestion along with diffused type II pneumocyte growth, thickening of interalveolar septa and pulmonary congestion were observed in lungs of sheep poisoned with *T. micrantha*. In accordance to study conducted by Quevedo *et al.* (2022), the

histopathology of the sheep lungs poisoned with *T. micrantha* had mild to moderate diffuse type II pneumocyte hyperplasia with thickened alveolar septa, which caused congestion and oedema within exaggerated diffused alveoli with the development of hyaline membranes.

This histopathological changes in lungs of earlier studies could be attributed to the changes in lungs of present study where the section of lungs indicated pulmonary oedema, inter-alveolar septal thickening, congestion and emphysema of alveoli. As per Ibrahim *et al*. (2020), trematoxin, a glycoside containing steroidal saponin, is the poisonous principle of *T. orientalis* plant. It could be severe hepatotoxic, slightly nephrotoxic and pneumotoxic in nature. Additional toxic phytoconstituents include tannins, flavonoids, phytosterols, triterpenes and various xanthone components, which could be responsible for the pathophysiological characteristics of plant.

As per the reports of Graydon *et al*. (1991); Diaz (2011); Oelrichs (1968) the possible mechanism of toxicity of trematoxin glycoside (steroidal saponins) in ruminants were attributed to the typical breakdown of steroidal saponins in the rumen leading to their toxic action. After rapid hydrolysis in the rumen, appropriate sugars and aglycones would be released, this would be the first stage in the metabolism of steroidal saponins (sapogenins). In the liver, they would be conjugated with glucuronic acid and eliminated in the bile, the sapogenins are subsequently absorbed and transported. Once they reach the bile, they would be forming sapogenin glucuronate insoluble calcium ions, which precipitate in and around the biliary ducts. The glucuronate crystals prevent bile from secreting normally, which might interfere with regular secretion of phylloerythrin which might cause secondary photosensitization. In the present study, there were no mortality and morbidity up to 1000 mg/kg dose of METO, which indicated that LD_{50} of methanolic extract of *T. orientalis* could be more than 2000 mg/kg and could be administered safely up to 2000 mg/kg in laboratory rats without appreciable clinical signs of toxicity.

CONCLUSIONS

The objective of the current study was to assess the toxicological characteristics of the methanolic extract of aerial parts of *T. orientalis* plant. Wistar albino rats were used for the safety evaluation of METO by repeated dose 28-day oral toxicity study. Repeated dose 28-day oral toxicity study of methanolic extract of *T. orientalis* aerial parts was performed in male Wistar albino rats following broader outlines of OECD guideline 407. The experimental rats were divided into four groups (n=6 per group), group I served as control, group II, II and IV rats were administered with METO at doses of 250, 500 and 1000 mg/kg respectively for 28 days. In the experimental rats no notable changes were observed with respect to bodyweight. The maximum tolerable dose of METO in rats was investigated to be more than 2000 mg/kg histopathological examination of the liver, kidneys and heart in the METO treated rats revealed dose-dependent and organ-specific circulatory, degenerative and inflammatory changes. The changes caused by the phytochemicals in the extract were evidenced by very mild to moderate vascular abnormalities, including congestion, haemorrhages, degenerative changes and infiltration of inflammatory cells in vital organs.

Trematoxin found in *T. orientalis* seeds, might be the possible contributing factor that would have major role in the pulmonary, hepatic and renal damage caused by continuous administration in rats. However, due to various factors such as management, nutrition and environmental conditions, the concentration of the phytoconstituent could be possibly toxic to domestic animals in animal husbandry. The present study concluded that *T. orientalis* would exhibit analgesic and antioxidant actions from therapeutic point of view, without completely ruling out the possible significant hepatic and pulmonary damages, which would have caused toxicities in goats.

FUTURE SCOPE

In order to understand better the phytochemical components causing the liver, kidneys and lungs injury produced by *T. orientalis*, there exists a vast scope with future studies in experimental animals. The current study concluded that the seeds and leaves could be toxic to domestic animals, including goats, depending upon the quantity consumed, the extent of exposure and other associated factors like pre-existing hepatic and renal dysfunction that may negatively affect the extent of toxicity. The current experimental study demonstrated that *T. orientalis* methanolic extract might be safer in lower doses for the therapeutic benefits. The identification of the unique phytochemical components would be helpful to assess the extent of potential liver, lungs and renal damage and the suitable agents or method of administration to be used for the possible amelioration of the damage.

Acknowledgement. I extend my sincere thanks to Prof. (Dr.) N.B Shridhar (major advisor) and to my advisory committee members for giving me proper guidance throughout the course of study.

Conflict of Interest. None.

REFERENCES

Andersen, H., Larsen, S., Spliid, H. and Christensen, N. D. (1999). Multivariate statistical analysis of organ weights in toxicity studies. *Toxicology*, *136*(3), 67-77.

Bandarra, P. M., Pavarini, S. P., Raymundo, D. L., Corrêa, A. M. R., Pedroso, P. M. O. and Driemeier, D. (2010). *Trema micrantha* toxicity in horses in Brazil. *Equine veterinary journal*, *42*(5), 456-459.

Bandarra, P. M., Bezerra Júnior, P. S., Oliveira, L. G. S. D., Correa, G. L., Borba, M. R., Reck Júnior, J. and Driemeier, D. (2011). Experimental *Trema micrantha* (Cannabaceae) poisoning in horses. *Pesquisa Veterinária Brasileira*, *31*, 991-996.

Diaz, G. J. (2011). Toxic plants of veterinary and agricultural interest in Colombia. *International Journal of Poisonous Plant Research*, *1*(1), 1-19.

Dubey, S., Maity, S., Singh, M., Saraf, S. A. and Saha, S. (2013). Phytochemistry, pharmacology and toxicology of *Spilanthes acmella*: A review. *Advances in Pharmacological and Pharmaceutical Sciences*, *2013*, 1-9.

Graydon, R., Hamid, H., Zahari, P. and Gardiner, C. (1991). Photosensitisation and crystal-associated cholangiohepatopathy in sheep grazing *Brachiaria decumbens*. *Australian Veterinary Journal*, *68*(7), 234-236.

Hemalatha, T., Mary, D. A. and Ganthi, A. S. (2019). Acute and sub-acute toxicity study of *Trema orientalis* (L.) Bl. methanol extract in rats. *Journal of Drug Delivery and Therapeutics*, *9*(1), 307-311.

Ibrahim, N., Saleh, A., Usman, A., Yahaya, S. and Isa, H. (2020). Uses and toxicological potentials of *Trema orientalis* linn blume in livestock: A review. *Nigerian Journal of Scientific Research*, *19*(2), 124-132.

Kouakou, Y. K. F., Gnahoue, G., Yapi, H. F., Ayebe, E. A., N'guessan, J. D. and Djaman, A. J. (2014). Toxicological and phytochemical screening study of *Trema guineensis* (Ulmaceae), plant of Côte d'Ivoire (West Africa). *World Journal of Pharmaceutical Research*, *3*(8), 12-23.

Oelrichs, P. B. (1968). Isolation and purification of trematoxin from *Trema aspera*. *Phytochemistry*, *7*(9), 1691-1693.

Philippe, G. and Angenot, L. (2005). Recent developments in the field of arrow and dart poisons. *Journal of ethnopharmacology*, *100*(2), 85-91.

Quevedo, L. S., Cristo, T. G., Cunha, A. L., Hemckmeier, D., Marian, L., Medeiros, A. L. and Casagrande, R. A. (2022). Toxic pneumopathy by *Trema micrantha* in sheep in the State of Santa Catarina, Brazil. *Pesquisa Veterinária Brasileira*, *42*, 1-6.

Rao, M. R., Palada, M. C. and Becker, B. N. (2004). Medicinal and aromatic plants in agroforestry systems. *In New Vistas in Agroforestry*: A Compendium for 1st World Congress of Agroforestry, 2004 (pp. 107-122). Springer Netherlands.

Saleh, A., Zainal-Ariffin, S. M., Yahaya, S. F. and Khaleel, A. G. (2020a). Antioxidant activities and estimation of phenol and flavonoid contents in the extracts of *Trema orientalis* Linn Blume. *Nigerian Veterinary Journal*, *41*(2), 73-84.

Saleh, A., Usman, A., Ibrahim, N. B., Abalaka, S. E., Sani, N. A., Mohammed, A. and Zainal-Ariffin, S. A. (2020b). Clinicopathological effects of oral administration of ethanol leaf extract of charcoal–tree (*Trema orientalis* Linn Blume) in Jamnapari Crossbred Goats. *Nigerian Veterinary Journal*, *41*(3), 223-233.

Whitley, B. F. (2022). Phylogenetic, Morphometric, and Biogeographic Investigations of *Trema micrantha* (Cannabaceae). Southern Illinois University at Carbondale.

Wouters, F., Wouters, A. T. B., Watanabe, T. T. N., Soares, M. P., Cruz, C. E. F. and Driemeier, D. (2013). Pneumotoxicosis in sheep caused by ingestion of *Trema micrantha*. *Veterinary pathology*, *50*(5), 775-778.

How to cite this article: Niranjan D., Shridhar N.B., Vinuta M.H., Manjunatha S.S., Sunilchandra U., Pradeep B.S., Manju G.U. and Patel Suresh Revanna (2023). Toxicological Evaluation of *Trema orientalis* in Rats with Reference to Histopathology of certain Tissues. *Biological Forum – An International Journal*, *15*(8a): 317-325.