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ORIGINAL ARTICLE

Clinicopathological Effects of Oral Administration of Ethanol Leaf Extract of Charcoal–Tree (*Trema Orientalis* Linn Blume) in Jamnapari Crossbred Goats

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SUMMARY

The present study was aimed at evaluating the clinicopathological changes due to oral administration of ethanol leaf extract of *Trema orientalis* (ELETO) in Jamnapari crossbred goats. The clinical manifestations, gross and histopathological changes in the major vital organs were used as indices of the toxicity. The severity of gross and microscopic changes were evaluated by scoring technique. Twenty goats weighing between 15-20kg were divided into four groups with five goats in each group in a completely randomized design. The test groups I, II, III were administered ELETO at the dosages of 0.5, 1.0 and 2.0g/kg b.wt *per os*/day respectively, for 14 days while, group IV served as a control. Groups II and III showed decreased appetite whereas, group III showed bilateral congestion of ocular mucous membrane, lacrimation, rectal tenesmus and a significant decrease in body weight compared to control. The main gross and microscopic changes were mild to moderate and included; engorgement of the gall bladder, congestion and icteric liver, hepatocellular degeneration, vacuolation, necrosis and renal congestion observed mainly in group III goats. The results indicate that the ELETO was hepatotoxic and nephrotoxic at continued oral doses equal to or more than 2.0g/kg b.wt in goats but no significant toxicity when used at lowers doses. Therefore, special caution should be taken when keeping goats in areas with *T. orientalis*.

Keywords: Ethanol extract, *Trema orientalis,* Clinicopathological changes, Goats

INTRODUCTION

One of the major constraints in developing ruminant livestock industry is the difficulty to provide sufficient feed, both in quality and quantity throughout the year (Islam *et al.,* 2000). Goats usually feed on grasses or tree leaves (Morand–Fehr, 2005) including *Trema orientalis* in most humid tropics. *Trema orientalis* is a species of evergreen flowering plant in the hemp family, Ulmaceae. It grows up to 1.5m tall in the Savannah and up to 18m high in forest regions with leaves varying from 2.0-20.0cm long, 1.2-7.2cm wide and taper from the base to the apex (FAOSTAT, 2008). The fruits are small (3-5mm long), round, and dark green or purple drupes that change to black when ripe; carried on very short stalks (FAOSTAT, 2008). *Trema orientalis* most probably originated from low land humid tropics of Asia. It is also found throughout the South East Asia and tropical Africa and grows on a wide range of soils from light sand to heavy clay (Smith, 1966). The plant is commonly called *charcoal–tree* or *pigeon wood* (English)*, telemukwu* (Igbo)*, Afefe* (Yoruba) and *menarong* (Malay) (Malan and Notten, 2005; GRIN, 2007). Motooka *et al.* (2003) reported that cattle and game animals relish *T. orientalis* leaf due to its abundance and high palatability. Despite its toxic content; the leaves, seeds, and pods are widely used as fodder for buffaloes, cattle and goats due to the high crude protein content (Orwa *et al.,* 2009; Motooka *et al.,* 2003; Holmström, 2013). Recent reports have recorded animal toxicity due to ingestion of *Trema spp.* (Traverso and Driemeier, 2000; Bernal and Galeano, 2006; Gava *et al.,* 2010). Toxicological evaluations of all plants consumed by animals are essential to establish a scientific evidence of its safety for consumption (Weingand *et al.,* 1996). The primary aim of toxicological assessment of any plant is to identify adverse effects and to determine limits of exposure levels at which such effects occur (Asare *et al.,* 2012). Little research has been conducted on *T. orientalis* and its toxicity effects in goats. The aim of this study is to characterize the clinicopathological features in goats following oral administration of different dosages of ELETO.

MATERIALS AND METHODS

Chemical Sources

All reagents and solvents used in this study were of analytical grade. The ethanol and Disodium hydrogen phosphate (Na$_2$HPO$_4$) was obtained from R&M Chemicals Co., Essex, UK. Sodium dihydrogen phosphate, monohydrate (NaH$_2$PO$_4$) was obtained from Sigma–Aldrich Chemicals Co., St Louis, USA. Sodium bicarbonate (NaHCO$_3$) was purchased from Loughborough, Leics, UK. Formaldehyde (37 to 41%) was sourced from Fisher Scientific, UK. Lithium carbonate (Li$_2$CO$_3$), hydrochloric acid (HCl), Xylene, Eosin, and Hematoxylene were sourced from Emsure Chemicals Co., Germany.

Leaf Collection and Identification, and Crude Extract Preparation

Trema orientalis leaf sample was procured from University Sultan Zainal Abidin (UniSZA) Farm, Terengganu, Malaysia. The sample was authenticated by Prof. Dr. Nashriyah Binti Mat, a botanist from School of Agricultural Sciences and Biotechnology, Faculty of Bioresources and Food Industry (FBIM), UniSZA. The voucher specimen (Voucher No.: 00267) was deposited at the herbarium of School of Agricultural Sciences and Biotechnology, FBIM, UniSZA. The leaf sample was gently washed using tap water to remove impurities and air dried at room temperature until completely dried. The dried sample was then crushed and pulverized to obtain homogeneous fine powder using a Laboratory Blender (HGB550, USA) in order to improve the kinetics of analytic extraction and also increased the contact of sample surface with the solvent system. Proper measures were taken to ensure that the potentially active constituents were not distorted, lost or destroyed during the preparation of the extract as described by Sasidharan *et al.* (2011). Approximately 500g of powdered leaf sample of *T. orientalis* was placed

in 5L conical flask. The sample was completely soaked with 3.5L of 95% ethanol and then covered with aluminum foil. The mixture was allowed to stand at room temperature (25°C ± 1) for a period of 72 hours with frequent agitation in order to facilitate dissolution of soluble matter. The mixture was then strained and pressed using muslin cloth to remove solid material. The extraction was repeated by soaking the solid material using 1.5L of ethanol. The strained liquids were then clarified by filtration using Smith Filter Papers. The combined filtrate was concentrated under reduced pressure (180m/bar) at 40°C using Rotary evaporator (BUCHI Rotavapor R–210, Switzerland). The ELETO obtained was dark–green in colour. The dried extracts obtained were then stored in a deep freezer at –20°C (DW–40L508, China) and aliquots of the concentrations were prepared immediately before use and was found to be stable throughout the duration of the experiment.

Ethical Clearance
The experimental protocol and procedures used in the current study were approved by the Animal Ethics Committee (AEC) UniSZA, Tembila campus, Terengganu with Permit Number: UniSZA/AEC/14/002 in conformity with the guide and care of the use of animals in research and teaching of the university (published by the AEC of UniSZA).

Experimental Animals and Design
Twenty male and female Jamnapari crossbred goats weighing 15-20kg were randomly selected from a flock of 260 goats in UniSZA Farm, Pasir Akar, Besut Terengganu. The goats were randomly divided into four groups with each group consisting of five goats. The experimental groups consist of I, II and III whereas, the controls made up of group IV. Routine physical examination was conducted to ascertain the general health status of the goats. The goats were fed twice daily at 10 am and 6 pm with commercial goat pellet and Napier grass (*Pennisetum purpureum*). Water was given *ad libitum.* The animals were allowed to acclimatize

under room temperature and humidity for 2 weeks. All experimental goats were kept in hygienic and well ventilated goat pens. The test groups I, II, III were given ELETO dissolved in 1% DMSO orally at the dosages 0.5, 1.0 and 2.0g/kg/day respectively. Group IV served as a control and was given 1% DMSO orally. Administration of ELETO was carried out between the hours of 8 am and 10 am daily for 14 days using stomach tube.

Observation of Toxicity Signs
All goats were observed at 6 h intervals post oral administration of ELETO for clinical signs for up to 17 days. The goats were observed for diarrhea, salivation and changes in the body weight, mucous membranes, hair coat, circulatory, respiratory, autonomic and central nervous systems, behavioral pattern, coma and death as described by Nwosu *et al.* (2008). The body weight of each animal was recorded at day 0 and day 14 of the experiment. Changes in body weight were calculated using the following equation;

$$\text{Percentage change in body weight} = \left(\frac{\text{Change in body weight}}{\text{Initial body weight}}\right) \times 100$$

***Post–Mortem* and Histopathology**
All goats were euthanized at day 21 for a full gross examination (Bafor and Igbinuwen, 2009). During the *Post–mortem*, the liver, lung, kidney, brain and heart were examined by visual examination, palpation, and incision. Gross pathological changes in the liver were scored and recorded, according to Dowling *et al.* (2002) and Bafor and Igbinuwen (2009). Score 0 indicates no abnormality, 1 = mild focal changes (or 5–10% of the organ surface is affected), 2 = moderate multifocal changes (or 11–25% of the organ surface is affected) and 3 = severe diffuse changes (or above 25% of the organ surface is affected). Representative tissue samples for histopathology were collected from the vital organs and fixed in 10% neutral buffered formalin at room temperature for one week.

The tissues were sliced laterally and longitudinally to place them into the cassettes and processed using Leica rotary Histokinette (EV1160, Germany). In the Histokinette, the tissues were run through formalin, followed by a series of graded ethanol, xylene, and paraffin wax. After processing overnight, they were embedded in molten paraffin. Paraffin sections were cut at 4μm of thickness except the brain tissue that was cut at 6μm using a Microtome processor (Leica RM2035, Germany), mounted on glass slides and left to air dry. The paraffin sections were heated at 60°C for 60min. All sections were routinely stained with H&E for assessment of the tissue morphology. Sections were deparaffinised by immersion in xylene (I, II, III), and then rehydrated in a graded series of alcohol (100% [trice], 90%, 80% and 70%), before washing in running tap water with a final rinsing in distilled water (GAD I and II), each change was approximately 3min. The sections were immersed in hematoxylin for 20sec followed by washing in running tap water and then rinsed in distilled water (GAD I and II). The sections were decolourized with acid alcohol (0.5% HCl + alcohol) for 2sec and then neutralized with 0.1% Li_2CO_3 for 2sec, before rinsing in water. The sections were counter stained in 0.5% eosin for 5 min. After completion of the staining, sections were dehydrated by sequential immersion in distilled water (GAD I and II), through graded ascending alcohol solutions (70%, 80%, 90% and 100% [twice]) before being cleared in xylene I, II &III and mounted with dipex mountant. The histopathologic features were assessed according to changes in the liver, lung, kidney, brain and heart. The severity of the changes in the liver were scored and recorded according to the modified version of Dagleish *et al.* (2010). The liver changes included hepatic degeneration, cytoplasmic vacuolation, and cellular infiltration. All sections were examined under a light microscope (Olympus DP80, BX53F, UK).

Images of the sections were captured by screen shots.

Statistical Analysis

Independent T–Test was used to determine the differences between the initial weight and final weight in each group; One–Way Analysis of Variance was used to differentiate changes in bodyweight among groups using SPSS version 2 0.0. Meanwhile, mean total gross and histopathol ogical scores were summarized and subjected to a descriptive statistic.

RESULTS

The mean initial body weight of the group I, II, III, and IV goats were 17.3±1.92, 16.5±1.22, 19.1±1.03 and 17.1±1.7 respectively (Table I) whereas, the mean final body weight of the same groups were 16.9±1.25, 16.1±1.25, 18.0±1.30 and 17.4±1.20 respectively (Table I). Only the mean body weight in group III was significantly different (P < 0.05) compared to the control group (Table II). Groups II and III manifested the first signs of toxicity as early as day 10 and day 7 following ELETO administration respectively. Changes observed were apathy and decreased appetite. However, group III goats also manifest bilateral congestion of ocular mucous membrane, lacrimation (Plate I) and rectal tenesmus (Table III).

TABLE I: Changes in mean body weight Mean±SD (n= 5) in goats following oral administration of ethanol leaf extract of *Trema orientalis*

Groups	Initial weight (kg)	Final weight (kg)	Weight difference (kg)
I	17.3±1.92	16.9±1.25	0.4±0.65[ab]
II	16.5±1.22	16.1±1.25	0.4±0.89[ab]
III	19.1±1.03	18.0±1.28	1.1±0.35[a]
IV	17.1±1.70	17.4±1.20	0.3±0.67[b]

Means with different superscripts (a,b) differ significantly (p < 0.05)

TABLE II: Number (n/N) and percentage of goats showing toxicity signs following oral administration of ethanol leaf extract of *Trema orientalis*

Toxicity signs	Groups			
	I	II	III	IV (Control)
Bilateral congestion	0/5 (0%)	0/5 (0%)	2/5 (40%)	0/5 (0%)
Lacrimation	0/5 (0%)	0/5 (0%)	2/5 (40%)	0/5 (0%)
Apathy	0/5 (0%)	0/5 (0%)	3/5 (60%)	0/5 (0%)
Decreased appetite	0/5 (0%)	2/5 (40%)	5/5 (100%)	0/5 (0%)
Rectal tenesmus	0/5 (0%)	0/5 (0%)	2/5 (40%)	0/5 (0%)

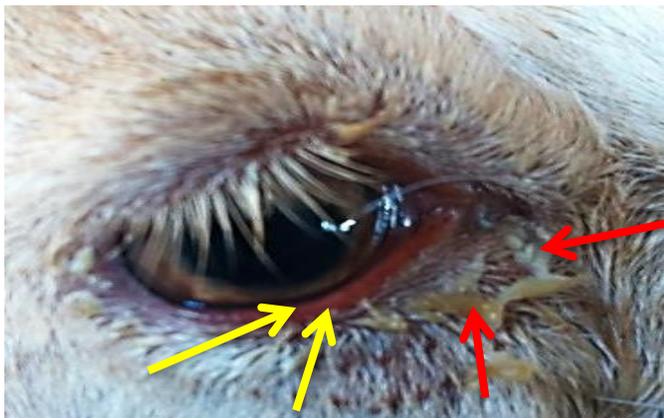n = goats affected, N = total number of goats in the group.

PLATE I: Congestion of ocular mucous membrane (yellow arrows) and lacrimation of the eye (red arrows) following oral administration of ethanol leaf extract of *Trema orientalis* (Group III)

Gross Pathological Findings

Gross pathological examinations were performed on 20 goats. No visible changes were observed in the brain, heart, lungs and intestine in all the groups. Detail of pathological changes observed in the liver and kidneys were summarised in Table III below**.**

Histopathological Findings

For the 20 liver samples that were assessed from test and control groups. Livers in group I were similar to control (Plate V). The severities of the liver changes were mild and moderate hepatocellular degeneration, vacuolation and necrosis in groups II and III (Plate VI) respectively. However, mild renal congestions were observed in group III. Detailed scored histopathological changes are shown in Table IV below.

DISCUSSION

Plant toxicity in animals is usually accidental and most frequently occurs during unfavorable conditions when pastures are scarce due to overstocking, drought, veldt fires, and livestock trampling. The toxicity depends on the condition, part, growth stage of the plant, the amount consumed and the species of the animal involved. In the present study, decrease in body weight observed can be associated with the reduced feed intake, consequently resulting in decreased cellular metabolism leading to lipid peroxidation (Hispard *et al.,* 2007; Ige *et al.,* 2011). Studies have shown that toxicity can be revealed by reduction in the body and organ weights following exposure to a particular substance (Anderson *et al.,* 1999; Nadir and Suat, 2007). Similarly, Gurr and James (1980) reported that oxidation of polyunsaturated fatty acids in the mitochondria, erythrocytes, and platelets leads to the formation of lipid peroxide with consequent cell damage. The decreased body weight could also be as a result of hepatocellular injury which might lead to a decrease in liver weight following oral administration of ELETO. Amna *et al.* (2013) reported that subacute oral administration of ethanol extract of *Cosmos*
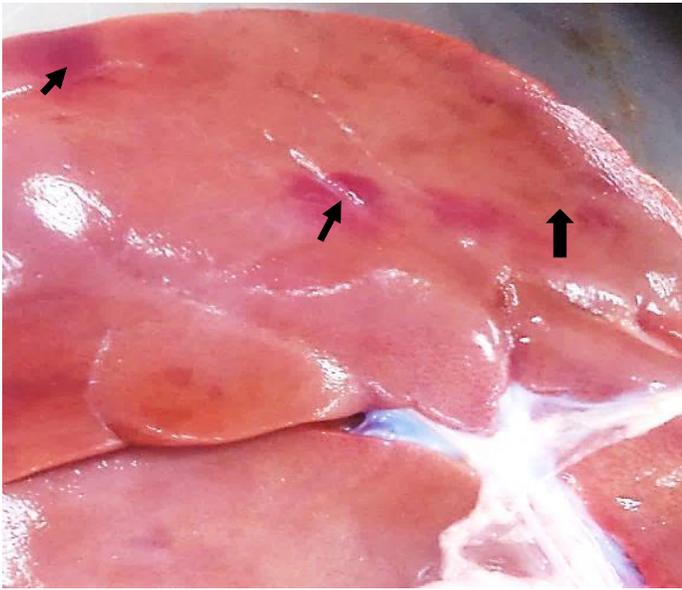
PLATE II: Section of the liver of goat showing multifocal haemorrhages (arrows) following oral administration of ethanol leaf extract of Trema orientalis (Group III goats).
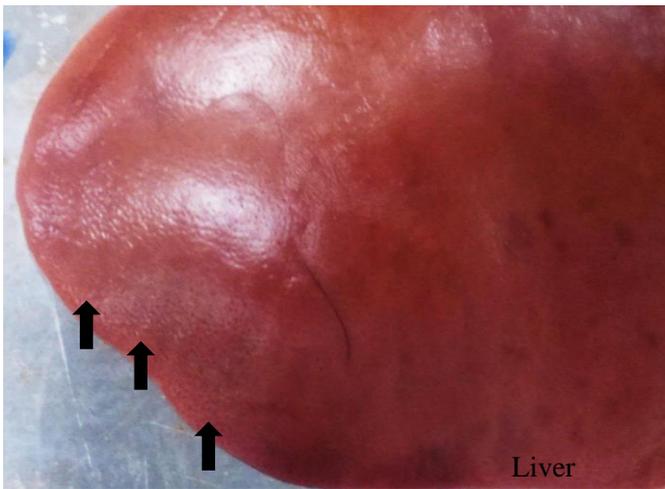

PLATE III: Section of the liver of goat showing enlargement –blunt edge (arrows) with prominent lobular pattern (Group III)

PLATE IV: Section of the liver of goat showing distended and engorged gall bladder with deep greenish bile (red arrows) following oral administration of ethanol leaf extract of *Trema orientalis* (Group III)

audatus leaf causes a significant decrease in weight of the liver in rats. A decrease or increase of the mass of organs may indicate systemic toxicity, and they can affect the vital functions in the organism (Christybapita *et al.,* 2007). Both direct losses (death) and indirect losses (e.g., reduced weight gains) may have high economic consequences on livestock production for the farmer.Rectal tenesmus is a spurious feeling of the need to evacuate the bowels, with little or no stool passed which could be constant or intermittent, and is usually accompanied by cramping and involuntary straining efforts. The tenesmus observed in the goats might have resulted from irritant constituents in the plant.
Liver enlargement could be as a result of the liver dogged up with toxic blood from the portal vein which cannot flow easily, leading to swelling and congestion. The distension of the gall bladder might be due to mild intrahepatic cholestasis as a result of hepatocellular injury caused by ELETO as reported by Carton and

McGavin (1995). Liver discolouration observed in the present study was also reported in *T. micrantha* toxicity in horses (Traverso *et al.,* 2002; Gava, 2010). Microscopically, liver appeared to be the most affected organ probably because about 80% of its blood supply is from the portal vein that drains blood from the gastrointestinal tract (GIT). Liver also has the greatest enzymatic activity of the mixed–function oxidases, capable of activating compounds into toxic forms, thereby causing hepatic injury (Carton and McGavin, 1995; Cullen, 2007). Microvesicular droplets observed could be as a result of fatty degeneration which may occur following accumulation of triglyceride (Saukkonen *et al.,* 2006). Hepatocellular degeneration and necrosis are particularly common findings associated with hepatotoxic insult as portion of the lobules receives less oxygenated blood leading to hypoxia (Zachary, 2017). Congestion of the liver produces stasis of blood and hypoxia (Carton and

TABLE III: Dowling score (n/N) of gross pathological features of vital organs of goats administered with ethanol leaf extract of *Trema orientalis*

Organs	Lesions	I	II	III
Liver	Focal haemorrhages (Plate II)	0 (0/5)	0 (0/5)	1 (3/5)
	Enlargement (Plate III)	0 (0/5)	1 (1/5)	2 (4/5)
	Congestion	0 (0/5)	0 (0/5)	1 (3/5)
	deeply greenish bile (Plate IV)	0 (0/5)	0 (0/5)	1 (3/5)
Kidney	Congestion	0 (0/5)	1 (1/5)	1 (2/5)

n = goats affected, N = total number of goats in the group

TABLE IV: Dowling score (n/N) of histopathologicallesions of some organs in goats

Organs	Lesions	I	II	III	IV (Control)
Liver	Hepatocellular vacuolation	0 (0/5)	1(3/5)	2(5/5)	0 (0/5)
	necrosis	0 (0/5)	1(3/5)	2(5/5)	0 (0/5)
	Biliary duct hyperplasia	0 (0/5)	0 (0/5)	1 (2/5)	0 (0/5)
Kidney	Congestion	0 (0/5)	0(0/5)	1 (2/5)	0 (0/5)

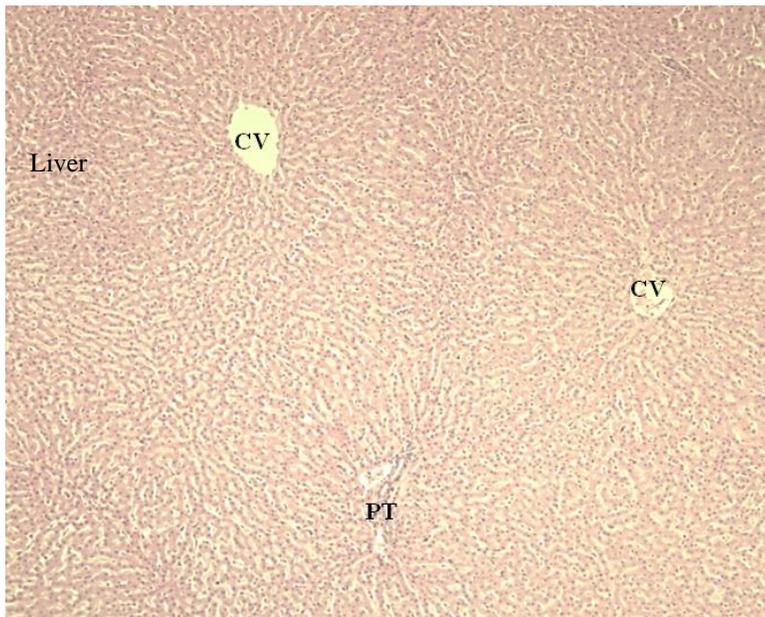n = goats affected, N = total number of goats in the group.

PLATE V: Photomicrograph showing liver of goat following oral administration of ethanol leaf extract of *Trema orientalis* (Group III). H&E stain, x40. Note the moderate (score 2) multifocal hepatocellular vacuolation and necrosis (arrows), and congestion (asterisks). CV = central vein, PV = portal vein
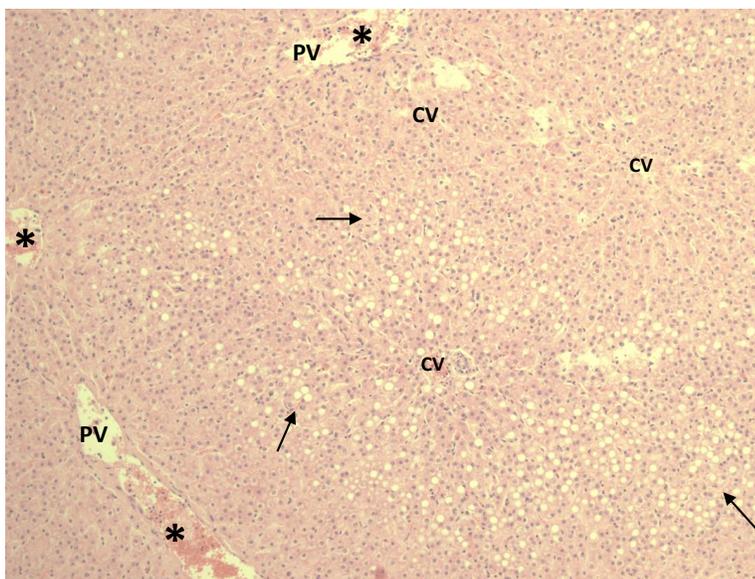

PLATE VI: Photomicrograph showing liver of goat following oral administration of ethanol leaf extract of *Trema orientalis* (Group III). H&E stain, x40. Note the moderate (score 2) multifocal hepatocellular vacuolation and necrosis (arrows), and congestion (asterisks). CV = central vein, PV = portal vein

McGavin, 1995). The necrosis observed might be due to the presence of anaemia in the goats following administration of ELETO. Anaemia results in low oxygen transport to the liver especially hepatocytes nearer to the central vein that may be due to stagnation of the circulation with consequent anaemic anoxia, resulting in more cell death nearer to the central vein thereby, leading to necrosis. The mild appearance of the inflammatory cell infiltration might be for the removal of the necrotic debris. The presence of hepatocellular vacuolation and fatty degeneration, biliary duct hyperplasia, congestion, and necrosis were also reported in *T. micrantha* and *T. aspera*, *Lantana camara, Microcystis species*, *Aphanizomenon spp*, and *Senecio spp* (Carton and McGavin, 1995; Traverso *et al.,* 2002; Cullen, 2007). Plants toxicity depends on the quantity consumed and duration. Toxicity may result from the direct effect of the primary compound, reactive metabolite or an immunologically mediated response affecting hepatocytes, biliary epithelial cells and liver vasculature. The hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. The liver is involved in the synthesis of plasma–clotting proteins, and urea, and Bile acids for the removal of toxic substances and serves as a filter that separates out harmful substances from the blood stream for excretion. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat–soluble vitamins.

CONCLUSION

Our studies indicate that the ELETO was hepatotoxic and nephrotoxic at continued oral doses equal to or more than 2.0mg/kg b.wt in goats but no significant toxicity when used at lower doses. Therefore, special caution should be taken when keeping goats in areas with *T. orientalis*.

RECOMMENDATIONS

Emphasis should be based on research of the bioactive components identification, isolations, purification, characterization and elucidation of the structure of the bioactive compounds. Furthermore, chronic study is required to establish the possible long term toxic effects of the various parts of the plant. Also, work is needed to determine the mechanism of actions of the various bioactive chemical components of *T. orientalis* plant.

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