

Full Length Research Paper

Acute oral toxicity of *Euphorbia heterophylla* Linn. ethanolic extract in albino mice

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This study was carried out to determine acute oral toxicity and histopathological effects associated with consumption of *Euphorbia heterophylla* ethanolic extract using 9 to 10 weeks old Albino mice randomized in six groups. The five groups were orally administered with single graded doses of plant extract at 1500, 2000, 2500, 3500 and 4000 mg/kg body weight while the sixth group was administered 1 ml of physiological saline and the animals were observed for toxicity signs and death. Viscera organs were obtained after cervical dislocation for histopathological assessment. The graded extracts induced dose-dependent toxicity signs with major clinical manifestation prior to death including: polyurea, circling, paralysis, thirst, loss of appetite and gait, tachypnea, dehydration and stupor. The major dose-dependent histopathological lesions included: Hemorrhages, congestion, peri-vascular degeneration and necrosis in viscera organs in the groups that received 2000 to 4000 mg/kg body weight. The 24 h median lethal dose was 2831 mg/kg body weight and the 95% confidence interval of median lethal dose was 2490 to 3218 mg/kg body weight and R^2 is 0.96 indicating *E. heterophylla* is of low toxicity. The study demonstrated the toxicity potential associated with uncontrolled use of this plant by the communities. Toxicological studies of sub chronic and chronic toxicity, as well as *in vitro* mutagenicity and genotoxicity need to be conducted considering the well claimed prolonged use of the plant extract to assess the effect prolonged use on animals.

Key words: Acute oral toxicity, histopathological lessons, *Euphorbia heterophylla*.

INTRODUCTION

Medicinal plants play a significant role in the livelihoods of rural communities of developing countries especially where health and veterinary services are limited. About 80% of the world population depends partially or whole on medicinal plants for primary healthcare (WHO, 2010).

The medicinal plants are used in humans and livestock to control a multiplicity of diseases and conditions. For instance, *Euphorbia heterophylla* Linn belong to the family Euphorbiaceae, has been used for treatment of several diseases and conditions of man and livestock.

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Although most members of Euphorbiaceae family are reportedly poisonous, some are of economic and medicinal value (Burkill, 1994). The leaves and stem of Euphorbiaceae species exude a milky sap when injured and contain latex in all parts of the plant (Parsons and Cuthbertson, 1992).

In Uganda, the *E. heterophylla* is used to treat a number of diseases and conditions like helminthes and constipation in man and livestock in addition to feed the plant to animals like pigs and rabbits (Nalule et al., 2011). In Africa and India, *E. heterophylla* is used to treat constipation, bacterial and inflammatory disease conditions such, arthritis and rheumatism, migraine and wart cures (Falodun et al., 2008; Anilkumar, 2010; Karimi et al., 2010). The plant lattices are used as fish poison, repulsive, insecticide and ordeal poisons (Rodriguez et al., 1976; Falodun et al., 2003) and medically for fungal infection and gonorrhoeal treatment (Moshi et al., 2007). The plant is also reported to have diuretic and purgative action in addition to treating asthma by causing bronchial relaxation (Johnson et al., 1999, Edeoga and Okafor, 2005). In 2010, Oluduro and Olumide demonstrated anti-typhoid activity of aqueous and methanolic leaf extracts. According to Edeoga et al. (2005), the plant vegetable and latex are used in insect bites, treatment of erysipelas, cough, bronchial paroxymal asthma by causing bronchial relaxation hay fever and catarrh. *E. heterophylla* was reported useful in rheumatism, dropsy, gout, neuropathy, deafness and cough (Kirtikar and Basu, 1975). A study by Okoli et al. (2009) revealed alcoholic extract of *E. heterophylla*, was effective against *Streptococcus* sp. In Nigeria, the plant extract is used to treat ear pain, and induces milk flow in addition to increasing sperm quality (Okoli et al., 2009). A study of anthelmintic activity of *E. heterophylla* used by Ugandan pastoralists revealed the plant is very effective and potent (Nalule et al., 2013). In the same study, phytochemical screening revealed presence of tannins, alkaloid, saponins, flavonoids, steroids glycosides, triterpenes, coumarin derivatives, anthocyanocides, anthracenocides and reducing sugars whose intensity varied with solvent used for extraction.

However, despite the several uses of medicinal plants, herbal medicine popularity has raised concerns over the quality and safety with adverse effects resulting from taking herbal medicines having been reported (Chan, 2003; Fennell et al., 2004). For instance, toxicity has been reported in most members of genus *Euphorbia* where individuals sensitive to latex show strong reactions, including dermatitis and anaphylaxis to the sap exuded by this plant (Wilken and Schempp, 2005). Milkweed has been recorded as toxic to stock (Parsons and Cuthbertson, 1992) despite reports that root bark is used as medicines. There has been a tendency of generalized reports of euphorbia species latex toxicity to animals and humans (Burkill, 1935; Wattman and Breyer-Brandwijk, 1962). Considering the medicinal importance of this plant, the current study was undertaken to determine acute oral toxicity and histopathological effects of single

graded doses of ethanolic extract of whole plant over a twenty four hour period.

MATERIALS AND METHODS

Plant collection and preparation of plant extract

The plant material was collected from Nakasongola district and identified in Makerere University herbarium where a sample specimen was deposited. The whole aerial plant part material was oven dried and milled into fine powder. Two hundred fifty grams (250 g) was macerated in 2 L of 70% ethanol for 72 h in a dark room with intermittent shaking. Filtration through cotton wool was done to remove coarse particles (residues) and finely through filter paper 12.5 mm (Whatman®, England). This was followed by concentration on Rota-vapor type Buchi-R, Switzerland under reduced pressure at 50°C. The extracts was then put into an oven to dry completely at 50°C and finally packed into universal bottles and kept at 4°C till needed for toxicity studies.

Experimental animals

Thirty six 9-10 week old mice of either sex, of an average body weight of 19.4 g were randomly divided into six animals per group and put in a cage for easy observation. The animals were kept at 24 to 28°C and at a relative humidity of 60 to 70% for one week to acclimatize to experimental laboratory conditions. During this period, the mice were fed on a standard diet and given ordinary tap water *ad libitum*. After one week of acclimatization, the animals were starved for 18 h but water provided *ad libitum* prior to administration of extracts. The individual body weights were taken shortly before drug administration to guide dose and drug volume for each animal.

Acute oral toxicity and determination of median lethal dose (LD₅₀)

The studies were carried out according to Good Laboratory Practice (GLP) Regulations of Organization for Economic Cooperation and Development (OECD) (UNDP/World Bank/WHO, 2001). Acute oral toxicity of ethanolic *Euphorbia heterophylla* extract was determined orally in mice following the procedure used by Ghosh (1984) and Toma et al. (2009). The five animal groups were orally administered with single graded doses of plant ethanolic extract at 1500, 2000, 2500, 3500, and 4000 mg/kg body weight as treatments 1 to 5. The mice in the control group (group 6) were each orally administered 1ml of physiological saline. After applying the treatments, the animals were observed for six hours continuously and thereafter at six hour intervals up to 24 h. The animals were observed for signs of toxicity: motor activity, tremors, convulsions, posture and spasticity, ataxia, righting reflex, lacrymation, salivation, urination, urine output, diarrhoea, sensation, respiratory rates and mortality. The LD₅₀ of the ethanolic extract was determined using Graph pad prism version 5.01 computer programme and also calculated using the arithmetic method of Karber as described by Ghosh (1984) using the formula;

$$LD_{50} = \frac{X - \sum(D \times \bar{D})}{N}$$

Where, LD₅₀, is the median effective dose; X, is the least dose that killed all the animals; D, is the dose difference; \bar{D} , is the mean dose and N, is the number of animals in a group.

Histopathological examination

Collection of organs samples

After the experimental regimen (24 h) post oral administration of the extract, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia and their viscera organs including intestine, liver, brain, lung and kidneys were collected and preserved in 10% formalin immediately after removal from the animal.

Tissue processing for histopathology

The tissue processing was conducted in an automatic processor model Leica TP 1020 in the college of veterinary medicine animal resources and Bio security, Pathology laboratory. The procedures described by Drury and Wallington (1980) and Prophet et al. (1994) were followed with slight modification. The tissues were placed in 10% formalin (10 parts of formalin and 90 parts of distilled water) for 1 h to rectify shrinkage due to high concentration of formalin. The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in a series of isopropyl alcohol including 70 and 80% for an hour each, then through 90 and 96% for 1.5 h each to avoid shrinkage of cells then in three 100% each for 1½ h each to ensure no traces of water in the cells. The dehydrated tissues were cleared in two changes of xylene for 1½ h each. The tissues were impregnated in two changes of molten paraffin wax for two hours each to provide an internal support. The wax impregnated tissues were embedded in paraffin wax using the same grade wax. The paraffin wax were mounted and cut with rotary microtome machine model Leica RM 2235 at 5µ thickness. The sections were floated on a tissue floatation water bath model Leica HI 1210 at 44°C and taken on plain glass slides. The cut sections were then transferred to an incubator at 53°C for overnight, and thereafter cooled and ready for staining.

Tissue staining

The staining procedures described by Prophet et al. (1994) were followed. The sections were deparaffinised by immersing in two changes of xylene for 2 min each in horizontal staining jar. The deparaffinised sections were washed in two changes of 100% isopropyl alcohol, then in 95% alcohol thereafter dipped in cold water and stained in Meyers hematoxylin for 8 to 12 min in horizontal staining jar. After staining in Meyer's hematoxylin, the sections were blued in tap water for 10 to 15 min to wash out excess hematoxylin in others structures and make the nuclei distinct. The sections were counter stained with eosin for 5 min (the cytoplasm and other organelles stain varying shades of pink to red and nucleus stain blue). The sections were counter stained in 1% aqueous eosin (1 g in 100 ml tap water) for 5 min and the excess stain was washed in tap water. Complete dehydration of stained sections was ensured by placing the sections in graded alcohols including 95, 100 and 100% for 5 min each. The sections were then mounted in DPX having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip). The slides were observed at low power objective under microscope. The cell injury and over aspects were observed under high power dry objective (Dunn, 1974). After mounting the slides were left on table to air dry for microscopic observation and evaluation. The slide imaging was carried out using Carl Zeiss photographic microscope. Model: German-176045 and Canon camera PC 1200. The haematoxylin and Eosin stained slides images were read and the pathological lesions described at magnification x5.

Data analysis

The Graph pad prism computer programme version 5.01, software was used to transform the data and determinate LD₅₀ and the 95% confidence interval of median lethal dose and the slope.

RESULTS

Toxicity signs in mice dosed with whole plant ethanolic *Euphorbia heterophylla* crude extract

The animals to which the different doses of ethanolic crude extract of *E. heterophylla* were administered displayed graded clinical signs whose severity increased with increasing dose before death (Table 1).

Median lethal dose (LD₅₀)

The survival and mortality results of mice dosed with ethanolic extract of *E. heterophylla* are given in Table 2. The 24 h post treatment LD₅₀ by graph pad prism computer programme was determined to be 2831 mg/kg body weight and the 95% confidence interval of median lethal dose (LD₅₀) was 2490 to 3218 mg/kg body weight and R² was 0.96 indicating the plant is of low toxicity. Table 2 also provides data used in calculation of median lethal dose using arithmetic method of Karber for comparison. The LD₅₀ of *E. heterophylla* was 2958 mg/kg when the Karber arithmetic method (Ghosh, 1984) was used. The calculated LD₅₀ still indicate the plant is relatively of low toxicity.

Gross and histopathological findings

The histopathological examination was conducted to determine the damages caused by ethanolic extract to the tissues. At gross examination, the animals in groups treated with 4000 mg/kg of extracts looked dehydrated and weak. There were hemorrhages on the brain meninges and peritoneum, while their liver looked congested. However, the kidneys apparently appeared to be of normal colour and consistence.

The organs from the both groups administered 2500 and 4000 mg/kg body weight, showed histopathological lesions or alterations in histology including congestion of blood vessels, hemorrhages, degenerative changes and cell damage (Table 3). The effects obtained from both doses were the same although varied in magnitude with 4000 mg/kg causing severe injuries compared with 2500 mg/kg. However, the kidney, liver, brain, intestine and lungs of mice from the control groups showed normal architecture and normal cells. The stomach and intestines from treated groups revealed severe inflammation of the walls of the stomach and intestine, ulcers, erosive necrosis and sloughing off of mucosa though the basement remained intact. The histopathological representations of light micrograms of intestine, liver, kidney, brain and lungs lesions are illustrated in Figures 1 to 5 respectively.

Table 1. Toxicity clinical signs in mice treated with a single dose of ethanolic crude extract of *E. heterophylla* Linn.

Dose (mg/kg of body weight)	Noticeable toxicity signs post <i>E. heterophylla</i> ethanol extract administration (N = 6)													
	IR	CV	ATX	IUR	DHD	THT	LOA	DEP	IST	MLT	STP	CHN	DTH	TTD
Normal saline (1 ml)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1500	-	-	-	+ (2)	-	-	+ (2)	-	-	-	-	-	-	-
2000	+ (2)	+ (1)	+ (1)	+ (3)	+ (3)	+ (3)	+ (3)	+ (2)	+ (1)	+ (1)	+ (3)	+ (1)	+ (1)	22
2500	+ (3)	+ (2)	+ (3)	+ (3)	+ (2)	+ (2)	+ (3)	+ (2)	+ (2)	+ (2)	+ (3)	+ (1)	+ (2)	22
3500	+ (4)	+ (4)	+ (5)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (4)	+ (6)	+ (6)	+ (4)	+ (4)	18
4000	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	+ (6)	8

+, Present; -, absent; (), number of animals showing the signs; IR, increased respiration; CV, convulsions; ATX, Ataxia; IUR, increased urination rate; DHD, Dehydration; THT, thirst; LoA, loss of appetite; Dep, depression; IST, insensitivity; MLT, misuse of limbs and tail; STP, stupor; CHN, circling and head nodding; DTH, death; TTD, time to death (h).

Table 2. Twenty four hour dose -response relationship and Median Lethal dose (LD₅₀) of mice dosed with ethanolic crude extract of *Euphorbia heterophylla*.

Group	Dose (mg/kg)	Dose difference (a)	Log dose (mg/kg)	No. of dead animals	Mean death (b)	% of dead animals	Dose difference × mean death (a × b)
1	0.0	-	0.0	0	0	0	0
2	1500	-	3.176	0	0	0	0
3	2000	500	3.301	1	0.5	16.67	250
4	2500	500	3.398	2	1	33.33	500
5	3500	1000	3.544	4	3	66.67	3000
6	4000	500	3.602	6	5	100.00	2500
Total							6250

N= 6 mice; LD₅₀ = 4000 - (6250/6) mg/kg body weight. Therefore, LD₅₀ = 2958 mg/kg.

Table 3. Histopathological lesions of mice treated with ethanolic crude extract of *E. heterophylla*.

Organ	Histopathological Lesions	Microscopic pathological diagnosis*
	Control (Physiological saline)	Animals treated with 2500 and 4000 mg/kg body weight ¹
Liver	Normal cell architecture	Haemorrhages, distension of hepatic sinusoids, congestion of vessels, perivascular necrosis
Intestine	Intact mucosa and basement	a) Sloughing off of mucosa and mucosa membrane b) Haemorrhages, ulcers, erosion of mucosa, glands not affected
Brain	Normal cells	a) Renal cortical haemorrhages, degeneration of neurons, b) Haemorrhages
Kidney	Normal architecture	Haemorrhages on renal cortex, congestion and perivascular necrosis (nuclear pycknosis, and tubule degeneration)
Lung	Normal architecture	Necrosis of lung tissue, haemorrhages, Emphysema, and congestion of vessels

¹The two doses displayed similar lesions but varied in severity; *overall pathological diagnosis: Severe vascular degeneration leading to haemorrhagic shock that caused death of the animals

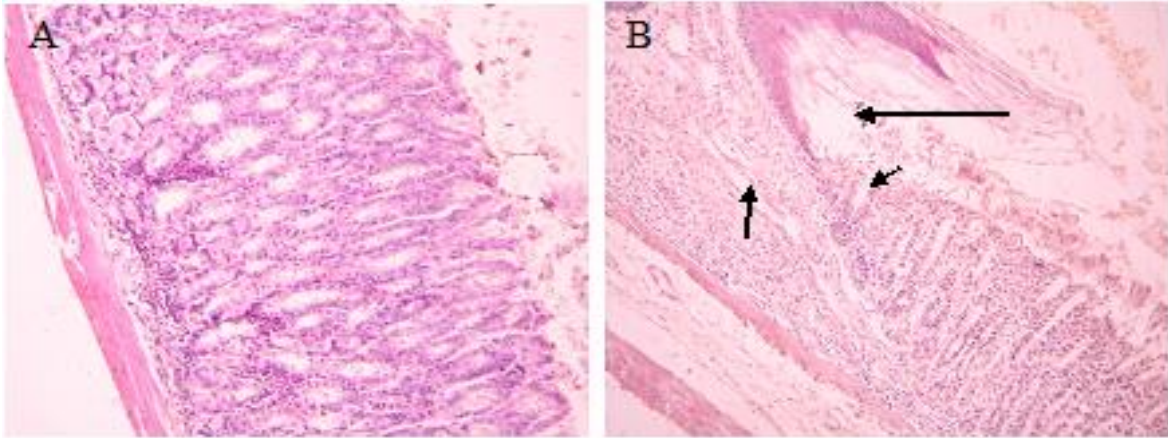


Figure 1. Histopathological presentation of the intestine from mice orally administered with 4000 mg/kg of ethanol crude extracts of *E. heterophylla* (B) at 24 h post exposure, magnification x10. The architecture of organs were severely affected indicated by sloughing off of intestinal mucosa and ulcerative lesions compared with intact mucosa of untreated control (A). Note the loss of lamina propria and lamina muscularis mucosa, basement membrane and damage of epithelial cells (B).

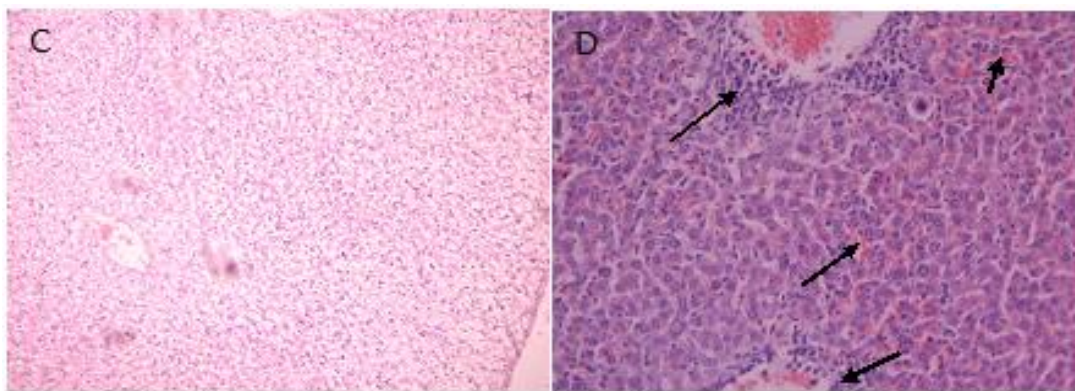


Figure 2. Histopathological presentation of the liver from albino mice orally administered with 4000 mg/kg of ethanol crude extracts of *E. heterophylla* (D) at 24 h post exposure, magnification x 10. The architecture of liver from treated mice shows hemorrhages, distension of hepatic sinusoids and cell degeneration around blood vessel compared with untreated control (C). The arrows point to haemorrhages and cell degeneration.

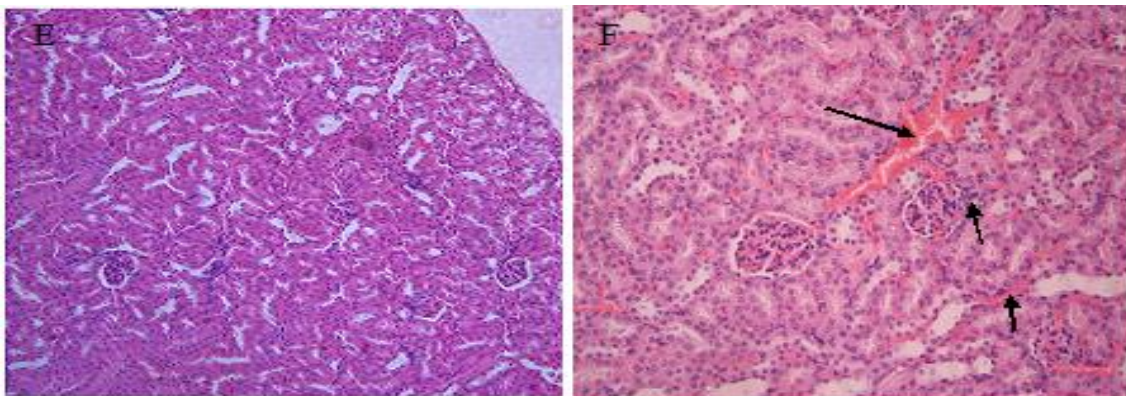


Figure 3. Histopathological presentation of the kidney from albino mice orally administered 4000 mg/kg of ethanol crude extracts of *E. heterophylla* (F) at 24 h post exposure, magnification x 10. The kidney cortex from treated mice shows hemorrhages, renal corpuscles with cellular infiltration, blood between ducts of nephron cells and cell degeneration compared with untreated control (E).

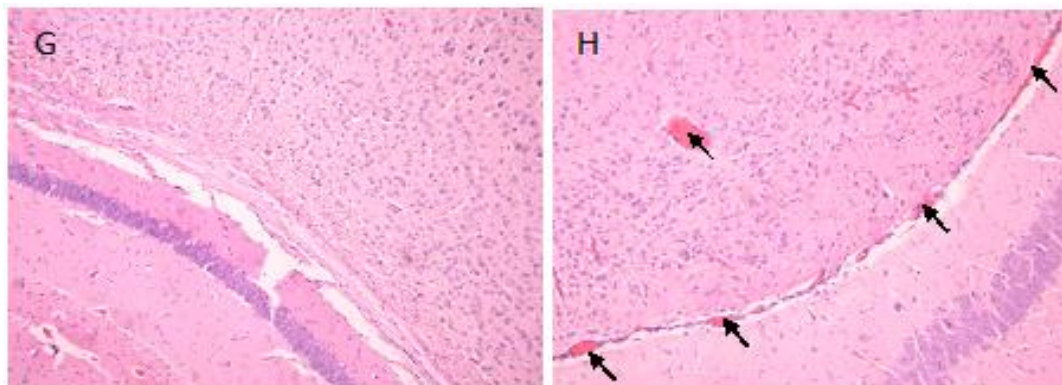


Figure 4. Histopathological presentation of the brain from mice orally administered 4000 mg/kg of ethanol crude extracts of *E. heterophylla* (H) at 24 h post exposure, magnification x 10 compared with untreated control (G). The architecture of brain from treated mice shows exposures to *E.heterophylla* produce hemorrhages in different parts of the brain indicated by arrows.

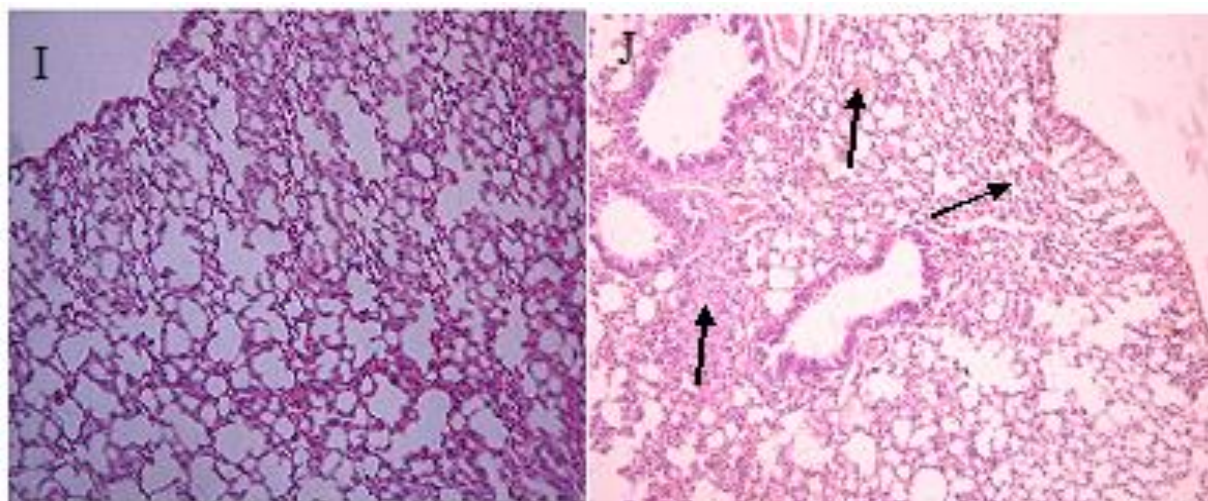


Figure 5. Histopathological pictures of the lung from albino mice orally administered 4000 mg/kg of ethanol crude extracts of *E. heterophylla* (J) at 24 h post exposure compared with untreated control (I), magnification x 10. The architecture of lung from treated mice shows exposures to *E.heterophylla* cause hemorrhages, congestion and edema reducing the alveoli space in the lungs.

DISCUSSION

This study has established that the ethanolic extract of *E. heterophylla* is slightly toxic with LD₅₀ of 2831 mg/kg according to Matsumura (1975) and Ghosh (1984) classification of toxicity. This is in agreement with the report by Moshi et al. (2007) who also established an LC₅₀ of 80.2 µg/ml using brine shrimp model; and a study by Ologe and Sogbesan (2007) that established an LC₅₀ of 1.8 g/L of the aqueous extract on *Barbus occidentalis* fingerlings. The difference in the LC₅₀ could be attributed to the source of the plant, maturity, species of the animals on which it is tested and solvent of extraction. Kuppasamy and Murugan (2008) reported an ovicidal

and pupicidal activity of ethanolic extract of *E. heterophylla* on mosquito development stages with LC₅₀ ranging 14.98 to 25.72 ppm. Adedapo et al. (2004) reported macrocytic normochromic anaemia and a total white blood cell count resulting from 14 day's administration of aqueous fresh extract at 1 g/100 g in rats.

The study further indicated that the ethanolic extract has potential to cause severe clinical signs in the mice (Table 1) and damage to viscera organs exhibiting as severe haemorrhages, cell degeneration, ulcerations and necrosis (Figures 1 to 5). The severe haemorrhages observed indicate that animals feeding on the plant or unregulated use of the plant may cause anaemia and

reduced immunity. These results agree with the findings of Adedapo et al. (2004) who reported anaemia and reduction in total white cell count. The haemorrhage could be due to a plant lectin, phytohaemagglutinins reported by Nsimba-Lubaki et al. (1983). The observed damages in the viscera organs of animals is agreement with earlier observations that euphorbia species induces inflammation on mucous membranes in animals (Karimi et al., 2010). The clinical manifestations observed in the mice may be linked to presence of polyphenols such as tannins and triterpenes such as saponins present in the plant (Nalule et al., 2013). Damage to the brain and kidney could have caused excessive urination probably due to the effect on the anti-diuretic hormone (ADH). Alldredge (1993) and Kongvongxay et al. (2011) attributed reduced feed intake in animals to tannin containing diets that have strong astringent property and cause induction of internal malaise in mammals, which may contribute to reduce feed intake.

Haemorrhages, cell infiltration and cell degeneration present in all organs assess could be attributed to presence saponins and tannins which damage cells. A plant lectin (phyto-haemagglutinins) has been isolated from this plant (Nsimba-Lubaki et al., 1983). The authors reported that *E. heterophylla* agglutinins agglutinate the human and animals erythrocytes in a non blood group specific. The observed mucosal erosion and ulceration (Figure 1) in the stomach and intestine of the test mice administered high doses are consistent with a website report that a post-mortem examination of people killed by *Euphorbia* latex have severe inflammation of the walls of the stomach and intestine while in some patients, the wall of the stomach are perforated (<http://www.theamateur.digest.com?epoisons.htm>, accessed April, 2011).

The excitement and increased urination observed in the mice could be due to the damage caused to the brain and kidney that were characterized by severe haemorrhages and cell degeneration. Thirst was observed in all animals and it could also be associated with the excessive urination that directs to the diuretic effect of the plant extract on the anti-diuretic hormone. The diuretic activity observed in this study agrees with report by Johnson et al. (1999) who reported that euphorbia species have diuretic activity. Similar observations were reported by community who said that humans taking the aqueous plant extract need to drink a lot of water due to the excessive thirst the extract cause to the patient (Nalule et al., 2011).

The histopathological lesions in the viscera organs suggest the level of toxicity and the affected body systems targeted by the *E. heterophylla* extract. Beside these perilous effects, *Euphorbia* species have numerous therapeutic beneficial effects including anti-inflammatory and purgative, antioxidant and hepato-protective activity (Jyothi et al., 2008). These effects may be attributed to the rich phytochemical composition present in this plant

which include but not limited to alkaloids saponins, glycosides, flavonoids, tannins (Falodun et al., 2006; Karimi et al., 2010). The many secondary metabolites are said to have a positive or negative effect on animals and as parasites/disease causing agent.

Tannins and anthraquinones are said to have both prooxidant and antioxidant effects on the animal. While antioxidant act as an anti-inflammatory (Ogueke et al., 2007; Anilkumar, 2010; Karimi et al., 2010) and protect body, the prooxidants cause damage to animal tissues and organs. The tannins and saponins are responsible for the antibacterial activity of the plant extracts (Gloor, 1997). Saponins have been used in veterinary vaccines such foot-and-mouth disease vaccines as adjuvant and helping to enhance immune response. The presence of anthraquinones in the extracts could be contributing to the damage observed. Huang et al. (1992) reported that anthraquinones produce free radicals and hydrogen peroxide during its oxidation to semiquinone in the body that damages the body cells. The terpen ester composition in the plant determines how caustic and irritating to body tissues such as the skin and mucous membranes (Karimi et al., 2010). Karimi et al. (2010) reported the sap from euphorbia species cause keratoconjunctivitis and that the latex (milky sap) of spurge act as deterrent for the herbivores as well as wound healer. A study by Adedapo et al. (2004) showed slight reduction in packed cell volume (PCV) level of the test mice but a significant decrease in the levels of red blood cells (RBC) and haemoglobin concentration, indicating that the leaves of this plant could also cause anaemia in animals.

Flavonoids are well known for ant-oxidant activity and in this study they may have acted to reduce some of the inflammatory reactions in the animal. Flavonoids have also been reported to show anti-inflammatory, anti-allergic, antimicrobial and anticancer activity (Balch and Balch, 2000). The nitrogen containing alkaloids are physiologically active with sedative and analgesic properties and are reported to relieve pains, anxiety and depression (Jisika et al., 1992). However, alkaloids are toxic due to their stimulatory effects that lead to excitation of cells and neurological dysfunction (Ekam and Ebong, 2007; Malu et al., 2009).

The excitatory behaviour is consistent with previous findings of Adedapo et al. (2004) who reported that the aqueous crude extracts of *E. heterophylla*, caused an excitatory effect followed by dullness in addition to anaemia and leucocytosis. These effects could be explained by the severe haemorrhage that was observed in all internal organs in the current study. Adedapo et al (2004) study also reported increase in the level of albumin, ALT and AST. Although the *E. heterophylla* lower doses, caused no observable effects, the hemorrhages caused indicate that prolonged consumption of this plant could also cause anaemia in animals that feed on it like the pigs and rabbits. Gross and

histopathological findings, which included, increased urine output, hemorrhages, depression, cellular degenerative loss of appetite among others, were indicative of poisoning by this plant that were also consistent with findings of Adedapo et al. (2004).

The significant change in the architecture of the viscera organs observed in this study is a clear indication of toxicity potential associated with this plant. Adedapo et al. (2004) reported increase in the levels of aspartate aminotransferase (AST) and alanine associated with the liver damage observed in this study. It therefore evident that with continuous administration of this plant extracts to animal, the principal function of vital organs would be compromised with consequent failures of a number of body systems. The toxicity of the plant, especially of the root and latex, was also recognized in East Africa (Burkill, 1985).

CONCLUSIONS AND RECOMMENDATIONS

It should be concluded that although *E. heterophylla* possess medicinal benefits to humans and their livestock, this study has shown that using the extract at a high dose can cause fatal vital organs' damage. It is imperative to note that continuous exposure of livestock to these plants may lead to morbidity and/or mortality leading to reduced livestock productivity. Caution should therefore be exercised in the use of *E. heterophylla* for medicinal purposes and feed. Patients, whether human or animal taking the extract should equally be availed with enough fluids due to severe loss of fluids. Understanding the toxic principle and its mechanism of action would greatly guide on the protection of the patients. Toxicological studies of sub chronic and chronic toxicity, as well as in vitro mutagenicity and genotoxicity need to conduct considering the well claimed prolonged use of the plant extract to assess the effect prolonged use on animals.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Ethical approval

Authors hereby declared that the experimental protocol was reviewed and approved by the competent members of graduate research and ethics of Faculty of Veterinary medicine and re-reviewed by the University graduate research and training committee of Makerere University.

All ethical consideration for using animal subjects were considered. Experimental protocol was followed according to OECD Guidelines 2000. All rules were followed as well as specific national laws where applicable.

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