Journal of Complementary and Alternative Medical Research
1(3): 1-10, 2016, Article no.JOCAMR.29520

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Acute Toxicity of Aqueous Leaf Extract of *Euphorbia heterophylla* L. in Sprague Dawley Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOO designed the study, wrote the protocol, and wrote the manuscript. Authors OI and AA managed the experimental process. Author NV assisted in design protocol and analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2016/29520
Editor(s):
(1) Ahmed Moussa, Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Algeria.
Reviewers:
(1) Rita Maneju Sunday, National Biotechnology Development Agency, Ogbomosho, Nigeria.
(2) Erhirhie Earnest Oghenesuvwe, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
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(4) Moke Emuesiri Goodies, Delta State University, Abraka, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/16707

Original Research Article

Received 15th September 2016
Accepted 20th October 2016
Published 28th October 2016

ABSTRACT

Aims: The present study, aim to investigate the acute toxicity of *Euphorbia heterophylla* leaf (EHL) aqueous extract.
Study Design: Female Sprague Dawley Rats were divided into five experimental groups consisting of four EHL treatment groups (50 mg/kg, 150 mg/kg, 300 mg/kg and 2000 mg/kg) and control group. The animals received their respective treatments once orally, observed for 14 days and sacrificed.
Place and Duration of Study: Food technology Department, Federal Industrial Institute of Research Oshodi (FIRRO), Nigeria, between 16th May 2016 and 31st May, 2016.
Methodology: Healthy female Sprague Dawley rats (80-100 g) were used. Weights of the animals were recorded before and after EHL extract administration. The feed consumption pattern, relative

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organ weight, hematological parameters, clinical biochemistry and histology of the liver were carried out.

Results: There was a significant decrease ($p< 0.001$, $p< 0.01$) in mean weekly percentage increase in body weight of rats that received 50 mg/kg, 150 mg/kg and 300 mg/kg in either weeks of treatment, although there was no significant change in the food consumed. The relative weight of the liver, kidney and brain significantly increased ($p< 0.05$) especially at 2000 mg/kg. There was also a significant increase in hematocrit (HCT) and hemoglobin (HB) at 50 mg/kg ($p< 0.05$) and 150 mg/kg ($p< 0.001$). However, red blood cells (RBC) ($p < 0.05$), platelets (PLT) ($p< 0.001$) and white blood cells (WBC) ($p< 0.05$) significantly decreased mostly at 2000 mg/kg. There was significant elevation in either aspartate transaminase (AST), alanine transaminase (ALT) or alkaline phosphatase (ALP) at 50 mg/kg, 300 mg/kg and 2000 mg/kg. Moreover, EHL caused mild inflammation or portal congestion in all treatment groups.

Conclusion: EHL possess toxicity potentials clinically, especially at higher doses and safe use of the plant extract is recommended in regards to its common traditional use.

Keywords: Euphorbia heterophylla; acute; toxicity; leaf; extract; rat.

1. INTRODUCTION

The use of herbs as medicine is the oldest form of health care known to humanity and has been practiced as traditional medicine in all cultures throughout history. Currently, there is a global expansion in the use of herbal medicine for the treatment of various disease conditions [1]. In fact, medicinal herbs are beginning to gain preference over conventional drugs because of the perceived safety, affordability, availability and efficacy of medicinal herbs [2]. Due to the increasing interest in the use of medicinal plants in the treatment of disease conditions, there is need for thorough evaluation of toxicological profile as several toxicity relating to the use of herbs have also been documented [3].

Euphorbia heterophylla Linn, commonly referred to as spurge weed or milk weed is a medicinal shrub that belongs to the family Euphorbiaceae and is widely distributed in tropical and temperate regions in the world as a weed or ornamental plant [4,5]. The plant has a characteristic milky latex present in all part of it. The latex is used as fish poison, insecticide and arrow poison. The toxicity of latex has been recognized in Africa, although the latex is also used as antidotes against irritation caused by latex of other Euphorbia species [6]. *Euphorbia heterophylla* leaves serves diverse ethnomedicinal purpose in different cultures as they have been used in treating constipation, epilepsy, respiratory diseases, gonorrhea, diabetes mellitus and malaria. *Euphorbia heterophylla* is rich in phytochemicals such as flavanoids, alkaloids, tannins and sterols that are responsible for its pharmacological activities [7,8]. Some of the reported pharmacological activities include anti-inflammatory, anti-bacterial, antioxidant, anti-diabetic, anti-sickling activity and uterine contractile activity [9,10,11]. Despite reports on the pharmacological activities of *Euphorbia heterophylla*, there have been limited reports on its safety. This study aims to investigate the acute toxicity of the aqueous extract of *Euphorbia heterophylla* leaf.

2. METHODS

2.1 Plant Material

Fresh leaves of *Euphorbia heterophylla* were collected from a crop field in Federal University of Agriculture, Abeokuta. Dirt from the plant was removed by proper rinsing. The plant was then sent for identification and authentication at the Herbarium of the Department of Botany, University of Lagos, Akoka, Nigeria. A voucher specimen of the plant with voucher number of LUH 6958 was kept in the herbarium of the department.

2.1.1 Preparation of ageous leaf extract of plant

The aqueous extract of the plant was prepared according to the method adopted by Okolie et al. [12] with slight modification. The plant was dried at room temperature (27℃) and the dried leaves pulverized in a blender. The dry leave sample (50 g) was weighed into 1 L of boiling water at 100℃. It was filtered hot and the filtrate evaporated to dryness with the aid of a water bath and drying cabinet. It was kept refrigerated at a temperature of -4℃ until use.

2.2 Experimental Animals

Healthy female Sprague Dawley rats (80-100 g) were procured from animal house, College of Medicine, University of Lagos, Nigeria. The animals were kept and maintained under standard conditions (12 h light and dark cycle and room temperature at 25℃). The animals were fed on standard feed diet and given water *ad libitum*. The animals were then used for the experiment after an acclimatization period of seven days.

2.2.1 Experimental design for acute toxicity

The acute toxicity of the aqueous leaf extract of *Euphorbia heterophylla* was evaluated in rats using the procedure described by Organization for Economic Cooperation and Development (OECD) guidelines 423 with slight modification [13]. A total of 25 female rats were divided into five dosage groups with 5 animals per dose. The control group received normal distilled water. The other four groups were administered with aqueous leaf extract of *Euphorbia heterophylla* by oral gavage at 50 mg/kg, 150 mg/kg, 300 mg/kg and 2000 mg/kg. The animals were fasted overnight prior to administration of the aqueous leaf extract. After dosing, the animals were observed during the first 30 minutes and periodically (with special attention given during the next 4 hours) for 24 hours and next 14 days for signs of toxicity and behavioral changes such as loss of coordination, convulsion, writhing, loss of fur and mortality.

2.2.2 Body weight and food consumption

The weight of the animals was recorded before the experiment (day 0) and subsequently weekly (day 7 and day 14). The weight of the feed consumed by rats in each group were measured daily as the difference between the quantity of feed supplied and amount remaining after 24 hours. The mean weekly percentage increase in body weight was then calculated.

2.2.3 Relative organ weight

All the rats were sacrificed by cervical dislocation on day 15. A comprehensive gross observation was carried out on the internal organs namely lungs, heart, liver and brain. They were observed for any signs of abnormality and presence of lesions. The organs were carefully dissected out, cleaned of fat and weighed (absolute weight). The relative organ weight (ROW) of each organ was then calculated according to the method adopted by Halim et al. [14].

ROW= Absolute organ weight (g) / Body weight of rat on sacrifice day (g) × 100.

2.2.4 Hematology

The blood of the animals were collected from the retro orbital sinus and dispensed in EDTA bottles for estimation of hematocrit (HCT), haemoglobin concentration (Hb), red blood cell count (RBC), platelets (PLT), white blood cells (WBC), neutrophils (NEUT), monocyte (MONO), lymphocytes (LYMPH) and eosinophils (EOS), using an automated hematology analyzer (Mindray, BC -2800, Shenzhen, China).

2.2.5 Clinical biochemistry

The blood of animals were collected from the retro orbital sinus, dispensed in plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The serum was separated and stored at -4℃ to be used for evaluation of biochemical parameters which include; alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) levels. The samples were analyzed according to the principle of Reitman and Frankel using the commercial kits obtained from Randox laboratories, UK [15].

2.2.6 Histology

The liver of the rats was fixed in 10% formalin for 48 hours. They were processed routinely, and the tissues were embedded in paraffin wax. Histological sections of the liver was cut at 5-6 µm and stained with hematoxylin and eosin. It was then examined by a consultant histopathologist that had no idea which groups the animal belonged. The stained sections were examined for any cellular damage or change in morphology of the tissue.

2.3 Statistical Analysis

Statistical analysis of the differences between mean values obtained for experimental groups was calculated using Microsoft excel program and GraphPad prism (5.01). Data was subjected to one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. In all cases, p values ≤ 0.05 was regarded as statistical significant.

3. RESULTS

3.1 Acute Toxicity Studies

3.1.1 Physical observation and mortality

Acute toxicity studies revealed that oral administration of the aqueous extract of *Euphorbia heterophylla* leave (EHL) at 50 mg/kg, 150 mg.kg, 300 mg/kg and 2000 mg/kg did not cause death of the animals. However, EHL extract exerted sedative effects for 2 hours on the treated animals and caused the excretion of small and dark stools compared to brown stools excreted by controls especially at 2000 mg/kg, although there were no physically observed toxic effects. There were no changes on the skin, fur, eyes, mucous membrane, behavior patterns and there was absence of tremors, salivation, diarrhea and coma.

3.1.2 Effect of aqueous extract of EHL on food consumption

On the first day of feed administration there was an observed reduction of feed consumption in the 2000 mg/kg treated group compared to the control and other treatment groups. However, in the second week of treatment, there was an insignificant increase in food consumption in the 50 mg/kg EHL, 300 mg/kg EHL and 2000 mg/kg EHL treated groups similar to the control group, although the 150 mg/kg EHL treated group showed an insignificant decrease in feed consumption (Table 1).

Table 1. Food consumption (g) of different experimental groups

Group	Week 1	Week 2
Control	61.97 ± 8.00	62.81 ± 9.42
50 MG/KG	50.17 ± 14.56	51.73 ± 11.40
150 MG/KG	69.23 ± 19.80	64.17 ± 7.41
300 MG/KG	58.59 ± 5.91	66.70 ± 8.51
2000 MG/KG	60.63 ± 8.30	76.01 ± 8.83

Values are mean ± SD, N=5, EHL= Euphorbia heterophylla leaf

3.1.3 Effect of aqueous extract of EHL on percentage increase of body weight (% IBW)

The percentage (%) increase and decrease of body weight weekly in the treatment and control rats are shown in Table 2. In the first week after treatment, there was a significant decrease ($p < 0.001$) in the % IBW of rats treated with 50 mg/kg EHL compared to control while the 150 mg/kg, 300 mg/kg and 2000 mg/kg EHL treatment had no significant effect on % IBW of rats. However, in the second week of treatment there was a significant increase ($P < 0.001$) of % IBW in rats that received 50 mg/kg compared to control, although the % IBW significantly decreased in rats that received 150 mg/kg ($p < 0.001$) and 300 mg/kg ($p < 0.01$). There was no significant change in % IBW in rats that received 2000 mg/kg EHL.

Table 2. Mean percentage increase of body weight (%) weekly of different experimental groups

Group	Week 1	Week 2
Control	16.35 ± 3.20	2.02 ± 2.44
50 MG/KG	-4.25 ± 7.25***	11.75 ± 2.27***
150 MG/KG	20.26 ± 6.90	-17.72 ±0.77***
300 MG/KG	22.17 ± 4.24	-4.32 ± 3.57**
2000 MG/KG	9.08 ± 1.56	0.08 ± 2.85

*Values are mean ± SD, N=5, * p< 0.05, ** p< 0.01, *** p< 0.001, EHL= Euphorbia heterophylla leaf*

3.1.4 Effect of aqueous extract of EHL on relative organ weights

There was a significant increase ($p < 0.05$) in the relative weight of the liver, brain and kidney in rats treated with 2000 mg/kg EHL. Rats treated with 150 mg/kg EHL also showed a significant increase ($p < 0.05$) in relative weight of kidney. There was no significant change in the relative weight of the heart in all the treatment groups (Table 1).

Table 3. Relative organ weight (%) of different experimental groups

Organ	Heart	Liver	Brain	Kidney
Control	0.38 ± 0.04	3.37 ± 0.10	1.31 ± 0.01	0.67 ± 0.02
50 mg/kg EHL	0.38 ± 0.02	3.41 ± 0.21	1.26 ± 0.05	0.73 ± 0.06
150 mg/kg EHLS	0.37 ± 0.03	3.36 ± 0.13	1.42 ± 0.11	0.77 ± 0.05*****
300 mg/kg EHL	0.34 ± 0.01	3.61 ± 0.12	1.21 ± 0.06	0.72 ± 0.03
2000 mg/kg EHL	0.41 ± 0.03	3.90 ± 0.33*****	1.43 ± 0.10*****	0.78 ± 0.05*****

*Values are mean ± SD, N=5, * p< 0.05, ** p< 0.01, *** p< 0.001, EHL= Euphorbia heterophylla leaf*

Table 4. Hematological parameters of different experimental groups

Treatment groups	HCT (%)	HB (g/dl)	RBC (× 1012/L)	PLT (/cmm)	WBC (/cmm)	NEUT (%)	LYMPH (%)	MONO (%)	EOS (%)
Control	37.10 ± 2.72	12.39 ± 1.35	7.62 ± 0.48	494.00 ± 21.46	88.80 ± 9.28	52.80 ± 3.03	44.00 ± 4.18	2.00 ± 0.71	1.20 ± 0.45
50 mg/kg EHL	43.30 ± 0.16***	14.56 ± 0.33*	7.66 ± 0.40	484.20 ± 30.29	87.60 ± 6.15	55.80 ± 5.22	43.20 ± 4.32	2.40 ± 0.55	0.60 ± 0.55
150 mg/kg EHL	46.40 ± 1.49***	14.54 ± 0.59*	7.91 ± 0.61	391.60 ± 20.37**	80.40 ± 12.10	54.40 ± 3.65	41.20 ± 2.17	2.60 ± 0.89	0.60 ± 0.55
300 mg/kg EHL	38.30 ± 1.17	12.56 ± 0.98	6.87 ± 0.54	323.40 ± 38.57***	74.20 ± 7.6	51.00 ± 2.35	43.00 ± 1.23	3.40 ± 1.14	1.00 ± 0.71
2000 mg/kg EHL	36.08 ± 1.97	12.38 ± 0.62	6.32 ± 0.77*	331.00 ± 62.10***	69.40 ± 4.98*	51.20 ± 3.96	45.40 ± 3.65	2.40 ± 0.89	1.60 ± 1.14

*Values are mean ± SD, N=5, * p< 0.05, ** p< 0.01, *** p< 0.001, EHL= Euphorbia heterophylla leaf. HCT; Hematocrit, HB; Hemoglobin, RBC; Red blood cell; PLT; Platelet; WBC; White blood cell; NEUT; Neutrophils; LYMPH; Lymphocytes; MONO; Monocytes; EOS; Eosinophils*

3.1.5 Effect of aqueous extract of EHL on hematological parameters

Hematological values are reported in Table 2. The Hematological values measured showed a significant elevation ($p< 0.001$) of HCT in the rats treated with 50 mg/kg and 150 mg/kg EHL extract compared to the control group. There was also a significant elevation of HB ($p< 0.05$) in the 50 mg/kg and 150 mg/kg EHL treated rats compared to the control rats. However, the RBC was significantly reduced ($p< 0.05$) in rats treated with 2000 mg/kg EHL. Platelet was also significantly reduced in the rats treated with 150 mg/kg EHL ($p< 0.05$), 300 mg/kg EHL ($p< 0.01$) and 2000 mg/kg EHL ($p< 0.001$). There was also a significant reduction of WBC in rats treated with 2000 mg/kg EHL. Other hematological values, NEUT, LYMPH, MONO and EOS were not significantly different in the extract treated groups when compared to control group.

3.1.6 Effect of aqueous extract of EHL on serum chemistry

There was a significant increase ($p< 0.001$) in AST level of rats that received 2000 mg/kg EHL extract compared to the control. ALT level was also significantly increased ($p< 0.05$) only in the rats that received 300 mg/kg EHL extract. ALP was significantly elevated at 50 mg/kg EHL ($p< 0.001$), 300 mg/kg EHL ($p< 0.01$) and 2000 mg/kg EHL ($p< 0.001$) compared to control (Table 3).

Control group

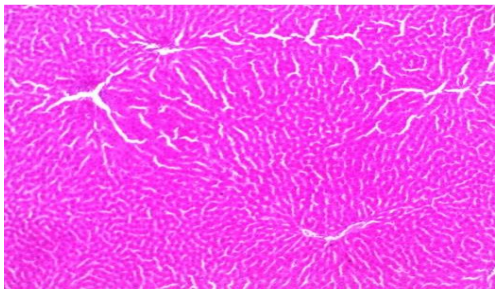

Fig. 1. Normal liver architecture with normal hepatocytes H distribution. No portal congestion or inflammation observed H & E X 100

50 mg/kg EHL treated group

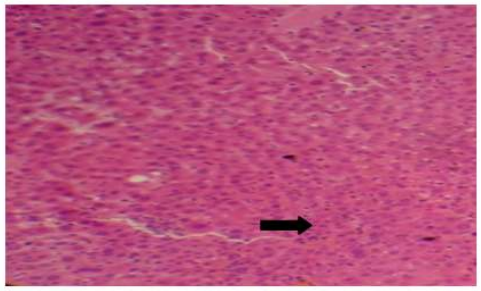

Fig. 2. Normal hepatocyte appearance, however there is mild sinosudal congestion and sparse diffuse inflammatory cell infiltrates, especially in the lower half of the field (see arrow). H & E X 100

150 mg/kg EHL treated group

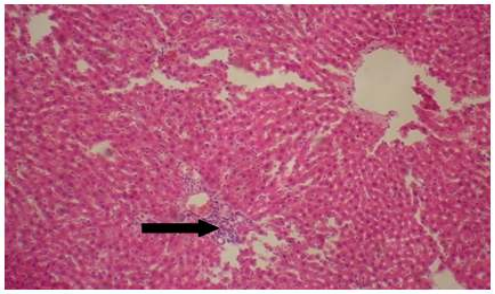

Fig. 3. Normal hepatocyte appearance although there is more intense portal tract inflammation (see arrow), with spilling of the inflammatory cells to the sinusoids (between hepatocyte plates). There is also portal congestion. H& E × 100

300 mg/kg EHL treated group

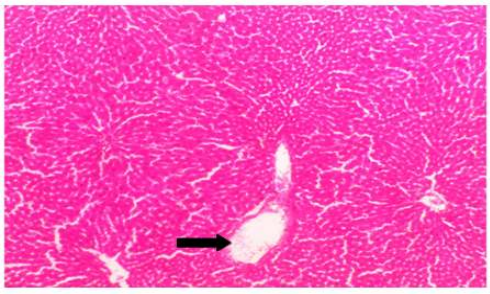

Fig. 4. Normal hepatocyte appearance. No portal tract (see arrow). Congestion and inflammation also observed. H & E X 100

Table 5. Serum chemistry of different experimental groups

Serum chemistry	AST (iu/l)	ALT (iu/l)	ALP (iu/l)
Control	10.7 ± 0.28	6.64 ± 0.98	131.0 ± 2.65
50 mg/kg EHL	12 ± 0.70	7.90 ± 0.16	142.2 ± 3.85***
150 mg/kg EHL	12.38 ± 0.26	6.98 ± 0.34	132.8 ± 1.91
300 mg/kg EHL	12.52 ± 0.88	8.39 ± 0.83*	138.0 ± 2.15**
2000 mg/kg EHL	13.6 ± 1.90***	7.59 ± 0.86	147.8 ± 2.45***

*Values are mean ± SD, N=5, * p< 0.05, ** p< 0.01, *** p< 0.001, EHL= Euphorbia heterophylla leaf. AST; Aspartate transaminase ALT; Alanine transaminase; ALP; Alkaline phosphatase*

3.1.7 Effect of aqueous extract of EHL on histopathology

The EHL extract treated rats showed mild inflammation and portal congestion in all groups. Though, the 50 mg/kg EHL, 150 mg/kg EHL and 300 mg/kg EHL reported normal hepatocyte appearance and distribution, there was still inflammation and sinusoidal or portal congestion observed while the 2000 mg/kg EHL treated rats showed intense diffused infiltration of the sinusoid by dense inflammatory cell aggregates and mildly congested central veins (Fig. 5).

2000 mg/kg EHL treated group

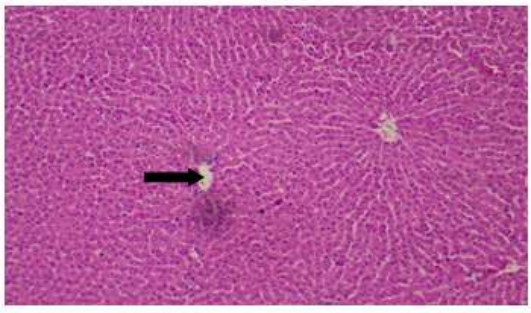

Fig. 5. Intense diffused infiltration of the sinusoid by dense inflammatory cell aggregates (see arrow). The central veins are also mildly congested

4. DISCUSSION

The use of herbal preparations in treating diseases and abnormal physiological conditions is very common in African and Asian countries. Herbs are used mostly as food and traditional medicine. Moreover the use of herbs as medicine is gradually replacing clinical therapies and demands for herbal medicine is increasing due to their perceived efficacy [16]. *Euphorbia heterophylla* leaf (EHL) extract has been used traditionally for treatment of respiratory tract infections, constipation, migraine, gonorrhea, epilepsy, malaria and skin infections [11,12]. Scientific evidences have also attested to the efficacy of *Euphorbia heterophylla* leaf extract, yet there has been limited report on its systematic safety [8,17]. Acute toxicity study is important to establish the systematic safety of test compounds. It involves the short term administration of the test compound at different doses to animals within 24 hours and identifying clinical signs or death, evoked by the test substance, as well as possibly estimating its therapeutic index [18]. Acute toxicity studies carried out on *Euphorbia heterophylla* will ascertain its safety and evaluate its potential for development into pharmacological compounds.

During the 14 day observation period, the sedative effects and changes in excretory pattern produced by EHL suggest that it may involve the CNS and digestive tract respectively in its toxic effect. However, the EHL extract demonstrated a high safety margin since the animals tolerated up to 2000 mg/kg of the extract orally administered, as there was no death recorded at all tested doses. This suggests that the LD_{50} value of EHL aqueous extract is greater than 2000 mg/kg. According to the OECD guideline and classification [13], EHL will be ranked at category 5, which represents a low acute toxicity hazard. Thus, this may justify the common use of EHL orally in traditional medicine. The suggested safety of *Euphorbia heterophylla* is similar to reports by Adaikpoh et al., where no death of animals was recorded up to 4000 mg/kg extract aerial portion of *Euphorbia heterophylla* [19]. Though, rats that received 50 mg/kg, 150 mg/kg and 300 mg/kg EHL showed a significant decrease in percentage increase of body weight (%IBW) in either the first or second week of treatment. These weight changes can be related to the food consumption pattern except for rats that received 300 mg/kg EHL extract. It is possible that EHL extract at certain doses might be metabolized into forms that interfere with gastric function and decrease food conversion efficiency.

At all the treatment doses, EHL extract showed no deleterious effect on the heart of animals. However, 2000 mg/kg EHL extract caused a significant increase in the relative weight of the liver, brain and kidney. Animal administered 150 mg/kg EHL extract also showed a significant increase in kidney weight. Changes in relative organ weight are often associated with treatment related effect and can be used as an indicator for unspecific adverse side effects as it reflects the pathological and physiological state of the animal or human [20]. Increase in organ weight has been associated with toxic effects involving inflammation and hypertrophy [21,22]. Thus, the elevation in kidney weight by 2000 mg/kg EHL extract may reflect that the plant has renal toxic, chronic progressive nephropathy and tubular hypertrophy effects [23]. The increase in liver weight may also suggest that at selected doses the plant extract causes hepatocellular hypertrophy (arising from enzyme induction or peroxisome proliferation) or inflammation of the liver. Moreover, the elevation of AST at 2000 mg/kg EHL extract suggests that the plants may be associated with necrotic effects on the liver, though ALT is a more specific parameter for predicting liver damage than AST. Only the rats administered 300 mg/kg EHL extract showed a significant elevation of ALT which was not dose dependent. The significant elevation of ALP was also non dose dependent in the treatment groups. Nevertheless elevations of AST, ALT and ALP are indicative that the extract has hepatotoxic potentials. Adedapo et al. [24] and Okolie et al. [12] also reported similar work on the hepatoxic effect of the aerial portion of *Euphorbia heterophylla*. The hepatoxic effect was due to elevated AST and ALT. Though Apiamu et al. [25] reported that *Euphorbia heterophylla* had no significant effect on liver enzymes.

The EHL extract showed a significant increase in HCT and HB at 50 mg/kg and 150 mg/kg EHL extract doses. This suggests that the extract may stimulate hemoglobin synthesis at lower doses. Moreover, *Euphorbia heterophylla* contains considerable quantity of iron [8]. Iron is important in the synthesis of hemoglobin as it remains the backbone of the heme structure, an essential part of hemoglobin that mediates the reversible binding of oxygen [26]. The deranged production of heme causes a variety of anemia. However, in this study, the significant reduction of RBC value at 2000 mg/kg indicates that at higher doses, EHL extract is toxic to circulating red blood cells and interferes with RBC production [27]. This suggests that the aqueous leaf extract of *Euphorbia heterophylla* may possess the potential to induce anemia at higher concentrations. Moreover, reports by Adedapo et al. [24] showed that 1 g/ 100 g of aqueous extract of *Euphorbia heterophylla* significantly reduced HB, PCV and RBC. Adaikpoh et al. [19] also reported that 250 mg/kg and 4000 mg/kg of *Euphorbia heterophylla* aerial parts reduced HB, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). In this study, the dose dependent reduction of WBC, especially at 2000 mg/kg suggests that at higher concentrations, the plant may have immunosuppressive effects. This may be due to its cytotoxic effects at higher concentrations which would destroy WBC [8]. Thus, considerations should be given to administration of plant extract at higher doses especially to immunocompromised individuals. The significant reduction of platelets at 150-2000 mg/kg EHL extract also suggests that the plant may be cytotoxic to platelets. Platelets consists of a mesh work of fibrin fibres that helps to mediate blood clotting as it plays a crucial role in reducing blood loss and repairing of vascular injury. Substances that reduce platelets may facilitate internal or external hemorrhage [28].

The histology of the liver showed that the plant has potential adverse effects on the liver. Though, 50- 300 mg/kg EHL reported normal hepatocyte appearance and distribution, there was still congestion and mild inflammation reported. Moreover, the 2000 mg/kg EHL showed intense diffused infiltration of the sinusoid and mildly congested central veins. The liver is the main organ responsible for the metabolism of drugs and toxic compounds, as a result it is the primary target organ readily exposed to toxic metabolites [29]. These toxic metabolites can change the normal liver architecture suggesting liver damage. Thus, the mild inflammation and congestion observed in the liver histology of all the EHL treated rats suggest that the plant has hepatotoxic potentials.

5. CONCLUSION

The acute toxicity study of EHL aqueous leaf extract at 50 mg/kg, 150 mg/kg, 300 mg/kg and 2000 mg/kg body weight administered orally to Sprague Dawley rats did not cause any death of animals. However, there were acute adverse effects on clinical observation mostly found in rats that received 150-2000 mg/kg EHL extract. With the wide ethno-medicinal applications of

Euphorbia herterophylla, the present toxicity study result suggests appropriate use of the plant especially at lower doses to prevent adverse effects. Nonetheless, sub acute and chronic toxicity studies are still necessary to fully ascertain the toxic potentials of *Euphorbia heterophylla* leaf extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

ACKNOWLEDGEMENT

This is to acknowledge the valuable help of Mr Dike in the animal sacrifice. Also not forgetting to mention Mr Sunday, who assisted in analysis of blood samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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