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ASSESSMENT OF ACUTE AND SUB-ACUTE TOXICITY STUDIES OF HYDRO-ETHANOLIC EXTRACT OF *Euphorbia hirta* Linn LEAVES EXTRACT

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Abstract

The present study aimed to evaluate the acute and subacute toxicity profiles of *Euphorbia hirta* leaves extract in albino rats using the methods described in the guidelines of the OECD. The acute toxicity was performed where the limit dose of 2000 mg/kg body weight used. Observations were made and recorded for 24 h, and once daily further for a period of 14 days. The rats were weighed and various observations, like mortality, behavior, injury, or any signs of illness were conducted once daily during the period. For subacute study, four groups of 6 animals (Male rats) received distilled water (control), and 100, 200 and 400 mg/kg of freshly-prepared extracts, respectively, every 24 h orally for 28 days. At the end of each study, hematological analysis and biochemical parameters were evaluated. Histopathological examination of vital organs (Liver and kidney) of the animals were taken for gross findings, compared to controls. There was no significant difference ($p > 0.05$) observed in the relative organs, body weights, hematological, biochemical parameters, and gross abnormalities, compared to the control. No mortality was recorded. Therefore, analysis of results may lead to the conclusion that the medium-term oral administration of the *Euphorbia hirta* leaves extract (EHLE) for 28 days does not cause toxicity. On the basis of acute and sub-acute toxicity studies, the minimal effective dose of 200mg/kg is taken for efficacy (anti-cancer) studies.

Keywords: *Euphorbia hirta* leaves extract, Acute and subacute toxicity; Biochemical analysis; Hematological parameters; Histopathology

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INTRODUCTION

Medicinal plants have been used in all civilizations and cultures; consequently, they have all the time performed an essential purpose globally in health-care systems. In mainly emergent nations, the native procedures of herbal management are components of the traditions and the principal method of remedial treatment. This remediation' s, with a significant scope of efficacy, are collectively established, cheaply feasible and, typically, are the simply accessible foundation (Patil *et al*., 2010). Plants synthesize a variety of metabolites, some of which may be beneficial or potentially toxic to mankind. Also, it has been true that pharmaceutical drugs may be therapeutic at one dose and toxic at another. In order to ensure safety, there must be a study to show safety profiles of herbs claimed to be beneficial to humans and the animals before deciding to use them (Dias and Takahashi, 1994; Nath *et al.,* 2015). This raises concerns about the potential toxic effect resulting from chronic use of such medicinal plants. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used clinically or preclinically, is a crucial part of its assessment of potential toxic effects.

The acute and subacute toxicity test is a method based on an evaluation of the harmlessness and safety of chemicals, as well as an analysis of their mode of action. Acute and subacute systemic toxicity studies are used for hazard disclosure and risk management in the context of the production, handling and use of chemicals. Acute toxicity is a single-dose test that identifies symptoms and the extent to which toxicity affects animals. Subacute toxicity studies at repeated doses can be carried out after obtaining preliminary information from acute toxicity tests, which provide information about the animal' s target tissue or organ (OECD, 2008a). The objective of the present study is to evaluate the acute and subacute toxicity of the hydro-ethanolic extract of *Euphorbia hirta* leaves.

MATERIALS AND METHODS

Animals

Acute toxicity study carried out accordance with The Organization for Economic Cooperation and Development (OECD) guidelines for the Testing of Chemicals. Male albino rats of Wistar strain approximately weighing 180-200gms were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hrs light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experimental protocols were approved (Approval number: XXV/VELS/PCO/L/14/2000/CPCSEA/IAEC/09.10.2021) by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Preparation of extract

10grams of *Euphorbia hirta* leaves powder were used for extraction. Extraction was performed with cold extraction using the maceration method into hydro-ethanol (70%) solvent for 24 hours using the " intermittent shaking" method to obtain an extracts. The extracts were filtered using Whatman filter No 1 paper and filtrate was used for toxicity studies.

Acute toxicity studies

Albino rats were randomly assigned into two groups of each six rats. Group 1 is control group, fed daily with only normal laboratory diet and water. Group II was treated with hydro-ethanol extract of *Euphorbia hirta* leaves at a single dose of

2000 mg/kg body weight for 14 days through an oral needle following a period of 10hrs fasting. All animals were maintained on standard laboratory diets with water *ad libitum*. After administration of the extract, animals were monitored continuously for every two hours for a day to detect acute changes in behavioral responses, spontaneous activity, irritability, corneal refex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.

Sub-acute toxicity studies

Albino rats were randomly assigned into four groups of each six rats. Group 1 is the control group, fed daily with only a normal laboratory diet and water. Group 2, Group 3 and Group 4 were treated with hydro-ethanol extract of 100, 200 and 400mg/kg of *Euphorbia hirta* leaves extract (EHLE) respectively. The rats were administrated *Euphorbia hirta* leaves extract orally daily for 28 days.

Group I: Normal saline (0.5ml).

Group II: *Euphorbia hirta* leaves extract 100mg/kg of body weight.

Group III: *Euphorbia hirta* leaves extract 200mg/kg of body weight.

Group IV: *Euphorbia hirta* leaves extract 400mg/kg of body weight.

Collection of samples

At the end of 14 days, overnight-fasted rats were sacrificed by cervical dislocation and blood was removed to obtain plasma and serum for analysis of various biochemical parameters, blood samples were used for the analysis of haematological parameters. In addition, the liver and kidney were rapidly removed, cleaned (using ice-cold saline), homogenized (in 0.25 M sucrose and 0.1 M Tris-HCl buffer solution, pH 7.4), centrifuged (at 3000 rpm for 10 min), and the supernatant was used for detection of various oxidative stress biomarkers. At the same time, kidney and liver tissues were carefully excised for histopathological examination.

Biochemical analysis

Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). RBC, RBC counted and PCV by the method of Ochei and Kolhatkar, (2000). Protein was estimated by the method of Lowry *et al.* (1951). Albumin was estimated by the method of Rodkey (1965). The serum total bilirubin was estimated by the method of Malloy and Evenlyn (1937). The serum SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King' s (1954). Urea was estimated by the method of Natelson (1957). Serum creatinine was carried out by alkaline picrate method of Boneses and Taussy (1954).Cholesterol and HDL were assayed by Allain *et al* (1974). Triglyceride was assaed by Werner *et al* (1981) method. HDL cholesterol was determined by the method of Allain *et al.* (1974). LDL cholesterol was calculated as per Friedewald' s (1972) equation. Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was assayed by the procedure of Kakkar *et al* (1984). The activity of catalase was determined by the method of Beers and Sizer (1952). Reduced glutathione was determined by method of Moron *et al* (1979). The activity of glutathione peroxidase was estimated by the method of Rotruck *et al* (1973). The level of ascorbic acid was assayed by the method of Omaye *et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980). Serum sodium was estimated by colorimetric method of Maruna and Trinders (1958). Serum potassium was estimated by method of Maruna (1957).

Histopathological studies

The organs, namely liver and kidney were carefully excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study Histological studies carried out by the method of Ochei and Kolhatkar, (2000). Slides were viewed on a photographic microscope to find out the histological changes in liver and kidney.

Statistical analysis

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant (^{NS}P>0.05).

RESULTS AND DISCUSSION

Acute toxicity study

General appearance and behavioral observations

Acute oral toxicity studies of herbal medicines are essential to identify the safety and the determination of dose level that could be used subsequently. It also helps in the investigation of the therapeutic index of drugs and xenobiotic. The clinical signs and symptoms exerted by drugs on vital body organs are considered as principal observations among toxicity indicators. On the 14 days treatment of hydro-ethanolic extract of *Euphorbia hirta* leaves extract, the rats were survived throughout the entire study period. No treatment-related toxic symptoms or mortality were observed after oral administration of tested extract. None of these rats had shown any abnormal behavioral responses in any dose range. There was no change in behavioral responses, spontaneous activity, irritability, corneal refex, tremors, convulsion, salivation, diarrhea and lethargy if any when compared to control group (Table 1).

Table 1: Acute toxicity study of extracts in wellness parameters of rats

Observations	Animal group	
	Control rat	Extract (2,000 mg/kg body wt)
Consciousness	+	+
Grooming	-	-
Touch response	+	+
Sleeping duration	+	+
Movement	+	+
Gripping strength	+	+
Righting re flex	+	+
Food intake	+	+
Water consumption	+	+
Tremors	-	-
Diarrhea	-	-
Hyper activity	-	-

Pinna reflex	+	+
Corneal reflex	+	+
Salivation	+	+
Skin color	+	+
Lethargy	-	-
Convulsion	-	-
Morbidity	-	-
Sound response	+	+

Note: + indicate normal - indicate absent

There were generally no significant differences observed in the relative body weights in this study (Table 1). From the present study it was seen that there was no significant change in the haematological and biochemical parameters in the *Euphorbia hirta* extract treated group compared to the normal control group (Tables 2 to 7). Gross examination at autopsy and histopathological evaluations of liver and kidney organs stained with haematoxylin and eosin revealed no significant differences (Figure 1). Acute oral toxicity effects of hydro-ethanolic extract of *Euphorbia hirta* leaves extract on rats were studied and no animal deaths in rats receiving 2000 mg/kg of extract. No sign of toxicity was observed in the wellness parameters during the 14-days observation period. Therefore, the approximate acute lethal dose (LD_{50}) of *Euphorbia hirta* extract in rat was estimated to be higher than 2000 mg/kg.

Table 2: Effect of *Euphorbia hirta* leaves extract on animal and organ weight of control and experimental rats (Acute toxicity)

Parameters	**Group I (Normal)**	**Group II (2000mg/kg)**	***P* Value**
Initial day (gm)	184.16±3.76	185.00±4.47	$^{NS}P>0.05$
Final day (gm)	196.66±7.52	198.33±6.83	$^{NS}P>0.05$
Liver weight (gm)	5.32±0.28	5.56±0.36	$^{NS}P>0.05$
Kidney weight (gm)	1.49±0.20	1.52±0.22	$^{NS}P>0.05$
Acute Oral Toxicity Effects (N = 6)			
Animal live (Nos.)	6±0	6±0	$^{NS}P>0.05$
Animal dead (Nos.)	Nil	Nil	
% of Mortality	Nil	Nil	

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. $*P<0.05$ significant and NS non-significant ($^{NS}P>0.05$).

Biochemical Analysis

The effects of acute administration of *Euphorbia hirta* leaves extract (EHLE) on biochemical parameters are presented in Table 3. The EHLE had no effect on serum electrolytes (Na and K). The kidney

function parameters, like urea, and creatinine, did not reveal any significant changes. No statistically significant differences in the liver function parameters like alanine aminotranferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were observed. Additionally, no relevant changes were found in total protein, albumin, and globulin content (Table 3).

Table 3: Effect of *Euphorbia hirta* leaves extract on Liver marker profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Protein (mg/dl)	7.40±0.24	7.45±0.13	$^{NS}P>0.05$
Albumin (mg/dl)	4.12±0.14	4.24±0.54	$^{NS}P>0.05$
Bilirubin (mg/dl)	0.69±0.03	0.71±0.05	$^{NS}P>0.05$
ALT (IU/L)	28.35±0.69	28.74±0.73	$^{NS}P>0.05$
AST (IU/L)	51.79±1.06	51.84±0.94	$^{NS}P>0.05$
ALP (IU/L)	50.69±1.14	50.71±1.83	$^{NS}P>0.05$
Creatinine (mg/dl)	0.90±0.03	0.91±0.03	$^{NS}P>0.05$
Urea (mg/dl)	24.93±1.97	25.13±1.58	$^{NS}P>0.05$
Sodium (Meq/L)	152.42±6.69	151.92±4.61	$^{NS}P>0.05$
Potassium (Meq/L)	4.38±0.22	4.51±0.31	$^{NS}P>0.05$

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. $*P<0.05$ significant and NS non-significant ($^{NS}P>0.05$).

Hematological Analysis

The effects of acute administration of EHLE on haematological parameters (Hb, RBC, WBC, PCV, MCV, MCH and MCHC) are shown in Table 4. Administration of EHLE (2000mg/kg) did not cause any significant difference in most of the hematological parameters when compared with the control group.

Table 4: Effect of *Euphorbia hirta* leaves extract on hematology profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Hb (gm/dl)	13.79±0.30	13.85±0.38	$^{NS}P>0.05$
RBC ($\times10^6$ /mm^3)	4.47±0.38	4.57±0.26	$^{NS}P>0.05$
WBC ($\times10^3$ /mm^3)	7.36±0.35	7.63±0.36	$^{NS}P>0.05$
PCV (%)	25.64±1.59	26.42±2.02	$^{NS}P>0.05$
MCV (famato litre)	57.79±7.21	57.90±5.53	$^{NS}P>0.05$
MCH (pico gram)	31.02±2.59	30.38±2.20	$^{NS}P>0.05$
MCHC (%)	53.99±3.89	52.67±3.76	$^{NS}P>0.05$

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant ($^{NS}P>0.05$).

Oxidative stress markers

The effects of acute administration of EHLE on oxidative stress parameters (MDA, SOD, Catalase, GPx, GSH, Vitamin C and E) are shown in Table 5. Administration of EHLE (2000mg/kg) did not cause any significant difference in all of the oxidative stress parameters when compared with the control group.

Table 5: Effect of *Euphorbia hirta* leaves extract on oxidative stress profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
MDA (nmol of MDA formed/L)	7.39±0.17	7.48±0.43	$^{NS}P>0.05$
SOD (U/ml)	4.35±0.13	4.31±0.25	$^{NS}P>0.05$
CAT (U/ml)	6.51±0.36	6.59±0.25	$^{NS}P>0.05$
GPx (U/ml)	8.44±0.28	8.55±0.22	$^{NS}P>0.05$
GSH (mg/dl)	4.73±0.19	4.62±0.25	$^{NS}P>0.05$
Vit-C (µg/dl)	3.90±0.18	3.80±0.32	$^{NS}P>0.05$
Vit-E (µg/dl)	2.44±0.19	2.39±0.21	$^{NS}P>0.05$

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. **P*<0.05 significant and NS non-significant ($^{NS}P>0.05$).

Table 6 showed the effect of *Euphorbia hirta* leaves extract on lipids profile of control and experimental rats (Acute toxicity). There is no significant (*P*>0.05) changes were observed lipid profile as cholesterol, triglyceride, HDL and LDL on acute administration of EHLE.

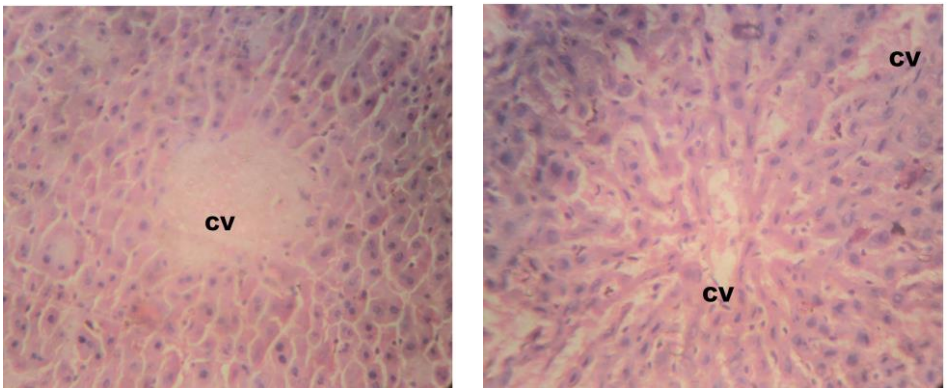
Table 6: Effect of *Euphorbia hirta* leaves extract on lipids profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	*P* Value
Cholesterol (mg/dl)	92.84±1.81	93.01±1.43	$^{NS}P>0.05$
Triglyceride (mg/dl)	113.83±4.76	114.69±4.37	$^{NS}P>0.05$
HDL (mg/dl)	33.09±2.60	32.45±1.21	$^{NS}P>0.05$
LDL (mg/dl)	36.98±3.86	37.61±1.92	$^{NS}P>0.05$

Values are expressed as Mean ± SD for six rats. Data was calculated by student *t*-Test (Independent sample, *P* value two tail) using SPSS ver. 20. Statistically significant level 0.05. *$P<0.05$ significant and NS non-significant ($^{NS}P>0.05$).

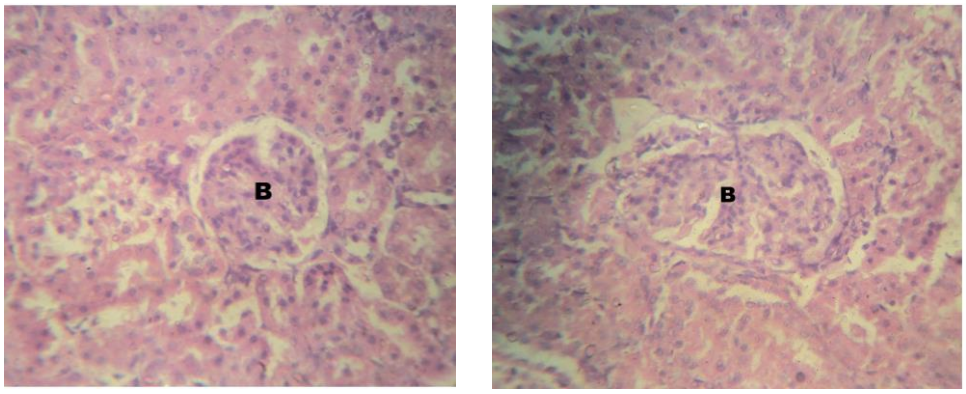
Histological observation

The microscopic examination revealed that none of the organs from the *Euphorbia hirta* leaves extract treated rats showed no alteration in cell structure or any unfavourable effects when viewed under the light microscope using magnification (10x40x) powers. No pathologies were recorded in the histological sections of the vital organs (liver and kidney) of the experimental group (Plate 1) and similar to the control group.



Group I (Normal) **Group II (2000mg/kg)**

Liver histopathology (10 × 40X)



Group I (Normal) **Group II (2000mg/kg)**

Kidney histopathology (10 × 40X)

CV: Central veins of liver

B: Bowman's capsule of kidney

Plate 1: Histopathology of liver and kidney in control and drug treated animal shows normal architecture

Sub-acute toxicity studies

A body weight was determined on initial (0) day and 28th days and the organs liver and kidney weight of four groups. The first one is the control, Group I is EHLE of 100 mg/kg, II EHLE of 200 mg/kg, and the last group, named as Group III, is EHLE of 400 mg/kg. No significant ($p > 0.05$) changes in the body weight were observed (Table 7)

Table 7: Effect of *Euphorbia hirta* on animal and organ weight of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
Initial day (gm)	186.20±3.13[a]	186.66±4.12[a]	185.01±3.48[a]	186.18±4.70[a]
Final day (gm)	234.16±3.78[a]	234.73±3.44[a]	231.21±4.18[a]	230.55±5.07[a]
Liver weight (gm)	5.55±0.26[a]	5.56±0.24[a]	5.39±0.09[a]	5.34±0.08[a]
Kidney weight (gm)	1.68±0.13[a]	1.70±0.12[a]	1.58±0.09[a]	1.53±0.09[a]
Sub-acute Oral Toxicity Effects (N = 6)				
Animal live (Nos.)	6±0	6±0	6±0	6±0
Animal dead (Nos.)	Nil	Nil	Nil	Nil
% of Mortality	Nil	Nil	Nil	Nil

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each

other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

The effect of sub-acute administration of EHLE on liver indices is presented in table 8. A non-significant (p > 0.05) changes observed protein, albumin, bilirubin content and enzymes AST, ALP and ALP activities were observed in 100, 200 and 400mg/kg treated groups as compared with control rats.

Table 8: Effect of *Euphorbia hirta* on Liver profile of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
Protein (mg/dl)	7.47±0.14[a]	7.48±0.10[a]	7.39±0.10[a]	7.37±0.10[a]
Albumin (mg/dl)	4.62±0.11[a]	4.60±0.09[a]	4.50±0.07[a]	4.49±0.10[a]
Bilirubin (mg/dl)	0.71±0.03[a]	0.72±0.04[a]	0.75±0.03[a]	0.76±0.01[a]
ALT (IU/L)	27.51±1.47[a]	27.47±1.51[a]	26.48±1.27[a]	26.03±1.18[a]
AST (IU/L)	51.96±1.62[a]	51.17±1.46[a]	50.19±2.16[a]	50.13±1.72[a]
ALP (IU/L)	48.96±1.97[a]	48.84±1.68[a]	46.61±2.50[a]	46.44±1.31[a]

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

Sub-acute administration of EHLE in the treated rats caused no significant difference (p > 0.05) in the kidney parameters (creatinine, sodium, potassium, and urea levels) investigated (Table 9).

Table 9: Effect of *Euphorbia hirta* leaves extract on kidney profile of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
Creatinine (mg/dl)	0.911±0.028[a]	0.918±0.027[a]	0.926±0.028[a]	0.936±0.024[a]
Urea (mg/dl)	23.61±1.99[a]	23.77±1.46[a]	24.18±1.93[a]	25.79±1.22[a]
Sodium (Meq/L)	153.89±2.59[a]	154.60±2.96[a]	155.04±5.29[a]	156.92±5.42[a]
Potassium (Meq/L)	4.51±0.12[a]	4.52±0.18[a]	4.66±0.11[a]	4.69±0.35[a]

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

The effects of sub-acute administration of EHLE on haematological parameters (Hb, RBC, WBC, PCV, MCH, MCHC and MCV) are shown in Table 10. Daily administration of EHLE for 28 days did not cause any significant difference in most of the hematological parameters when compared with the control group.

Table 10: Effect of *Euphorbia hirta* on hematology profile of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
Hb (gm/dl)	13.73±0.20[a]	13.64±0.12[a]	13.57±0.24[a]	13.55±0.22[a]
RBC ($\times10^6$ /mm^3)	4.55±0.15[a]	4.54±0.12[a]	4.52±0.13[a]	4.50±0.08[a]
WBC ($\times10^3$ /mm^3)	7.46±0.13[a]	7.49±0.18[a]	7.55±0.07[a]	7.58±0.10[a]
PCV (%)	26.03±1.38[a]	25.62±1.33[a]	25.07±1.10[a]	24.35±1.03[a]
MCV (famato litre)	57.33±4.89[a]	56.38±2.44[a]	55.46±2.06[a]	54.04±2.51[a]
MCH (pico gram)	30.19±1.13[a]	30.04±0.85[a]	30.03±1.01[a]	30.06±0.75[a]
MCHC (%)	52.87±3.10[a]	53.37±2.79[a]	54.21±2.40[a]	55.74±3.10[a]

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

The effects of sub-acute administration of EHLE on haematological parameters (MDA, superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione (GSH), Vitamin C and E) are shown in Figure 5. Daily administration of EHLE for 28 days did not cause any significant difference in MDA parameters when compared with the control group. The enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase activities of both control and EHLE -fed rats are indicated in Figure 5. The results indicated no significant difference in these enzymes activity after sub-acute treatment with different doses of EHLE for 28 days when compared to control set. The non-enzymatic antioxidants reduced glutathione (GSH), Vitamin C and E content of both control and EHLE -fed rats are indicated in table 11. The results indicated no significant difference in non-enzymatic antioxidants after sub-acute treatment with different doses of EHLE for 28 days when compared to control set.

Table 11: Effect of *Euphorbia hirta* on oxidative stress profile of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
MDA (nmol of MDA formed/L)	7.30±0.10[a]	7.29±0.12[a]	7.25±0.08[a]	7.35±0.12[a]
SOD (U/ml)	4.78±0.08[a]	4.79±0.06[a]	4.81±0.06[a]	4.85±0.08[a]
CAT (U/ml)	6.77±0.08[a]	6.79±0.11[a]	6.81±0.12[a]	6.82±0.3[a]
GPx (U/ml)	8.69±0.17[a]	8.70±0.07[a]	8.73±0.16[a]	8.71±0.17[a]
GSH (mg/dl)	4.72±0.12[a]	4.73±0.09[a]	4.76±0.08[a]	4.75±0.15[a]
Vit-C (µg/dl)	4.32±0.09[a]	4.33±0.08[a]	4.35±0.09[a]	4.31±0.14[a]
Vit-E (µg/dl)	2.36±0.09[a]	2.37±0.07[a]	2.39±0.08[a]	2.33±0.07[a]

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

Effects of sub-acute administration of EHLE on the lipid profile of experimental rats are shown in table 12. EHLE treatment resulted in non-significant changes (p>0.05) in TC and TG concentrations as compared to control rats. EHLE treatment at 100, 200 and 400 mg/kg both resulted in non-significant changes (p>0.05) in HDL and LDL levels when compared to the control.

Table 12: Effect of *Euphorbia hirta* on lipids profile of control and experimental rats (Sub-acute toxicity)

Parameters	Group I (Normal)	Group II (100mg/kg)	Group III (200mg/kg)	Group IV (400mg/kg)
Cholesterol (mg/dl)	95.14±0.97[a]	95.74±1.56[a]	96.51±1.47[a]	96.65±2.02[a]
Triglyceride (mg/dl)	112.58±5.26[a]	112.74±5.67[a]	115.02±3.89[a]	117.86±4.40[a]
HDL (mg/dl)	31.78±0.69[a]	31.89±1.40[a]	32.05±1.44[a]	32.12±0.90[a]
LDL (mg/dl)	40.84±1.39[a]	41.30±2.83[a]	41.45±1.94[a]	40.96±1.80[a]

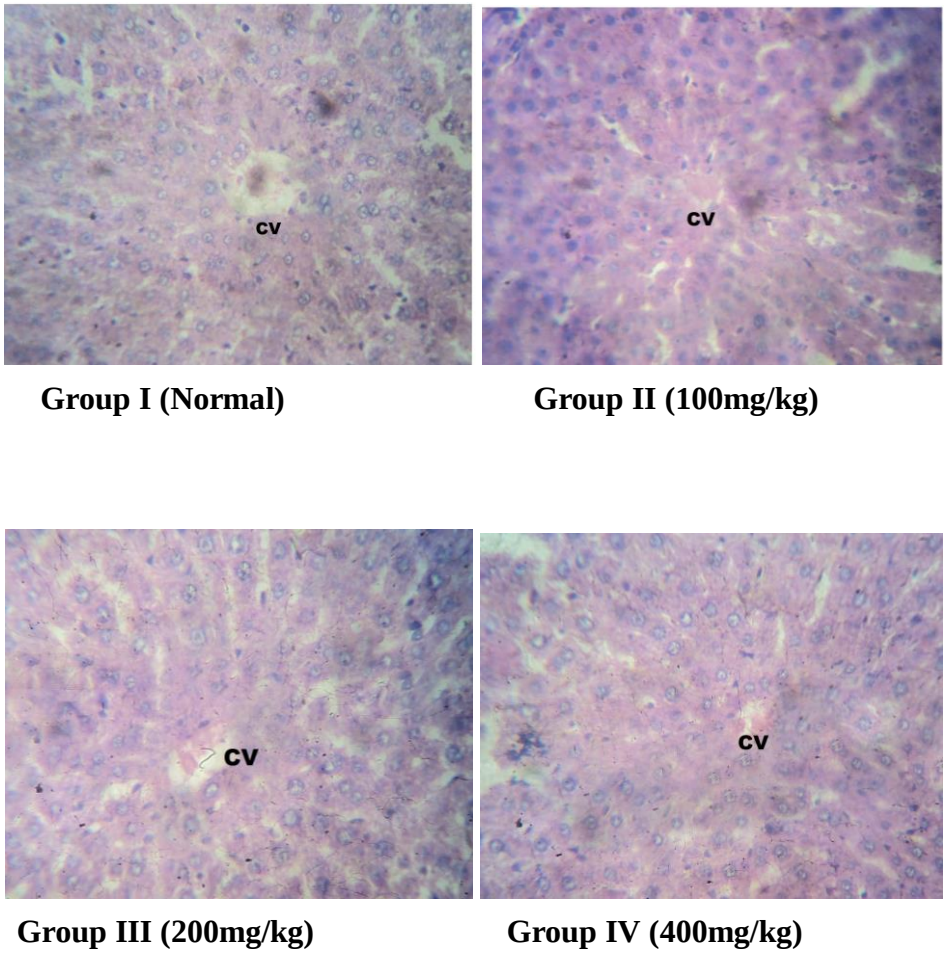
Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant (P<0.05) from each other group and same letter was statistically non-significant (P>0.05) were comparison by ANOVA, Duncan' s multiple range test (DMRT), significant level alpha 0.05.

Histopathological studies

Histological studies revealed no abnormalities in liver and kidney tissues in extract-treated rats. Thus, the histopathological evaluation indicated that the extract did not have any adverse effects on the morphology of the tissues and these observations supported the biochemical results mentioned. Therefore, it is concluded that the extract did not produce any toxic effects in male albino rats.

Liver

Histological studies of subacute toxicities of the liver in the control and experimental group of rats observed that the liver cells are arranged into lobules in both control and experimental groups (Plate 2). Liver cells of hepatocytes are arranged flat. A discontinuous layer of cells lines the sinusoids. The central vein is lined by epithelial cells' predominant nucleus. There are no abnormalities in the histology of the liver were observed in all the dose treated groups.



Group I (Normal) **Group II (100mg/kg)**

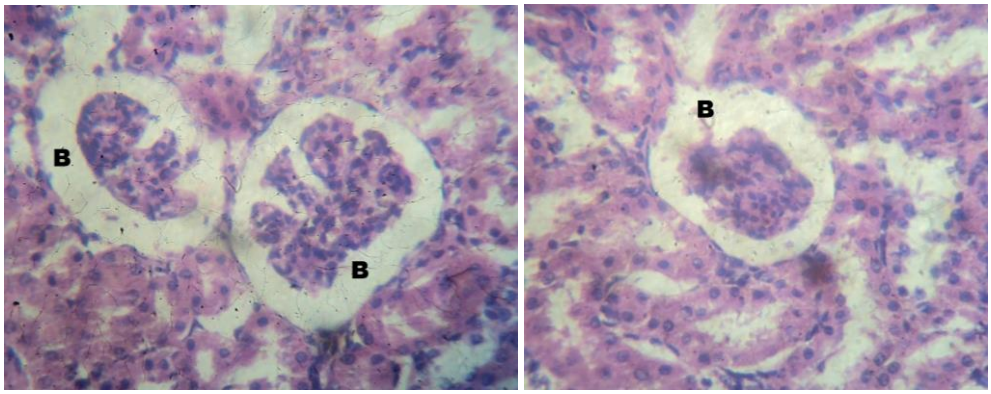
Group III (200mg/kg) **Group IV (400mg/kg)**

CV: Central veins of liver

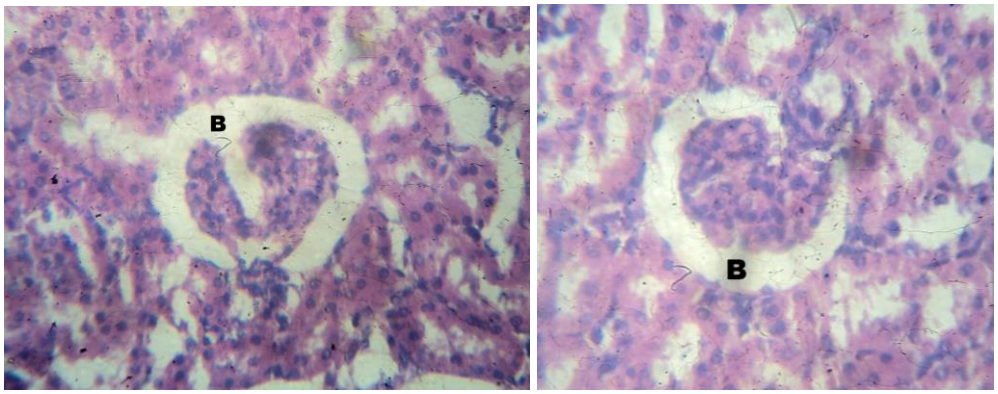
Plate 2: Histopathology of liver (10 × 40X) in control and experimental rats (Sub acute)

Kidney

The normal architecture of kidneys was observed in both the control and experimental group of rats (Plate 3). The Renal corpuscles in the centre display a slight shrinkage artifact and thus clearly demonstrate Bowman' s space. The renal corpuscles are surrounded by cross-sections of proximal convoluted tubules, distal convoluted tubules and macula densa. There are no abnormalities in the histology of the kidneys were observed in all the dose treated groups.



Group I (Normal) **Group II (100mg/kg)**



Group III (200mg/kg) **Group IV (400mg/kg)**

B: Bowman's capsule of kidney

Plate 3: Histopathology of kidneys (10 × 40X) in control and experimental rats (Sub acute)

DISCUSSION

Plants synthesize a variety of metabolites, some of which may be beneficial or potentially toxic to mankind. Also, it has been true that pharmaceutical drugs may be therapeutic at one dose and toxic at another. In order to ensure safety, there must be a study to show safety profiles of herbs claimed to be beneficial to humans and the animals before deciding to use them (Eran *et al.*, 2016). Therefore, scientific knowledge towards oral toxicity is much needed, which will not only help identify doses that could be used subsequently, but also to reveal the

possible clinical signs elicited by agents under investigation. Regardless of the pharmacological benefits of the *Euphorbia hirta* , detailed knowledge about subacute toxicity of this medicinal plant is lacking. Hence, the current study was undertaken to evaluate and focus on the acute and subacute toxicity of *Euphorbia hirta* leaves in an animal model.

Acute toxicity

Currently, medicinal plants are known for their pharmacological effects. However, less is known about the potential toxicity of their biologically active substances. A study of acute toxicity examines the adverse effects that occur in the short term after the administration of a single dose of a tested product. These tests are generally conducted on rodents and are carried out at the beginning of the development of a new substance in order to provide information on its toxicity (Yang et al., 2019). Moreover, if a high dose (e.g., 2000 mg/kg) is found to be survivable, no further acute testing will be conducted (NRC, 2006). In this study, *Euphorbia hirta* leaves at a dose of 2000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation. There were no significant changes in the weight and the organs of the rats. The bone marrow is a major location for novel blood cell manufacture and a vulnerable tissue targeted by toxic compounds in the hematopoietic system (Kifayatullah *et al*., 2015). The hematological parameters between control and treated groups showed the extract was non-toxic to the haemopoietic system. The liver biomarkers are specific tools in examining liver toxicity during drug biotransformation (Mukinda & Syce, 2007).

Additionally, most of the biochemical parameters were not altered. No relevant changes were found in levels of ALT, AST, ALP, creatinine, which are good indicators of liver and kidney functions. No gross lesions were found in histopathology examinations. Kidney disease can be detected by measurements of kidney indices like creatinine, uric acid, urea, potassium, sodium and chlorides and their normal levels reflect a reduced likelihood of renal problems (Dalle *et al.*, 2006). No statistically significant differences in the liver function parameters like ALT, AST, ALP were observed. In the present study, no significant alterations in ALT, AST, ALP, creatinine, urea, potassium, sodium. Additionally, no relevant changes were found in total protein, albumin and globulin levels in *Euphorbia hirta* extract fed rats when compared to the control was observed. This indicates that the functional integrity of the kidney was not compromised after treatment with graded doses of the extract. Similarly, *Euphorbia hirta* leaves extract oral administration non-significant changes ($p>0.05$) in total cholesterol (TC), serum triglyceride (TG), HDL and LDL levels were observed.

Effects of *Euphorbia hirta* extract on lipid peroxidation were evaluated by measuring MDA, SOD, Catalase, GPx, GSH, Vitamin C and E enzymatic and non-enzymatic activities. Elevation in oxidative stress in biological entities thereby interfering with the system' s antioxidant defence mechanisms (Pajero *et al.*, 2002). However, in this study, *Euphorbia hirta* administration at 2000 mg/kg bw did not cause any significant difference in all of the oxidative stress parameters when compared with the control group. Since no toxic stress were found during the acute toxicity study, further study was conducted to evaluate the subacute toxicity of *Euphorbia hirta* leaves extract up to 28 days to prepare inclusive toxicological records on this plant.

Subacute toxicity

Subacute studies provide information on dosage regimens, target organ toxicity and identify observable adverse effect that may affect the average life span of experimental animals. Consequently, in this study, the leaves of *Euphorbia hirta*

were evaluated in rats at doses of 100, 200 and 400 mg/kg for 28 days. The body weight changes serve as a sensitive indication of general health status of animals (Hilaly *et al.*, 2004). After 28 days of treatment of the extract, all the animals exhibited a normal increment in body weight. It can be stated that leaves of *Euphorbia hirta* did not interfere with the normal metabolism of animals. The significant increment in food and water intake is considered as being responsible for augmentation in body weight gain. Similarly, no significant changes in the weight of the liver and kidney were observed, suggesting that administration of *Euphorbia hirta* leaves at subacute oral doses produces no effect on the normal growth. The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it correlates well with histopathological changes (Kluwe, 1981). The results of this study revealed no significant changes in the relative organ weight of control and treated groups which showed that none of the organs were adversely affected, nor showed any signs of toxicity throughout the study.

The haematological parameters can be used to determine the blood relating functions of plant extract. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals. The extract indicated a significant difference on the RBC indices which suggested that the *Euphorbia hirta* leaves does not affect erythropoiesis, morphology, or osmotic fragility of red blood cells (Odeyemi *et al.,* 2009). WBC' s are the first line of cellular defines that respond to infectious agents, tissue injury, or any inflammation. Furthermore, significant changes were observed in PCV, MCH, MCHC and MCV in the leaves of *Euphorbia hirta* suggesting that the extract might not have exerted challenge on the immune system of the animals.

Evaluation of biochemistry was done to monitor the any alterations in renal and hepatic functions on treatement with extract. Total protein, albumin, globulin, and total bilirubin did not affecting the hepatocelluar and secretory functions of the liver. The non-significant in the levels of ALT, AST, ALP, creatinine, sodium, potassium and urea which are good indicators of liver and kidney functions (Olorunnisola, *et al.,* 2012), suggests that sub-chronic administration of extract did not alter hepatocytes and kidneys of normal metabolism of the animals. These observations were further confirmed by the histological assessment of the liver and kidney organs. Based on the results found in our study, we concluded that leaves of *Euphorbia hirta* hydro-ethanol extract was safer and non-toxic and could be well used for pharmacological and therapeutic purposes. The results indicated no significant difference in enzymatic and non-enzymatic antioxidants after sub-acute treatment with different doses of hydro-ethanol extract of *Euphorbia hirta* leaves for 28 days when compared to control set.

Euphorbia hirta leaves effects on lipid peroxidation were evaluated by measuring malondialdehyde (MDA) levels, enzymatic antioxidants SOD, catalase GPx and non-enzymatic antioxidants GSH, Vitamin C and E. Reduction of enzymatic and non-enzymatic antioxidants and increases in MDA levels connotes an elevation in oxidative stress in biological entities thereby interfering with the system' s antioxidant defence mechanisms (Pajero *et al.,* 2002). However, in this study, EHLE administration at 100, 200 and 400 mg/kg bw non-significantly increased ($p>0.05$) the MDA and antioxidant levels in comparison to the control.. This suggests that hydro-ethanol extract of *Euphorbia hirta* leaves possesses beneficial properties due to its content of phytochemicals, in boosting the

body' s defence. *Euphorbia hirta* leaves extract oral administration non-significant changes ($p>0.05$) in total cholesterol (TC), serum triglyceride (TG), HDL and LDL levels were observed. This study recommended that EHLE administration may prove effective in the management of cardiovascular ailments, diabetes as well as deregulated blood pressure. Many researchers supported the biochemical parameters, haematological, oxidative stress parameters, lipid profile and histological studies of present study (Osafanme et al., 2017; Osafanme et al., 2020; Ashutosh et al., 2022).

Conclusion

In view of these results, leaves of *Euphorbia hirta* extract are non-toxic in all doses considered in this study and did not created any obvious signs in the acute and subacute oral toxicity studies. All the haematological, oxidative stress parameters, lipid profile, histological and biochemical parameters did not altered on experimental period. Moreover, the information of acute and subacute studies of toxicity on leaves of *Euphorbia hirta* leaf extract was acquired so that to augment the assurance in its well-being to humans for the employment in the preparation of medicaments. These findings indicate that the no observed adverse effect level of *Euphorbia hirta* leaf extract was greater than 2000 mg/kg/day. On the basis of toxicity studies, the minimal effective dose of 200mg/kg is taken for efficacy studies.

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