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Acute and sub-chronic 28-days oral toxicity study of aqueous extract of *Euphorbia tirucalli* stem bark (Euphorbiaceae) in Wistar Rats.

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ABSTRACT

Euphorbia tirucalli (Euphorbiaceae) is a plant used in traditional medicine for the treatment of various diseases. This study aimed to evaluate the acute and sub-chronic oral toxicity of aqueous extract of *Euphorbia tirucalli* stem bark in rats. In acute study, rats were given a single administration of the extract at doses of 250, 500, 1000, 2000, 4000 and 8000 mg/kg; and they were observed for seven days. In sub-chronic study, the doses of 125, 250 and 500 mg/kg were administrated for 28 days. Food and water intake, body and organ weights, biochemical, hematological and histological parameters were monitory during the study. The acute study showed that LD50 of the extract is greater than 8000 mg/kg. In sub-chronic study, no significant variation in body and organ weights, food and water intake, total cholesterol, total and direct bilirubin, total protein, ASAT was noted. The extract significantly reduced the activity of ALAT; it also reduced serum creatinine level ($p<0.05$) and increased ($p<0.01$) urinary creatinine at 250 mg/kg. A significant increased in white blood cells was observed. Histological analysis revealed no abnormalities in the structure and morphology of the heart, liver and kidneys. The results suggest that *Euphorbia tirucalli* may have relatively low toxicity.

Keywords: *Euphorbia tirucalli*, acute toxicity, sub-chronic toxicity, rats.

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INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern medicines in a number of studies[1]. The World Health Organization (WHO) reported that 80 % of emerging world's population relies on traditional medicine for therapy[2]. Plants which are commonly used in traditional medicine are frequently promoted as natural and, therefore, harmless[3]. However, the latest surveys have indicated that many medicinal plants also showed adverse effects[4]. This raises concerns about the potential toxic effect resulting from the 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 assessment of its potential toxic effects.

Euphorbia tirucalli (ET) is one of the 8000 species within the family Euphorbiaceae. Recently, there is increasing interest focusing on this plant because of the reports on its various medicinal properties: antibacterial, antioxidant, insecticidal, larvicidal, molluscicide, anti-mutagenic, proteolytic, anti-arthritic, anti-herpetic, antifungal activities and hepatoprotective effects[5]. Despite its wide usage in men, the data on its toxicity is lacking. Thus, the aim of this study was to evaluate the acute and sub-chronic oral toxicity of AEET stem bark in Wistar rats.

MATERIALS AND METHODS

Plant material

Fresh stem barks of ET were collected in November 2015 at Foumbot (West Cameroon). The plant was identified in National Herbarium of Cameroon on the reference number N° 6608/HNC.

The air-dried stem barks were ground into a fine powder which was used for the extraction.

Aqueous extract preparation

The aqueous extract was obtained by decoction of one Kilogram (1kg) of the dried powdered plant in 10 L of distilled water for 20 minutes. After filtration of the preparation, the filtrate was concentrated by evaporating water at 40°C in a drying oven for 72 hours to afford 46.90 g of the aqueous extract.

Animals

Both Male and female Wistar albino (weighing 135 - 165 g) were used in this study. They were segregated according to the gender and then bred and housed in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang/Cameroon, at room temperature, with adequate ventilation and under a naturally illuminated environment. The animals were maintained on standard diet and provided with water *ad libitum*, but fasted overnight prior to oral administration of the extract.

Acute test

The acute toxicity of AEET was evaluated in rats according the World Health Organization (WHO) guideline[6], where the limit test dose of 8000 mg/kg was used. The rats were randomly distributed into one control group and six treated groups, containing 6 animals per group. They were acclimatized for seven days to the laboratory conditions before the experiment. AEET was administered orally using intragastric tube at doses of 250, 500, 1000, 2000, 4000 and 8000 mg/kg body weight. The animals were individually observed during the first 30 min after dosing and periodically during the first 24 h following dosing (with the special attention during the first 3 h). Observation once daily was carried out for 7 days. Finally, percentage mortality was determined.

Sub-chronic toxicity test

The Sub-chronic toxicity of the AEET was conducted according to World Health Organization (WHO) guideline [6]. Thirty-two rats (135 - 165 g) were randomly divided into four groups of 8 animals (4 males + 4 females). The first group (GPI), serving as control, received distilled water. Groups II, III and IV received AEET at respective doses of 125, 250 and 500 mg/kg. Treatments were orally administered for 28 consecutive days. The body weight, food and water consumptions were measured weekly during the study period.

Collection of urine

After the last treatment, animals were put individually in the metabolic cages for urine collection. The urine of 24 h was collected and centrifuged at 3000 rpm for 15 min. the supernatant was separated for determination of urine creatinine.

Collection of blood and organ samples

On the 29th day, after overnight fast, the animals were anesthetized by diazepam (10 mg/kg) and ketamine (50 mg/kg) and blood was collected from each rat by the catheterization of the abdominal artery into ethylene diamine tetra acetic acid (EDTA) tubes for hematological analysis and tubes without anticoagulant for biochemical analysis. Following blood collection, organs (heart, liver, lung, and kidney) were quickly removed, cleaned with saline solution (0.9%) and weight for calculation of relative organ weight (ROW). Organ samples (Heart, kidney, and liver) were next fixed in 10% formalin for histopathological examinations.

Hematological parameters

White blood cell (WBC), red blood cell (RBC) and platelets (PLT) counts, lymphocytes, granulocytes and monocytes percentage, hemoglobin (HGB), hematocrit (HTC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and mean corpuscular volume (MCV), were determined using fully automated hematology analyser (PCE-210N).

Biochemical parameters

Blood samples in dry tubes were centrifuged at 3000 rpm for 15 min. Serum was separated and stored at – 20 °C until determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol and serum creatinine by using inmesco kit, total protein according to the methods described by Gornall et al.[7] and total and direct bilirubin by using semi- automatic biochemical analyser (AE-600N, ERMA.Tokyo).

Histopathology

Organs were fixed in 10% formal saline before embedding in paraffin wax. Each organ tissue of heart, kidney and liver was sectioned with micrometer at 5 µm and stained with haematoxylin and eosin (H and E) stain. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Data obtained were analyzed using one way or two ANOVA followed by Turkey's and Bonferroni tests respectively using Graph Pad Prism Version 5.03; the p values < 0.05 were considered significant.

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RESULTS AND DISCUSSION

Acute oral toxicity study

A single oral administration of AEET at doses of 250, 500, 1000, 2000, 4000, 8000 mg/kg does not produce neither mortality, nor visible signs of toxicity in animals during the seven days of observation. This result indicates that lethal dose 50 (LD50) of AEET is greater than 8000 mg/kg bw. According to Singh et al. [8], substances that present a LD50 higher than 8 g/kg via oral route may be considered as practically non-toxic. Therefore, the observations from acute toxicity studies suggest that AEET is relatively non-toxic.

Sub-chronic oral toxicity study

Effects of AEET on body and relative organ weights

Changes in organ weights have been used as indicators of adverse effects of drugs and chemicals [9]. In this study, there was no significant variation in organ weights of treated rats compared to control rats (Figure 1), suggesting that AEET treatment appeared to cause no detectable toxic effects. The body weight changes serve as a sensitive indication of general health status of animals [10]. After 28 days treatment, all animals exhibited a normal weight growth (Figure 1); this indicates that stem barks of ET did not interfere with the normal metabolism of rats.

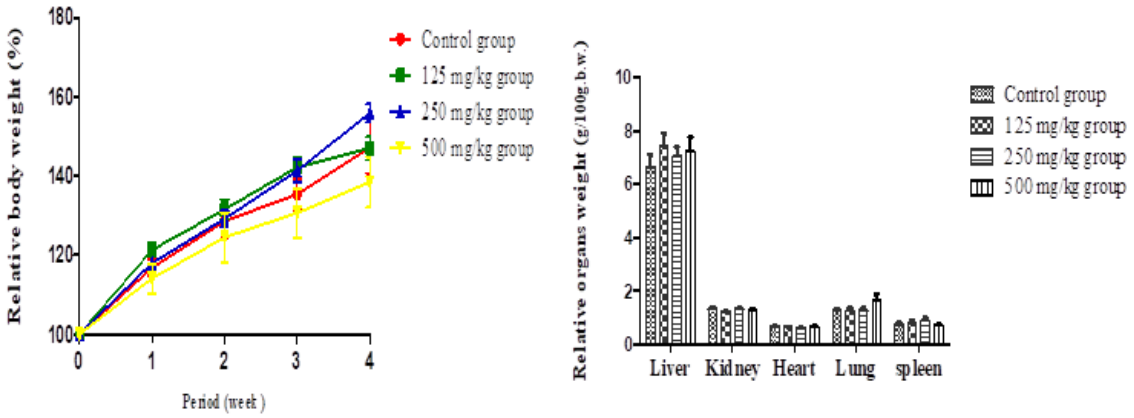

Figure 1: Body and organ weight of rats in the sub-chronic toxicity study of *Euphorbia tirucalli*. n=8; data are presented as mean ± SEM.

Effects of AEET on water and food intake

Figures 2 and 3 show that AEET did not have any effect on food and water intake, suggesting that it does not alters the functioning of hypothalamic receptors and the osmoreceptor which are known to regulate respectively hunger and thirst.

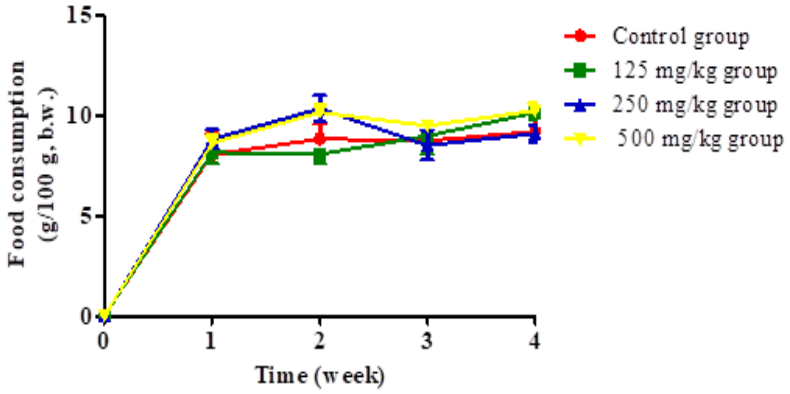

Figure 2: Food consumption of rats in the sub-chronic toxicity study of *Euphorbia tirucalli*. n=8; data are presented as mean ± SEM.

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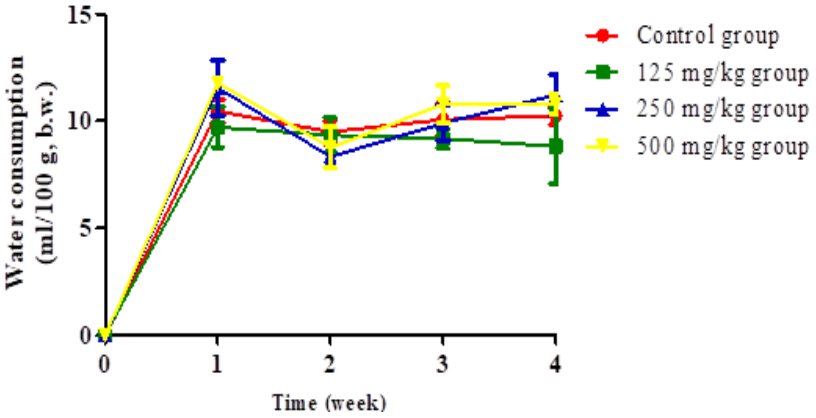

Figure 3: Water consumption of rats in the sub-chronic toxicity study of *Euphorbia tirucalli*. n=8; data are presented as mean ± SEM.

Effects of AEET on hematological and biochemical parameters

The data on table 1 present the effects of *Euphorbia tirucalli* on hematological parameters of rats. Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems [11]. The hematological profile of treated rats showed no significant difference with the control group, except WBC, MO, and LYM which significantly increased ($p<0.05$; $p<0.001$) (table 1). The role of white blood cells is to defend the body against invading pathogens [12]. The results suggest that the extract could stimulate the production of leucocytes and therefore may have immune boosting effect on rats.

Table 1: Hematological parameters of rats after 28 days of *Euphorbia tirucalli* administration

	GP I	GPII	GPIII	GPIV
RBC (x 10^6 /µl)	7,14 ± 0,41	7,23 ± 0,21	7,58 ± 0,21	7,42 ± 0,18
HGB (g/dl)	13,08 ± 0,34	13,10 ± 0,41	13,80 ± 0,39	13,52 ± 0,35
HTC (%)	39,87 ± 2,09	39,18 ± 0,79	42,59 ± 1,19	41,60 ± 1,43
MCV (fL)	55,88 ± 0,50	54,07 ± 0,81	56,02 ± 1,19	56,03 ± 1,03
MCH (pg)	18,45 ± 0,66	18,08 ± 0,25	18,15 ± 0,33	18,18 ± 0,18
MCHC (g/dl)	33,03 ± 1,07	33,37 ± 0,38	33,43± 0,48	32,48 ± 0,33
PLT (10^3/µl)	493,67 ± 42,89	500,83 ± 25,51	547,83 ± 32,74	509,33 ± 27,86
MPV (fL)	7,65 ± 0,11	7,50 ± 0,07	7,73 ± 0,03	8,10 ± 0,04
WBC (10^3/µl)	2,00 ± 0,43	4,88 ± 0,59*	6,21 ± 0,60***	6,36 ± 0,70***
MO (10^3/µl)	0,23 ± 0,06	0,68 ± 0,14*	1,85 ± 0,12***	1,73 ± 0,07***
GR (10^3/µl)	0,88 ± 0,05	0,74 ± 0,12	1,46 ± 0,07	1,30 ± 0,03
LYM (10^3/µl)	1,46 ± 0,35	2,40 ± 0,52*	3,71 ± 0,50***	4,53 ± 0,61***

*P <0.05, **P <0.01, ***P <0.001 vs control. n=8; data are presented as mean ± SEM n=8.

Effects of AEET on biochemical parameters

Assessment of liver and kidneys is a very vital index in evaluating the toxicity of drugs and plant extracts [13]. Kidney function was evaluated in this study by urine and serum creatinine. Increase blood creatinine is a good indicator of negative impact in kidney functions [14,15]. The results in table 2 indicate that, the extract at dose of 250 mg/kg significantly decreased ($p<0.05$) serum creatinine and increased ($p<0.01$) urine creatinine; whereas the other doses did not produce any effect on these parameters. These results suggest that the kidney functions were not altered in animals treated with AEET as confirmed by the histological analysis which showed no lesions. Alanine and aspartate amino transaminases are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage of liver [16]. In this study, serum ALT levels significantly decreased ($p<0.01$, $p<0.001$) in all treated groups while AST levels revealed no significant change (Table 2) compared to control group. The reduction of serum ALT concentration clearly suggests a hepatoprotective action of AEET.

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Table 2: Biochemical parameters of rats after 28 days of *Euphorbia tirucalli* administration

	GP I	GP II	GP III	GP IV
ALT (U/L)	2.50 ± 0.73	1.95 ± 0.37**	1.00 ± 0.24***	1.32 ± 0.66**
AST (U/L)	78.03 ± 1.20	71.20 ± 2.48	67.44 ± 4.01	74.10 ± 1.22
Conjugated Bilirubin (mg/dl)	0.079± 0.006	0.078± 0.008	0.055± 0.014	0.089 ± 0.007
Total Bilirubin (mg/dl)	0.223± 0.009	0.196 ± 0.009	0.205 ± 0.010	0.232± 0.017
Total Protein (mg/ml)	77.51 ± 1.82	78.50 ± 2.23	82.94 ± 2.33	85.32 ± 1.72
Total Cholesterol (mg/dl)	100.38± 13.33	106.20 ± 14.12	106.90 ± 8.50	102.90± 8.82
Serum creatinine (µg/ml)	5.10± 0.30	4.41 ± 0.52	3.04 ± 0.42*	4.72± 0.42
Urinary creatinine (µg/ml)	42.13± 3.65	50.77 ± 2.25	70.26 ± 2.67**	47.22± 1.77

*P <0.05, **P <0.01, ***P <0.001 vs control. n=8; data are presented as mean ± SEM

Results revealed no significant change in the total bilirubin, conjugated bilirubin, clearance and total protein level when compared to the control group (Table 2).

Results from the histological analysis

Microscopic examination revealed no remarkable abnormalities in heart, kidneys and liver in both control and treated groups (Figures 4, 5 and 6). This suggests that AEET did not affected structure and morphology of the organs.

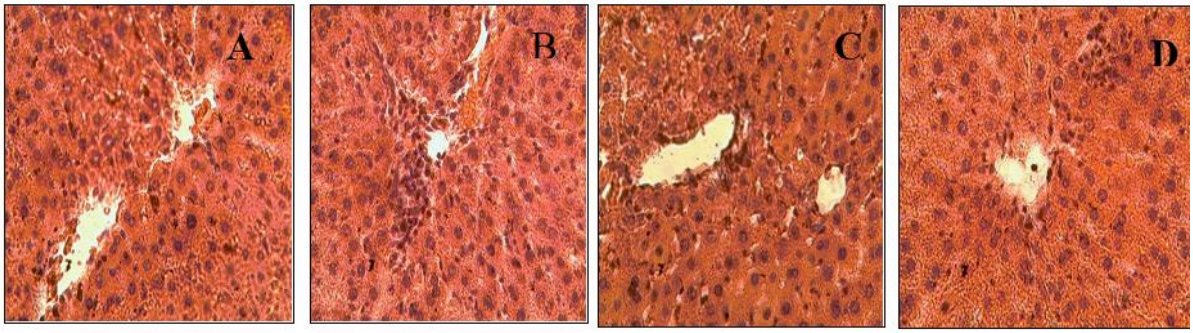

Figure 4: Photomicrographs of the liver sections of rats after the 28-days treatment. No change in all the treated groups. A: control; B: 125 mg/kg body weight aqueous extract *E. tirucalli*; C: 250 mg/kg body weight aqueous extract *E. tirucalli*; D: 500 mg/kg body weight aqueous extract *E. tirucalli*. 400X, H/E, 5 µm.

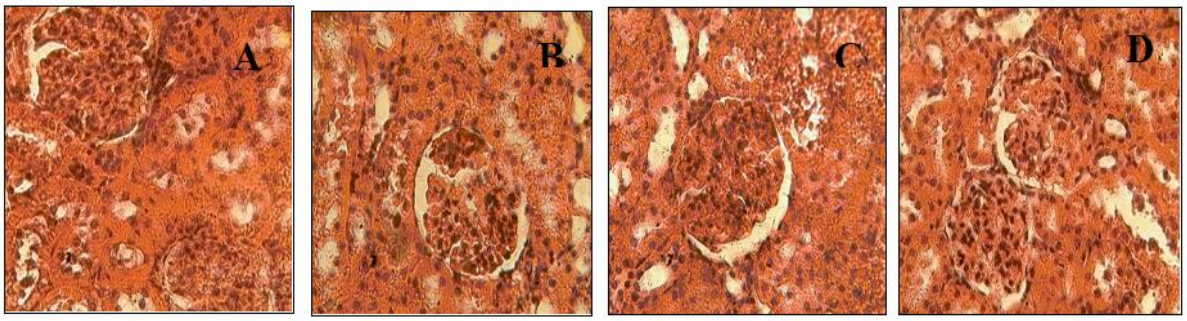

Figure 5: Photomicrographs of the kidney sections of rats after the 28-days treatment. No change in all the treated groups. A: control; B: 125 mg/kg body weight aqueous extract *E. tirucalli*; C: 250 mg/kg body weight aqueous extract *E. tirucalli*; D: 500 mg/kg body weight aqueous extract *E. tirucalli*. 400X, H/E, 5 µm.

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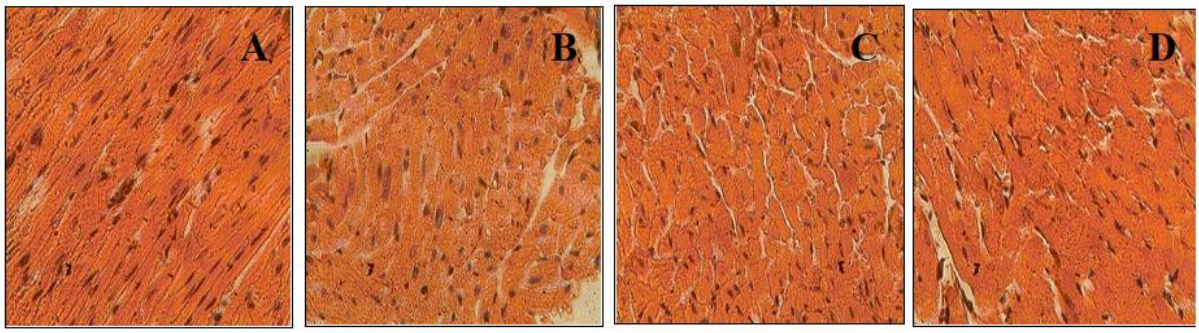

Figure 6: Photomicrographs of the heart sections of rats after the 28-days treatment. No change in all the treated groups. A: control; B: 125 mg/kg body weight aqueous extract *E. tirucalli*; C: 250 mg/kg body weight aqueous extract *E. tirucalli*; D: 500 mg/kg body weight aqueous extract *E. tirucalli*. 400X, H/E, 5 µm.

CONCLUSION

Based on the results obtained in acute (LD50 >8000 mg/kg) and sub-chronic oral toxicity study of aqueous extract of *Euphorbia tirucalli,* it may be suggested that the plant is safe. This justifies it use in traditional medicine.

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