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Effects of Oral Administration of Ethanol Extract of *Euphorbia hirta* Leaves on Haematological Parameters in Adult Male Wistar Rats

Izunya AM*[1], Akpamu U[2], Oaikhena GA[1], Ujaddughe OM[1,3], and Iyevhobu, K.O[4,5]

[1]*Department of Anatomy, Ambrose Alli University, Ekpoma, Nigeria*
[2]*Department of Physiology, Federal University Oye-Ekiti, Ekiti State, Nigeria*
[3]*School of Biomedical Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[4]*Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria*
[5]*Department of Medical Laboratory Science, Edo State University, Iyamho, Edo State, Nigeria*

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Corresponding Author: **Iyevhobu KO** | E-Mail: **(iyevhobukenneth@aauekpoma.edu.ng)**

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ABSTRACT

Euphorbia hirta (E. hirta) is a weed with several claimed medicinal potentials. Despite the ethnomedicinal benefits, few studies have described its haemato-toxicity and hence this study. The leaf was phyto-chemically screened. Using adult male Wistar rats (150g ±25g), acute toxicity was determined while sub-acute toxicity (14 days) was evaluated on the ethanol leaf extract at doses of 50mg/kg, 100mg/kg and 500mg/kg and judged against untreated group (control). Body weight was determined, blood sample collected and estimated for the parameters herein presented. The data was analyzed using one-way ANOVA and expressed as mean ± SEM at p< 0.05 significance. Phytochemical screening revealed abundance of alkaloids, flavonoids, and tannins while saponins, phenol, steroids, terpenoids, anthraquinones and cardiac glycosides were present in small amounts. Acute toxicity indicated LD_{50} greater than 5000mg/kg. Sub-acute toxicity showed dose dependent decrease in body weight in treated groups that is non-significantly lower compared with control. White Blood Cells (WBC) and neutrophil counts were significantly increased while lymphocyte counts were significantly decreased in a dose-dependent manner compared with control. In contrast, hemoglobin, Red Blood Cell (RBC), platelet, HCT, MCH, MCHC, and MCV levels were not significantly altered compared with the control; except for the 500m/kg-treated, where MCV was significantly higher. These findings suggest that crude E. hirta is safe during acute oral administration, but sub-acute oral administration of the ethanol leaf extract might be leuco-toxic. This assertion is based on the significant alterations in WBC, neutrophil and lymphocyte levels and thus, possible undesirable effects.

Keywords: *Ethanol leaf extract, Euphorbia hirta, hematological parameters, phytochemistry, toxicity.*

Introduction

Plants, with their numerous diversities, are essential sources of basic human needs (food, clothing and housing) and natural medicines (1). In fact, many conventional drugs originated from plant sources, such as aspirin (Salix alba), digoxin (Digitalis purpurea), quinine (Cinchona officinalis), and morphine (Papaver somniferum) (1). However, there is documented evidence of incidences of organs toxicity of these medicinal herbs and more from prolong ingestion (2, 3). Despite this reported toxicity, there is indiscriminate patronage of medicinal plants and related products, due to presumed safety, availability and affordability (4,5). This rising patronage comes with increasing tendencies for abuse, and hence necessitating safety of medicinal plants. In addition, the resounding calls for the integration of traditional medicine practice into conventional modern medicine (6,7), make the determination and documentation of the safety/toxic risk potentials of medicinal plants imperative.

Among the medicinal plants used in traditional practice and studied scientifically is *Euphorbia hirta* (*E. hirta*) (6).

This small annual herb belongs to the family Euphorbiaceae, grows to a height of 30–40 cm, and has a slender reddish stem. It is widely distributed worldwide, particularly in tropical and subtropical regions (8). The plant thrives in well-drained sandy and loamy soils and commonly grows in wastelands, along roadsides and riverbanks, and in gardens and pathways (9, 10).

Ethnopharmacologically, *E. hirta* is used to cure respiratory and bronchial disorders (hay fever, bronchitis and asthma), conjunctivitis and gastrointestinal diseases such as intestinal parasitosis, dysentery and diarrhea (11, 12). The whole plant has also been reported to possess antimalarial (13), anticancer (14), anti-asthmatic (15), antifungal and antibacterial (16), immunomodulatory (17), antihypertensive (18), antioxidant (19,20), anti-inflammatory (21,22,23) and antidiabetic (24) properties. Quantitative analyzing of *E. hirta* showed the presence of amino acids, a-amylase, β-amylase, carbohydrate, glutamine, protein, proline and phenolics (25) and phytochemicals such as alkaloids, anthraquinone, catachol, flavonoids, phenols, saponins, steroids, triterpenoids and tannins (25, 26).

Although medicinal plants are generally considered to be safe, they are not entirely free of side effects or toxicity (27) and their extracts could also be mutagenic or carcinogenic (28), causing acute toxicity and death of users (29,30). To the best of our understanding, there is a shortage of information on safety of *E. hirta*; hence the need to provide such useful information. Therefore, this present study seeks to assess the acute and sub-acute toxicity profile of the ethanol leaf extract of *E. hirta* by monitoring the body weight and hematological parameters in adult male Wistar rats.

Materials and Methods

Plant Collection, Ethanol Extract and Phytochemical Analysis

Samples of *Euphorbia hirta* leaf were collected from the College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. The leaf samples were identified and authenticated at the Herbarium Unit of Forestry Research Institute (FIH), Ibadan, Oyo State. The leaves were dried in the shade after being washed with distilled water and were then powdered by using a dry grinder and passed through a sieve. The ethanolic extract was prepared by cold maceration of 500 g of powdered leaves in 1500 mL of ethanol for 48 hours as previously reported (31, 32). Then, the extract was filtered, concentrated, air-dried (yield 6.20g) and the residue was stored in a refrigerator at room temperature for use in subsequent experiments. The extract was subjected to qualitative chemical tests for the identification of various phytochemicals following qualitative methods (32,33).

Animals

Male rats of the Wistar strain, weighing about 150–200 g, were obtained from the Animal Holding of AAU, Ekpoma for the study. Animals were kept in the Department of Anatomy Animal House at room temperature of 25–30°C, 45–55% relative humidity, and a 12-hour light-dark cycle. The animals were fed with rat pellets feed and sterile water *ad libitum*. Animal studies have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animal (CPCSEA) (34).

Experimental design

Acute Toxicity Study

Evaluation of the lethal dose (LD_{50})was carried out as by Lorke (35). The method entails two phases; in the first phase, rats were categorized into 3 consisting of 3 rats per group. Each rat was treated orally with the ethanol extract of *E. hirta* at doses of 10, 100 and 1000 mg/kg b.wt., respectively. In the second phase, another set of rats (3 rats per group) were treated orally with 1500, 3500 and 5000 mg/kg b.wt. of *E. hirta*. All animals were observed for 24 hours for mortality, general condition, and clinical signs including motor activity, appearance, and central and autonomic functions.

Sub-Acute Toxicity Study

Sub-acute toxicity studies was performed in twenty (20) male rats randomized into four groups of each consisting of five animals, as group A (control; administered with 0.5 ml of distilled water) while groups B, C and D were given 50, 100 and 500 mg/kg body weight of the ethanol extract, respectively. The administration was done repeatedly on daily basis for two weeks using metal oropharyngeal cannula. Body weight was obtained at commencement and end of the experiment using digital weighing scale. All animals were observed daily for mortality, general condition, and clinical signs throughout the dosing period.

Specimen Collection and Analysis

At the end of the 14 days experiment, on day 15, the rats in each group were euthanized by cervical dislocation and blood samples were collected from rats by cardiac puncture into sample bottles containing EDTA for the haematological analysis. The obtained body weights were used to determine percentage weight gain of each animal in each group. Hematological parameters including total count of Red Blood Cells (RBC), Hemoglobin concentration (HGB), Hematocrit (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Platelet count (PLT), total White Blood Cells count (WBC), and differential counts of each of the leukocytes: Neutrophils (NEU), lymphocytes (LYM), Basophils (BAS), monocytes (MON) and Esinophils (ESN) were estimated using haem automated analyzer (Cell Dyne: Model 331430, Abbott Laboratories, IL, U.S.A.).

Statistical analysis

The data obtained are expressed as mean ± standard error of the mean (SEM) and analyzed for statistical significance using student t test and one-way ANOVA followed by LSD test where applicable; all in Statistical Package for Social Sciences (SPSS version 21). $P < 0.05$ was considered significant.

Results

Phytochemical screening of ethanol leaf extract of *Euphorbia hirta*

The results of the present study show that the ethanol leaf extract of *Euphorbia hirta* contains an abundance of alkaloids, flavonoids and tannins (Table 1). Also present in small amount were saponins, phenol, steroids, terpenoids, anthraquinones and cardiac glycosides.

Table 1: Qualitative tests for the identification of secondary metabolites in the ethanol leaf extract of Euphorbia hirta

Phytochemicals	Sample extract
Saponins	+
Tannins	++
Flavonoids	++
cardiac glycosides	+
Anthraquinones	+
Terpenoids	+
Steroids	+
Alkaloids	++
Phenol	+

(+ present, ++ abundant)

Acute Toxicity Study

Table 2 shows the acute toxicity findings of the ethanol leaf extract of *Euphorbia hirta* in adult male Wistar rats. All the oral doses did not show any toxic signs of acute toxicity or death even at the highest dose of 5000mg/kg. The LD_{50} value was thus estimated to be higher than 5000 mg/kg b.wt. via oral passage.

Table 2: Acute toxicity reports the crude Euphorbia hirta leaf in adult male wistar rats

Groups	Number of rats	Dose of ethanol leaf extract of *Euphorbia hirta*(mg/kg body weight)	Mortality	Percentage mortality	Other symptoms
Phase 1					
A1	3	10	0	0%	No sign
B1	3	100	0	0%	No sign
C1	3	1000	0	0%	No sign
Phase 2					
A2	1	1500	0	0%	No sign
B2	1	3000	0	0%	No sign
C2	1	5000	0	0%	No sign

Sub-acute toxicity studies of EEEHL: Effect of EEEHL on body weight

Figure 1 shows the effect of ethanol leaf extract of *Euphorbia hirta* on percentage body weight gain of adult male Wistar rats after 14 days oral administration. Varying doses of the ethanol extract cause a dose-dependent non-significant decreased (p was >0.05) on percentage body weight gain compared with the control.

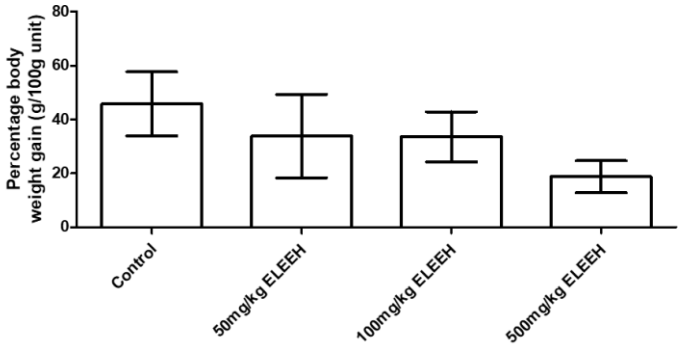


Figure 1. Effect of ethanol leaf extract of Euphorbia hirta on percentage body weight gain of adult male Wistar rats after 14 days oral administration.

Hematological analysis

Table 3 shows the effect of ethanol leaf extract of *Euphorbia hirta* on hematological parameters of adult male Wistar rats after 14 days oral administration. The majority of the hematological parameters (ESN, PCV, RBC, MON, MCH and MCHC) were not significantly altered compared with the control. All doses of the extract significantly increased WBC and NEU counts but decreased the level of LYM count compared with the control. At dose of 500 mg/kg, the extract significantly increased the level of MCV compared with the control (Table 3).

Table 3: Effect of ethanol leaf extract of Euphorbia hirta on haematological parameters of adult male wistar rats after 14 days oral administration

	Treatment Group			
Parameter	Control	50mg/kgbw	100mg/kgbw	500mg/kgbw
HB (g/dL)	14.63± 0.21	14.20± 0.56	14.17± 0.38	13.90± 0.52
RBC (x 10^6 A ^{BC})	7.63± 0.38	7.66± 0.49	6.45± 0.98	6.28± 0.14
HCT (%)	41.33± 2.52	37.00± 1.73	37.33± 2.52	39.67± 0.58
MCV (fL)	54.14± 1.26	48.53± 5.07	59.15± 12.79	63.17± 0.49$^+$
MCH (g/dL)	19.57± 0.31	18.59± 1.27	22.28± 3.02	22.15± 1.77
MCHC (pg)	36.16± 0.74	38.47± 3.19	38.07± 2.89	35.05± 1.63
WBC (x 10^3 A ^{BC})	4913.33±769.65	6933.33±493.2*	7833.33±929.1*	8800.00± 700.0*
LYM (%)	73.00± 2.00	68.00± 2.00*	61.00± 1.00*	60.00± 1.73*
NEU (%)	24.00± 1.73	28.33± 3.22	34.33± 1.53*	36.00± 1.00$^+$
MON (%)	1.33± 0.58	2.00± 1.00	2.00± 1.00	2.33± 0.33
ESN (%)	1.33± 0.58	1.67± 1.53	2.50± 0.50	1.67± 1.15
PLT (x 10^6 A ^{BC})	100033.33±458.06	118333.33±785.94	121000.00±4000.00	126833.33±225.46

*Values are the mean ± SD for 5 rats in each group. * indicates a significant difference from controls (P < 0.05). $^+$ indicates a highly significant difference from controls (P < 0.001)*

Discussion

Herbal medicines are widely perceived by the public as being natural, healthful and free from side effects due to their natural origins and are often considered as food supplements and not drugs (6,36,37). This is shown on the present study which indicates the ethanol leaf extract of *Euphorbia hirta* to contain alkaloids, flavonoids, tannins, saponins, phenol, steroids, terpenoids, anthraquinones and cardiac glycosides. The finding is consistent with reports of other study who reported the presence of these phytochemicals in various parts (6,11, 14, 38-40). The chemicals substances in medicinal herbs may be natural to the plant, but they are not natural to the human body and one of the stated problems of using plants as medicine is that in many cases no definite doses are prescribed often resulting in overdose and toxicity (37). Medicinal plants often possess numerous active pharmacological properties, and such has been the basis for the treatment of different diseases (14) and these constituents are no doubt responsible for their wide range of pharmacologic activities.

Alkaloids, flavonoids and tannins have been known to show medicinal activity as well as exhibiting physiological activity (33) with flavonoids known to have antioxidant effects and inhibit the initiation, promotion and progression of tumors (41). Tannins on the other hand have been shown to confer anti-diarrhoeic and anti-haemorrhagic properties (42) and saponins have been reported to have antifungal properties (43) as well as serve as an expectorant and emulsifying agent (44). The presence of these phytochemicals in the extract confers medicinal properties on the plant and this explains the use of this plant for treatment of different ailments.

The acute toxicity study is utilized to check the harmful effects of an agent to the organism given as a single or short-term exposure (25). The obtained LD_{50} of greater than 5000mg/kg for the extract in this study is an indication that the extract may be safe for human consumption, confirming the belief of the herbalists that the extract is not harmful.

Bearing in mind that herbal medicines are often used indiscriminately without recourse to the potential side-effects which could vary from being mild, moderate, and severe, to life-threatening (45), safety becomes a key issue that precedes the use of medicines be it orthodox or herbal.

Since no toxic effects were found during the acute toxicity study, further evaluation was conducted to evaluate the sub-acute toxicity up to 14 days in rats. Substances administered in chronic disease conditions may need repeated dosing toxicological evaluation (sub-acute toxicity study) since daily use may result in accumulation in the body with gradual effects on tissues and organs (46,47). In general, an increase or decrease in the body weight of an animal has been used as an indicator of an adverse effect of drugs and chemicals (48), and it will be significant if the body weight loss or gain that occurred exceeds 10% from the initial body weight (49). In the present study, the body weight of all treated rats did not differ significantly (p was > 0.05) from those of the control groups. This finding agrees with study by Kwan et al. (50) who reported that crude and ethanol extracts of *Euphorbia hirta* leaf had no significant effects on the body and relative organ weight of the rats used in their study. It indicates that the extract did not adversely affect food intake at all doses and had no harmful effect on body growth patterns.

The hemopoietic system is an important target for xenobiotics and is a sensitive index of toxic or pathological conditions. Haematological analysis reveals the physiological and pathological status of humans and animals in toxicological studies (51,52) and is an important aspect of toxicity study as it predicts the possible toxicity when translated, and provides useful information on inflammatory response, necrosis, and other infections (53-55). In this study, the varying doses of the extract had a significant effect on White Blood Cells (WBC), Neutrophils, and Lymphocytes.

The WBCs (leucocytes), neutrophils, lymphocytes, monocytes and eosinophils mediate the first line of defense against foreign substances, tissue injury and inflammation (56). Lymphocytes are dynamic cells and mediate immune response to foreign substances (57) and produce antibodies enabling the destruction of intracellular microbes and cancer cells (58). Neutrophils play a defensive role in microbial attacks and are also mobilised in acute inflammatory conditions (59). In this study, administration of *EH* led to a significant increase in the levels of WBC, which suggests that the extract stimulated the immune cells in the rats. Increase in the WBC production is always initiated either by infection, injury or toxic substance in the body (60). This shows that the *EH* may have immune boosting properties. The increase in WBC count may have been due to enhancement in the rate of entry of leucocytes into the blood from the bone marrow and a diminished rate of removal from circulation. These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extracts as some studies have reported saponins, alkaloids, tannins, phenolic compounds and flavonoids as immunostimulants (61,62). The immunostimulant effects of *E. hirta* had previously been documented (63).

The reduction in lymphocyte count in the present study could probably be due to cell margination rather than destruction. Since lymphocytes play the key role in all immune reactions (64), they migrate to sites of inflammation while their number may concomitantly decrease from circulation in the blood as were observed in the present study. It is possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood.

Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria, and they provide the first line of defense against invading microorganisms (65). This effect on neutrophil count may be partly responsible for the claim that *E. hirta* has antibacterial actions (66,67). An increase in neutrophils suggests enhancement of neutrophil migration and an anti-inflammatory effect of the extract, which could be due to flavonoids contained in the plant extract. In line with the findings of this study, the anti-inflammatory effect of *E,hirta*leaves have been reported by many researchers (6,21,22,23),

Platelet count was not significantly elevated. Platelets play an important role in blood haemostasis but an abnormally high platelet count may point to haemostatic disorders and eventually lead to thromboembolic diseases (68) and result in cardiovascular risks (51), while a decrease may infer liver disease (69). *E. hirta* is known to contain flavonoids, polyphenols, tannins and β-Sitosterol which are well-known compounds with platelet-increasing potential (6,70,71). In accordance with this study finding, platelet increasing potential of *E. hirta* has been reported by various authors (72-74). An increase in platelet count after 14 days of oral administration of ethanol leaf extracts of *E. hirta* may indicate that the extract had a megakaryopoiesis-stimulating activity.

Conclusion

The results of the present study indicated thatthe ethanolic extract of *E. hirta* leaves contains appreciable number of phytochemicals which include alkaloids, flavonoids, tannins and small amount of saponins, phenol, steroids, terpenoids, anthraquinones and cardiac glycosides.The acute oral administration of the crude extract showed no toxic symptoms or death even at the highest dose of 5000mg/kg. However, sub-acute oral administration of EEEHL caused significant variations in WBC, Neutrophil, and Lymphovyte counts which suggest that extract would have toxic effect on the hematological parameters and may not be completely safe as an oral remedy in male rats. There is greater need to take caution and avoid abuse and indiscriminate use of the leaves of *E.hirta.* This is more important giving the potential for cumulative toxic effects from continuous usage.

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