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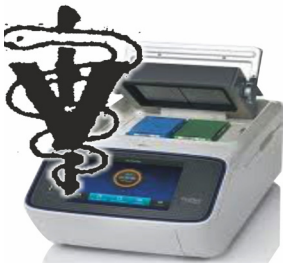

Effect of *Euphorbia hirta* Ethanol Extract on Hepatic Enzymes and Oxidative Stress in Albino Rats (Wistar Strain).

Aiyeyika, M.K[1]., Ifenkwe D.C[2]., Chima M. U[2]., Akomas, S.C[3]. and Egu, U.N[3].

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria.

[2]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

[3]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

*Corresponding Email: moses.aiyeyika@uniben.edu, Phone: +2347030380742.

ABSTRACT

Euphorbia hirta (commonly known as «Asthma weed»), is a plant widely used in traditional medicine to treat liver and kidney related conditions, however, there is paucity of scientific information on the impact of Euphorbia hirta leaf ethanol extract (EHLEE) on liver enzymes and antioxidant of male albino rats at lower doses. Hence, this study evaluated the hepatoprotective and antioxidant effects of EHLEE in albino rats. Forty-eight adult male albino rats were randomly divided into four groups and administered 0 (control), 50, 100, and 150 mg/kg body weight of the extract. The extract was administered orally for 66 days. The phytochemical results revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids and glycosides, it also showed a dose-dependent reduction in ALP and selectively, dose-specific decreases in AST (significant at 50 mg/kg) and ALT (significant at 150 mg/kg), indicating hepatoprotection. However, EHLEE exhibited minimal impact on systemic antioxidants, with no significant alteration in the mean serum catalase, SOD, or MDA level *Notably, a significant reduction in serum glutathione was observed at 100 mg/kg, suggesting a complex, potentially pro-oxidant interaction at this dose. In conclusion, this study has scientifically confirmed E. hirta's hepatoprotective potential via non-linear, biomarker-specific mechanisms, but not through broad systemic antioxidant upregulation. This study therefore recommends extensive mechanistic studies on biphasic responses, hepatic antioxidant assays, and effective dosing before therapeutic application.*

Keywords: *Euphorbia hirta, Hepatic enzymes, Antioxidant, Albino Rats*

INTRODUCTION

The liver is a vital metabolic organ central to detoxification, protein synthesis, and biochemical homeostasis [1]. Hepatic injury, often induced by toxins, drugs, or infections, disrupts these critical functions, primarily manifesting as elevated serum levels of hepatic enzymes such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP), which are established biomarkers of hepatocellular damage [2]. Concurrently, such injury is frequently mediated by oxidative stress (an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system), leading to lipid peroxidation, protein modification, and DNA damage, which further exacerbates tissue injury [3]. Given the limitations and potential side effects of conventional synthetic hepatoprotective drugs, there is a growing global interest in exploring medicinal plants with suspected hepatoprotective and antioxidant properties as complementary or alternative therapeutic agents.

Euphorbia hirta (commonly known as «Asthma weed» or «Tawa-tawa"), a plant widely used in traditional medicine across Asia and Africa, is reputed for its diverse pharmacological activities, including anti-inflammatory, antimicrobial, and antidiarrheal effects [4]. . *Euphorbia hirta* is a plant belonging to the phylum Angiosperm and family *Euphorbiaceae.* It is an annual, branched herb with branches up to 50cm long. The plant is hairy with simple leave and unisexual flower and contains a high amount of milky white latex which is more or less toxic [5]. Phytochemical screenings have revealed that *E. hirta* is rich in bioactive constituents such as flavonoids, tannins, terpenoids, and phenolic compounds, which are known for their potent antioxidant capabilities [6]. While traditional use suggests hepatoprotective potential, there is a need for systematic scientific validation of its effects on specific biomarkers of liver function and oxidative stress in appropriate biological models. This study, therefore, seeks to provide scientific evidence validation on the influence of *E. hirta* ethanol extract on hepatic enzymes activity and oxidative stress markers in an animal model.

METHODS

Ethical consideration

All procedures in this study were performed according to the Animal Ethic Committee, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202322).

Experimental animals

Forty-eight (48) male albino rats (Wistar strain) weighing between 140 – 150g within 8 weeks of age were used for the study. They were divided into four treatment groups T1, T2, T3 and T4. Each treatment group consisted of 12 rats replicated 3 times with 4 rats in a Completely Randomized Design (CRD) with four levels of the *Euphorbia hirta* ethanol extract as the test sample. The study was carried out in the Research and Teaching Laboratory Unit, Department of Veterinary Physiology Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria. Situated between latitude of 5°29'N and longitude 7°32'E and altitude of 123m above the sea level with an annual rainfall of 2177m, average ambient temperature of 22.32°C and relative

humidity of above 50-90 [7].

Plant collection and identification

Fresh leaves of *Euphorbia hirta* were harvested at various parts of the University environment, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant was identified and authenticated at the Taxonomy Section, Forestry department, Michael Okpara University of Agriculture, Umudike by Prof M.C. Dike.

Plant preparation

The leaves were washed to remove dirt and air-dried for 3- 4 days until a constant weight was attained, it was then blended into fine powder using a mechanical grinder and stored in air tight plastic containers. The ethanol leaf extract of *Euphorbia hirta* (ELEEH) was prepared by method described by Azwainida [8], and administered at 50mg/kg, 100mg/kg and 150mg/kg.

Phytochemical screening of *Euphorbia hirta* ethanol extract

The qualitative phytochemical screening was carried out using the method of Harborne [9].

Acute toxicity study

Acute and sub-chronic oral toxicity of *Euphorbia hirta* were evaluated in Wistar rats by Yuet [10] and reported that the plant extract at a single dose of 5000 mg/kg did not produce treatment – related signs of toxicity or mortality in any of the any animals tested during the 14 days observation period. Therefore, the LD 50 of this plant was estimated to be more than 5000 mg/kg and this was adopted for the present study

The acute toxicity testing of the extract was however evaluated using the original Lorke's method [11]. The rats were divided into 7 groups designated A-G, of five rats each, in phase 1, three dose levels (10, 100, and 1000mg/kg) were each administered orally to a single rat (n = 1 per dose). the animals were observed continuously for 24 hours for signs of toxicity and mortality, base on the results of phase 1, three dose levels (1600, 2600, and 5000 mg/kg body weight) were selected for the main study. Each dose was administered orally to a group of three rats (n = 3 per dose), while the control group of five rats were orally administered with 10 ml/kg distilled water. The animals were monitored for clinical signs of toxicity and mortality at 30 minutes, 60 minutes, 24, 48 and 72 hours, and an extended period of 7 – 14 days for delayed toxicity.

Study design

Forty-eight (48) male albino rats were divided into four treatment groups, and each treatment group consists of twelve (12) rats replicated 3 times with 4 rats per replicate in a Completely Randomized Design (CRD). The rats in groups 2, 3 and 4 received 50mg/kg, 100mg/kg and 150mg/kg of the extract respectively. The control rats in group 1 received 10 ml/kg of distilled water/kg body weight. The extract was administered orally with gavage for 66 days.

Blood sample collection and Biochemical assays

At the end of the experiment, blood samples 3ml were collected from the medial canthus vein of 3 males selected at random from each replicate, allowed to clot, and centrifuged at 3000 rpm for 15 minutes to separate serum. The serum samples were used for the estimation of ALT, AST, ALP and oxidative stress biomarkers using standard methods.

Data analysis

The values are reported as mean ± SEM and analyzed using the student's t-test and ANOVA where necessary. Statistical significance were considered if $P< 0.05$, while Tukey's Honestly Significant Difference (HSD) test was used to separate the means.

RESULTS

Qualitative phytochemical screening of *Euphorbia hirta* leaf Ethanol extract

The qualitative phytochemical screening result revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids and glycosides as major phytochemical composition (Table 1).

Effect of *Euphorbia Hirta* Leaf Ethanol Extract on Serum Biochemistry of Wistar rats.

There was dose dependent reduction in the ALP serum levels as the doses were increased. The reduction in the ALP serum level (39.19±3.61 IU/L), was significant ($p < 0.05$) at 150 mg/kg dose treated rats when compared with the serum level of ALP (55.33±2.17 IU/L) of the control rats. The rats treated with the least dose (50 mg/kg) recorded significant ($p < 0.05$) reduction in AST serum level (35.83±2.61 IU/L), when compared with 56.86±4.98 IU/L AST level in the control rats, whereas, other extract (100, and 150 mg/kg) treated rats had similar AST level as the control rats. The serum level of ALT significantly ($p < 0.05$) reduced from 10.18±1.01 IU/L (control rats), to 8.00±0.97 IU/L in 150 mg/kg extract-treated rats, while there was no significant ($p > 0.05$) difference, in the ALT serum level of 50, and 100 mg/kg extract-treated rats when compared with the control rats (Table 2).

Effects of *Euphorbia hirta* Leaf Ethanol Extract on Antioxidant activity of Wistar rats

The result of effects of the graded doses of EHLEE on the serum antioxidant activity of the treated rats showed that apart from the serum glutathione, which was significantly ($p < 0.05$) reduced from 13.98±3.00 µg/L (control rat) to 7.73±0.59 µg/L in 100 mg/kg extract treated other antioxidant parameters (catalase, superoxide dismutase and malondialdehyde), were not by the extract administration (Table 3).

Table 1: Qualitative phytochemical screening of *Euphorbia hirta* leaf ethanol extract

Phytochemicals	Inference
Flavonoids	++
Alkaloids	++
Saponins	+
Phenols	+++
Glycosides	+
Terpenoids	+

Keys: + = present; ++ = highly present; +++ = very highly present

Table 2: Effect of *Euphorbia hirta* Leaf Ethanol Extract on Serum Biochemistry of Wistar rats

	Treatment group			
Parameters	**Distilled water**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
ALP (IU/L)	55.33 ± 2.17^{a}	53.41 ± 5.91^{ab}	40.98 ± 4.71^{ab}	39.19 ± 3.61^{b}
AST (IU/L)	56.86 ± 4.98^{a}	35.83 ± 2.61^{b}	51.26 ± 8.76^{ab}	41.13 ± 2.64^{ab}
ALT (IU/L)	10.18 ± 1.01^{ab}	12.74 ± 1.43^{a}	9.70 ± 0.61^{ab}	8.00 ± 0.97^{b}

Key: Different superscript letters across treatment group show significant ($p \leq 0.05$) differences. ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

Table 3: Effects of *Euphorbia hirta* Leaf Ethanol Extract on Antioxidants of Wistar rats

	Treatment group			
Parameters	**D/ water**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
CAT (IU/g protein)	1.40±0.78	1.15±0.10	1.70±0.32	0.74±0.19
SOD (IU/g protein)	3.20±0.20	2.79±0.46	2.80±0.26	3.00±0.17
GLU (µg/L)	13.98 ± 3.00^{a}	8.77 ± 0.14^{ab}	7.73 ± 0.59^{b}	10.71 ± 0.68^{ab}
MDA (nanomole/g protein)	11.65±4.07	7.95±1.16	13.84±2.67	14.55±1.28

Key: Different superscript letters across treatment group show significant ($p \leq 0.05$) differences. SOD = Superoxide dismutase; CAT= Catalase; MDA= Malondialdehyde; GLU= Reduced glutathione; D/w = Distilled water.

Discussion

Medicinal plants contain chemical substances known as phytochemicals, these phytochemicals are found in the leaves, vegetables and roots [12]. It is categorized into two primary and secondary metabolites. The primary metabolites are common sugar (glucose), amino acids, proteins, purines and pyrimidine of nuclei acids, while the secondary metabolites include alkaloids, terpens, flavonoids, lignans, plants steroid, saponins, phenolics and glycosides [12, 13]. *In-vitro* phytochemical analysis of *Euphorbia hirta* in this study revealed the presence of important bioactive compounds such as flavonoids, alkaloids, phenols, glycosides, terpenoids and saponins, which agreed with the findings by other researchers on *Euphorbia hirta* leaf [14, 15, 16, 17, 18].

The study also revealed a complex and biomarker-specific influence of *Euphorbia hirta* leaf ethanol extract (EHLEE) on the serum biochemistry and antioxidant status of Wistar rats. The most consistent hepatoprotective signal was observed in the dose-dependent reduction of Alkaline Phosphatase (ALP), with a significant decrease at the highest dose (150 mg/kg). ALP is a marker of cholestasis and biliary function, and its reduction suggests that EHLEE may promote bile flow integrity and/or protect the biliary epithelium, particularly at higher concentrations [19]. The effects on the transaminases, ALT and AST, were more intricate and followed non-linear, biphasic dose-response patterns. The significant reduction of AST exclusively at the lowest dose (50 mg/kg) is indicative of a hermetic response, a phenomenon where a low dose of a stressor agent induces an adaptive beneficial effect that is absent at higher doses [20]. This suggests that low concentrations of EHLEE's bioactive compounds may optimally stabilize hepatic mitochondrial and cytoplasmic membranes or modulate inflammation, preventing enzyme leakage. In contrast, ALT, a more liver-specific cytosolic enzyme, was significantly reduced only at the highest dose (150 mg/kg). This divergence implies that the mechanisms governing the release and clearance of these two enzymes differ and are differentially sensitive to the phytochemical constituents of the extract. The overall trend of reduced hepatic enzymes aligns with the traditional use of *E. hirta* for liver ailments and can be attributed to its rich content of flavonoids and phenolics, known for their membrane-stabilizing and anti-inflammatory activities [6]. Contrary to expectations, the extract's impact on the systemic antioxidant profile was limited and, in one case, potentially adverse. The lack of significant change in catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels suggests that, at the administered doses and duration, EHLEE did not induce a systemic upregulation of these key enzymatic antioxidants or significantly reduce lipid peroxidation in the serum.

More notably, the significant reduction in serum glutathione (GSH) levels at the 100 mg/kg dose is a critical finding. GSH is the primary non-enzymatic intracellular antioxidant, and its depletion in serum could be interpreted in two ways. First, it may indicate a pro-oxidant shift or a metabolic burden at this specific dose, where the extract's compounds transiently consume GSH during metabolic conjugation, as part of a biphasic adaptive response [21]. Alternative-

ly, and more optimistically, this depletion could reflect an accelerated utilization of GSH in hepatic tissue for detoxification and cytoprotection, effectively "shuttling" the antioxidant activity to the site of need (the liver), which would not be reflected in serum levels. This hypothesis would require confirmation by measuring hepatic tissue GSH and related enzyme activity.

Conclusion

In conclusion, the ethanol extract of *Euphorbia hirta* leaves demonstrated a significant modulatory effect on hepatic function markers in Wistar rats, evidenced by the dose-dependent reduction in ALP and the selective dose-specific reductions in ALT and AST. These findings support its traditional hepatoprotective claims. However, its influence on the systemic antioxidant profile was minimal under the conditions of this study, with an unexpected reduction in serum glutathione at a mid-range dose, indicating a potential for complex, dose-dependent interactions with the body's redox system. Therefore, while EHLEE shows promise as a hepatoprotective agent, its effects are not universally antioxidative in the serum compartment but appear to follow biphasic patterns that necessitate careful dose consideration.

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Conflict of interest

The author declares that there is no conflict of interest.

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