

Biochemical and Haematological changes in Albino Wistar rats following administration of *Eleophorbiadрупifera* leaves extract

ABSTRACT

Different parts of the plant *Eleophorbiadрупifera* have been reportedly used for treatment of various ailments. This study aimed at evaluating the toxicological potential of the leaves of this plant in order to caution or encourage its use in traditional medicine. Water and ethanol-water extracts of *Eleophorbiadрупifera* leaves were administered orally in graded doses of 0.5ml (147.06mg/kg body weight), 1.0ml (303.3mg/kg body weight) water extract and 0.5ml (157.66mg/kg body weight), 1.0ml (301.72mg/kg body weight) ethanol-water extract to experimental animals for 3 weeks. The effect of the extracts on some biochemical and haematological parameters were evaluated in albino rats. At the end of 21 days, the AST and ALT levels of the test groups were significantly lower than the control ($p < 0.05$) and total bilirubin showed significantly high values in test groups when compared with the control ($p < 0.05$). The WBC was significantly increased while the RBC was decreased when compared with the control ($p < 0.05$). There were no significant changes in PCV and Hb of the experimental animals ($p < 0.05$). There was no significant difference between the effects of the water extract and that of the ethanol-water extract on the albino rats ($p < 0.05$). The results suggest that there may be no adverse effect associated with the use of this extract in phytotherapy.

Keywords: *Eleophorbiadрупifera*, biochemical, haematological, albino rats

INTRODUCTION

Medicinal plants are plants which have been claimed and confirmed to possess medicinal properties. They can also be referred to as herbal medicines. The healing power of herbs had been recognized and botanic medicine is one of the oldest practiced professions by mankind. Most medicinal plants have not been thoroughly evaluated for their toxicity profiles though medicinal plants and their products are relatively safer than their synthetic counterpart drugs [1]. This is due firstly to the fact that medicinal plant constituents mimic more closely the natural constitution of the human somatic system and following the lock and key hypothesis, it is expected that they will fit better into such system. In terms of environmental friendliness, medicinal plants and their products are far more advantageous than orthodox medicines since they constitute a lesser form of pollution menace and are renewable [2]. However, a blanket assumption should not be made about the safety of medicinal plants and their products, since seemingly innocuous plants may turn out to be toxic.

Eleophorbiadрупifera belongs to the family Euphorbiaceae which is one of the largest families of the angiospermae. It holds about 300 genera and over 600 species. *Eleophorbiadрупifera* looks like a tall Euphorbia with long fleshy leaves. When young, the branches are succulent and angular. Old trees have a clear trunk and densely crowded ascending branches with the leaves in the tufts at the end. Plants of the Euphorbiaceae are frequently used in the indigenous system of medicine. Little had earlier been known about the pharmacological actions of the specie *Eleophorbiadрупifera*, albeit it is listed among the plants that 'heal' [3] [4]. The leaves, stem, bark, roots and latex are used depending

on the type of ailment. Some herbal medicine practitioners posit that the leaves in particular are anti-hypertensive, antidiuretic, skeletal muscle relaxant and antidibetic [5]. Given the traditional use of the plant in the management of various ailments, it becomes necessary to evaluate its toxicological potential in order to caution or encourage its use in traditional medicine.

MATERIALS AND METHODS

Collection and preparation of samples

The leaves of *Eleophorbiadрупifera* were collected from the botanical garden of the University of Calabar, Cross River State and were authenticated by a taxonomist in the Department of Botany, University of Calabar. The leaves were prepared by washing with distilled water and dried under shade. It was afterwards dried in the oven at 50-60°C for 2 days, ground into powder with an electric blender and stored in airtight containers for analytical use.

Extraction of sample

The ground sample (10g) was weighed out into a volumetric flask. Distilled water was added and made up to the 100ml mark and the mixture vigorously agitated for 1 hour before it was filtered. The filtrate was stored for use as the water extract after determination of concentration. 10g of the blended leaves sample was again weighed out into a volumetric flask. 80% ethanol was added and made up to the 100ml mark. The mixture was vigorously agitated and filtered. The filtrate obtained was poured into a graduated beaker and evaporated on a hot plate. On evaporation to the 20ml mark, the beaker was removed from the hot plate and distilled water was added to the 75ml mark. This was used as the ethanol-water extract.

Experimental Animals

Thirty (30) albino rats weighing 100-141g were obtained from the disease free stock of the animal house, Department of Biochemistry, University of Calabar and used for the study. The 30 animals were housed in plastic cages with stainless still mesh at the bottom to ensure that faeces and feed droppings were inaccessible to the experimental animals. The environment was under standard conditions of temperature (28±2°C) and relative humidity (46±5%) with a 12h light- dark cycle and adequate ventilation. The animals were fed daily with commercial rat mash and water was given from bottles with stainless steel nozzles throughout the study [6].

Grouping and Treatment of animals

The animals were assigned into five groups of six rats each. Group 1 animals (control) were fed the commercial rat mash only. Groups 2 and 3 (experimental group) were placed on 0.5ml and 1.0ml water extract respectively,

Groups 4 and 5 (experimental group) were respectively treated with 0.5ml and 1.0ml ethanol-water extract. The extracts were daily administered to the rats orally for a period of 21 days.

Collection of Serum sample

Twenty four hours after the last administration, the animals were anaesthsized under chloroform and dissected. Blood was obtained via cardiac puncture into plain sample tubes and allowed to stand for 2 hours and thereafter centrifuged at 2000g for 10 minutes to separate serum from the blood cells. The blood serum obtained was used for assay of ALT, AST, total bilirubin, WBC, RBC, PCV and Hb.

The supernatant was separated and immediately used for assay of AST and ALT activities.

Biochemical Assay

Serum and liver activities of ALT and AST were determined using the methods of Stroev and Makarova[7]. Total bilirubin were estimated according to instructions from laboratory kits obtained from Randox Laboratory Ltd., United Kingdom and absorbance were read using a UV-vis spectrophotometer -SP-300 [8].

Haematological Assay

The WBC and RBC were counted by microscopic visual identification using Turk's fluid and Hayem's fluid respectively. The PCV was determined by capillary method using haematocrit centrifuge. Hb concentration was determined using Drabkin's reagent [9].

Statistical Analysis

Data are presented as mean \pm SD and analyzed for statistical significance among group means by t-test. P- values less than 0.05 were considered statistically significant.

RESULTS

As shown in table 1, the treatment groups showed significant increase in WBC and decrease in RBC when compared with the control group ($p < 0.05$). There were no significant differences in PCV and Hb concentrations between the treatment groups and the control ($p < 0.05$).

Table 1: Haematological Parameters of Rats fed Extracts of Eleophorbiadrupifera

Experimental group	WBC(X109)	RBC(X109)	PCV (%)	Hb (g/dl)
Group 1(Control)	2.46 \pm 0.28	2.47 \pm 0.06	39.4 \pm 4.34	12.88 \pm 2.24
Group 2	3.15 \pm 0.34	2.33 \pm 0.07	39.2 \pm 9.15	11.82 \pm 2.09
Group 3	3.95 \pm 0.28	2.34 \pm 0.01	42.75 \pm 2.50	13.03 \pm 2.07
Group 4	3.84 \pm 0.23	2.29 \pm 0.05	40.75 \pm 3.77	12.35 \pm 1.08
Group 5	3.36 \pm 0.64	2.17 \pm 0.20	38.5 \pm 7.15	12.18 \pm 1.82

Results are presented as mean \pm SD

As presented in table 2, the AST and ALT activities were found to be significantly lower in the treatment groups than in the control ($p < 0.05$). There was however no significant difference in effect on increasing dose of each extract and between the effects of the water extract and ethanol-water extract respectively ($p < 0.05$). The Total bilirubin of the treatment groups showed significant increase when compared with the control ($p < 0.05$).

Table 2: Biochemical Parameters of Rats fed Extracts of Eleophorbiadrupifera

Experimental group	AST (U/L)	ALT (U/L)	Total Bilirubin (μ mol/l)
--------------------	-----------	-----------	--------------------------------

Group 1 (Control)	126.16± 2.98	32.32± 5.02	11.29± 0.99
Group 2	68.5± 16.15	18.00± 2.94	16.19± 1.48
Group 3	69.2± 23.85	19.58± 4.44	18.70± 1.41
Group 4	59.38± 8.26	28.83± 3.57	1.61± 0.61
Group 5	69.93± 20.15	24.17± 2.60	14.06± 2.07

Results are presented as mean ±SD

DISCUSSION

This study examined the effects of *Elophorbiadrupifera* on the blood biochemistry and haematology of rats. Specifically the impact of treatment of rats with varying doses of *Elophorbiadrupifera* on serum levels of AST, ALT and total bilirubin, WBC, RBC, PCV and Hb concentrations, in an attempt to evaluate the hepatotoxic potential of this plant extract. One of the organs usually affected by ingestion of xenobiotics is the liver. Hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP. While some phytochemicals are hepatotoxic, others are hepatoprotective. The significant decrease in both ALT and AST activities of the experimental animals and the ALT/AST ratio of each group being below one indicates that there was no liver damage. This could be due to the liver's role in the storage, biotransformation and detoxification of toxic substances [10]. In contrast to the present study findings, Konya et al. [11], reported that aqueous extract of *Eleophorbiadrupifera* were toxic to Wistar rats. According to Hall and Cash [12], ALT/AST ratio greater than one are indicative of liver damage. The decrease in both ALT and AST activities might be due to active participation of the enzymes in tissue and cellular activities [13]. The increase in total bilirubin though significant was still within normal range as Hall and Guyton [14] posits that normal individuals have less than 20.56Umol/L of bilirubin circulating in their blood. The increase might have been due to destruction of some red blood cells as indicated by the decrease in RBC of the test animals. RBC though significantly decreased was still within normal range. Okon et al [15], also observed alterations in haematological parameters of Wistar rats following administration with *Carica papaya* leaves and seeds.

Some herbal medicine practitioners posit that leaves of *Eleophorbiadrupifera* in particular are anti-hypertensive, antidiuretic, skeletal muscle relaxant and antidibetic. *Eleophorbiadrupifera* can thus be said to have little or no toxicological effects on biological systems and could be used internally for medicinal purposes without fear of hepatotoxicity.

CONCLUSION

The effect of the extracts of *Eleophorbiadrupifera* on some biochemical and haematological parameters were evaluated in albino rats. The AST and ALT levels of the test groups were significantly lower than the control and total bilirubin showed significantly high values in test groups when compared with the control. The WBC was significantly increased while the RBC was decreased when compared with the control. There were no significant changes in PCV and Hb of the experimental animals. There was no significant difference between the effects of the water extract and that of the ethanol-water extract on the albino rats. The results suggest that there may be no adverse effect associated with the use of this extract in phytotherapy.

REFERENCES

1. Eno, A, Ofem, O, & Itam E. (2006). *Blood pressure lowering agent(s) in the leaves of a deciduous shrub-Elaeophorbiadrupifera.. The Internet Journal of Nutrition and Wellness*. 2006; (1).
2. Hall, JE, & Guyton, AC. *Guyton and Hall textbook of medical physiology* (12th ed.). Saunders/Elsevier. (7th Ed.) W. B. Saunders Company, U.S.A. p 42-53. 2011.
3. Jendrassik, L. and Grof, P. (1997). Determination of Bilirubin. *Clin. Path.* 297:81.
4. Nelson, DL, Cox, MM., & Hoskins, AA. *Lehninger Principles of Biochemistry*. 8th Edition. W.H. Freeman & Co. Ltd 1237PP. 2021.
5. Stroeve, E, Makarova, V. *Laboratory manual for Biochemistry*. Mir Publishers, Moscow. p 162-164. 1984
6. Van Kampen, EJ, Zijlstra, WG. Spectrophotometry of haemoglobin and haemoglobin derivatives. *Advances in Clin. Chem.* 1983; 23:199-202.
7. Josephine Ozioma, E.-O., & Antoinette Nwamaka Chinwe, O. Herbal Medicines in African Traditional Medicine. *IntechOpen*. 2019; doi: 10.5772/intechopen.80348
8. Okaiyeto, K, Oguntibeju, OO. African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. *International Journal of Environmental Research and Public Health*. 2021; 18(11): 5988. <https://doi.org/10.3390/ijerph18115988>
9. Okon AJ, Aluko OO, Tom UE, & Essien NE. Bioaccumulation of Heavy Metals in Liver of Albino Wistar Rats Exposed to Single and Heavy Metal Mixture. *African Journal of Environmental Health Sciences*. 2020; 7(November, 2020): 101-109. Available: <http://www.ehsanonline.org>
10. Dimmitt, MA. Euphorbiaceae (spurge family) Arizona-Sonora Desert Museum 2021 N. Kinney Rd., Tucson AZ 85743 U.S.A. 520.883.2702. Available: info@desertmuseum.org
11. Chaudhary, P, Singh, D, Swapnil, P, Meena, M, & Janmeda, P. Euphorbia neriifolia (Indian Spurge Tree): A Plant of Multiple Biological and Pharmacological Activities. *Sustainability*. 2023; 15(2): 1225. <https://doi.org/10.3390/su15021225>
12. Hall, P, Cash, J. What is the real function of the liver 'function' tests?. *The Ulster Medical Journal*. 2012; 81(1): 30-36
13. Okon, AJ, Etim, DJ, Daniel, A, Bobson, PM, Asuquo, AE. Effect of ethanolic extracts of Persea americana seed and Zea mays silk on blood glucose levels, body and

organ weights of alloxan- induced hyperglycemic albino wistar rats. *Global Journal of Pure and Applied Sciences*. 2018; 24: 153-159.doi:<https://dx.doi.org/10.4314/gjpas.v24i2.5>

14. Okon, AJ, Aluko, OO, Okon, IJ, Inah, SI, Dania, AV, Achi, FI-O. Effect of *Carica papaya* Leaves and Seeds on Electrolyte and Hematological Parameters in Albino Wistar Rats Exposed to Lead Nitrate. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2023;13(4) :15-27.

UNDER PEER REVIEW