

## Effects of *Eleophorbiadrupifera* leaves extract on some Biochemical and Haematological parameters of Albino rats.

Comment [H1]: Change the title

### ABSTRACT

Water and ethanol-water extracts of *Eleophorbiadrupifera* leaves were administered orally in graded doses of 0.5ml (147.06mg/kg body weight), 1.0ml (303.3 mg/kg body weight) water extract and 0.5ml (157.66 mg/kg body weight), 1.0ml (301.72 mg/kg body weight) ethanol-water extract on 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 growth response was higher in the test groups than in the control ( $p < 0.05$ ). 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 the leaf extract of *Eleophorbiadrupifera* may possess the capacity to stimulate appetite and there may be no adverse effect associated with the use of this extract in phytotherapy.

Comment [H2]: Write the brief introduction and objectives of the study

Comment [H3]: its two word give space in throughout the manuscript

**Keywords:** *Eleophorbiadrupifera*, 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 (Okon, 2012). 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 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 (Samorinia, 2016). Secondly, 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 (Gamaniel, 2021, Akpabio *et al.*, 2012; Akpakpanet *al.*, 2012). 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. Though some of the medicinal plants have both medicinal and industrial applications (Akpakpanet *al.*, 2020)

*Eleophorbiadrupifera* belongs to the family Euphorbiaceae which is one of the largest families of the angiospermae. It holds about 300 genera and over 600 species. *Eleophorbiadrupifera* 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 *Eleophorbiadrupifera*, albeit it is listed among the plants that 'heal'. The leaves, stem, bar, 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 anti diabetic (Comley, 2019). The leaf is also used as filaricide and against guinea worm infestation. Apart from the use of *Eleophorbiadrupifera* for treatment of various ailments, traditional herbalists claim that it is also effective in aiding or inducing labour or abortion. The latex is used to remove warts though very injurious to the eyes (Samorini, 2016). 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.

**Comment [H4]:** Mention in which species it is effective..?

## MATERIALS AND METHODS

The leaves of *Eleophorbiadrupifeara* 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 Uyo, Uyo.

The leaves of *Eleophorbiadrupifeara* were prepared by washing with distilled water and dried under shade. It was afterwards dried in the oven at 50-60<sup>0</sup>C for 2 days, ground into powder with an electric blender and stored in airtight containers for analytical use.

**Comment [H5]:** Phytochemical analysis...? mention result of phytochemical analysis .

## Extraction of sample

The ground sample (10g) was weighed out into a volumetric flask. Distilled water was added and made up to 100 ml 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 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, 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 100-141 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 cage with stainless still mesh at the bottom to ensure that feces and feed droppings were inaccessible to the experimental animals. The environment was under standard conditions of the temperature ( $28\pm 2^{\circ}\text{C}$ ) and relative humidity ( $46\pm 5\%$ ) 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.

### Grouping and Treatment of animals

The animals were assigned into five groups of six rats each. Group 1 animals (control) were fed with 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

**Comment [H6]:** As it is laboratory animal study, whether any approval taken from the animal ethical committee. If so mention it. What is the acclimatization period that also mention. Mention the year of study. Usually for toxicity study either 14 day or 28 days for acute or sub acute study. But here only 21 days any supporting guidelines for this period.

**Comment [H7]:** How the dose was fixed, whether followed OECD guidelines . It is acute or subacute toxicity study. As your manuscript mainly highlighting the toxicity study but nowhere guidelines are not mentioned

**Comment [H8]:** Correct it as after the last day of the study, animals were fasted for 24 hr later euthanized by using.....

**Comment [H9]:** rpm or g.?.? What about the recovery study..?

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.

**Comment [H10]:** mention the importance of these enzymes and parameters in the introduction

### Biochemical Assay

Serum and liver activities of ALT and AST were determined using the methods of Reitman and Frankel (2017). 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 (Jendrassik and Grof, 2021).

**Comment [H11]:** Correct the sentence its not serum activities or add as biochemical parameters, what about kidney parameters ( ALP enzyme, creatinine, BUN etc) studied..? If so mention it also

### Haematological Assay

The WBC and RBC were counted by microscopic visual identification using Turk’s fluid and Hayem’s fluid respectively (Dacie and Lewis, 2015). The PCV was determined by capillary method using haematocrit centrifuge (Jones, 2015). Hb concentration was determined using Drabkin’s reagent (Van Kampen and Zijistra, 2014)

**Comment [H12]:** As this paper restricted to biochemical/Haematological assay give detailed parameters including complete blood count

### Statistical Analysis

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

## RESULTS

**Table 1:**Haematological and Biochemical Parameters of Rats fed Extracts of Eleophorbiadrupifera

Parameter/ group	WBC(X10 <sup>9</sup> )	RBC(X10 <sup>9</sup> )	PCV(%)	Hb(g/dl)	AST(U/L)	ALT(U/L)	Total Bilirubin (µmol/l)
Group 1 (Control)	2.46±0.28	2.47±0.06	39.4±4.34	12.88±2.24	126.16±2.98	32.32±5.02	11.29±0.99
Group 2	3.15±0.34	2.33±0.06	39.2±9.15	11.82±2.09	68.5±16.15	18.00±2.94	16.19±1.48

Group 3	3.95±0.28	2.34±0.01	42.75±2.50	13.03±2.07	69.2±23.85	19.58±4.44	18.70±1.41
Group 4	3.84±0.23	2.29±0.05	40.75±3.77	12.35±1.08	59.38±8.26	28.83±3.57	16.19±0.61
Group 5	3.36±0.64	2.17±0.20	38.5±7.15	12.18±1.82	69.93±20.15	24.17±2.60	14.06±2.07

### Results are presented as mean ± SD

As shown in table 1. Above, 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 ( $p < 0.05$ ). 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 ( $p < 0.05$ ).

### DISCUSSION

Medicinal plants contribute immensely in the healthcare of populations in various parts of the world (FAO,1999) example in Africa (Cunningham, 2003; Mashall, 2016, China (He and Sheng, 2019), Europe (Gamaniel, 2021) and North America (Brevoort, 2015). Many of our rural populations depend on a variety of plant materials for their wellbeing ( Akpakpanet *al.*, 2017). These plants do not only supply essential nutrients but contain active substances which have proven efficacious against many diseases (Gamaniel, 2021). This study examined the effects of *Eleophorbiadrupifera* on the blood biochemistry and haematology of rats.

Specifically the impact of treatment of rats with varying doses of *Eleophorbiadrupifera* 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. Generally, hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP (Whitby *et al.*, 2013) and studies with medicinal plant extracts have shown the varying effects of photochemicals on serum and liver enzyme levels. While some phytochemicals are hepatotoxic, others are

**Comment [H13]:** Results should be too elaborative each single parameter need to be discussed and give statistical significance in each group. It is very short . Mentioned only treatment group like ....but animals have been assigned different groups with different dose and different extract . So among treatment groups among extract group need to be mentioned the result and discuss properly.

hepatoprotective. The significant decrease in both ALT and AST activities of the experimental animals albeit with an ALT/AST ratio of each group below one indicates that there was no liver damage. According to strove and (Makarova, 2014), 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 (Lehninger, 2002). The increase in total bilirubin though significant was still within normal range as Guyton (2016) 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 ranged. According to Guyton (2016) normal RBC ranges from 4.7 to 5.2x10<sup>12</sup>/L.

From the statistical comparisons, the effects of the leaf extract are not dose dependent. Some herbal medicine practitioners posit that leaves of *Eleophorbiadrupifera* in particular are anti-hypertensive, antidiuretic, skeletal muscle relaxant and antidibetic. The leaf is also used as filaricide and against guinea worm infestation (Comley, 2018). Apart from the use of *Eleophorbiadrupifera* for treatment of various ailments, traditional herbalists claim that it is also effective in aiding or inducing labour or abortion. The latex is used to remove warts though very injurious to the eyes (Samorini, 2016). Eno and Itam (2017) found out that there is a patent hypoglycemic agent(s) present in leaves of *Eleophorbiadrupifera*.

**Comment [H14]:** Use recent reference of this plant

## Conclusion

*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.

**Comment [H15]:** Conclusion can not be ambiguous „like little if so mention the dose at which has medium/low toxic like.

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