EFFECTS OF EXTRACTS OF *Rhizophora mangle* ON SOME HAEMATOLOGICAL PARAMETERS OF WISTAR RATS.

ABSTRACT

Rhizophora mangle is a mangrove plant found mainly in coastal lands of tropical and subtropical regions. It is a medicinal plant applied in folklore remedies in the treatment of fever, toothache, sore throat, constipation, etc.. This study investigated the effects of the leaves of *Rhizophora mangle* on haematological parameters of male Wistar rats. The animals were divided into four (4) groups of five (5) rats each. Group one (1) served as control and received distilled water. Group two (2), group three (3) and group four (4) rats were treated with 200mg/kg bw, 400mg/kg bw and 600mg/kg bw of the ethanolic extract of the leaves of *Rhizophora mangle* respectively, for a period of 30 days. The results indicated that the extract did not significantly ($p<0.05$) affect the red blood cell count, packed cell volume and haemoglobin concentration. The white blood cell (WBC) count and platelet count were not significantly affected following extract administration. The levels of red blood cell indices were not altered. The differential white blood cell count showed that the neutrophils and eosinophils increased significantly in the tests groups but the lymphocyte level decreased significantly. This study have shown that the extract may not be capable to stimulate erythropoietic series because of the non significant changes in RBC count and Hb concentration in male Wistar rats.

Keywords*: Rhizophora mangle, haematological parameters, ethanolic extract, Wistar rats*

INTRODUCTION

"Plants and its products has formed a vital component of traditional medicine as their use for treatment of diseases and maintenance of health and wellbeing has become a common practice in most African societies" (Langlois-Klassen et al., 2007). Traditional medicine which employed the use of plants as its primary source or raw materials, used to be the only form of health-care

system available to the entire African population before the introduction of orthodox medicine (Abdullahi, 2011).

"The use of plants and its products in treatment of illnesses has grown tremendously due to several reasons including, the emergence of some illnesses that are resistant to synthetic drugs, high poverty rate in most developing countries, high cost of synthetic drugs etc. Phytopharmaceutical components are been discovered with the sole purpose of extracting its beneficial pharmacological effect on the human or animal body. These secondary metabolites account for the medicinal properties of these plants" (Dar *et al.,* 2017). There is a promising future with medicinal herbs with more than half a million plants around the world that are not yet studied in terms of their therapeutic potentials (Jamshidi-Kia *et al.,* 2018).

"*Rhizophora mangle,* also known as red mangrove belongs to the *Rhizophoraceae* family; a group of tropical or subtropical flowering plants found mainly in estuary habitats" (Ellison *et al,* 2015; Guo *et al,* 2017). In folklore, this mangrove plant play useful roles in the remedy of fever, toothache, sore throat, dysentery, malaria, constipation and rheumatism etc. (Bandaranayeke, 1998). A wide range of active compounds, including tannins, phenols, saponins, alkaloids, and flavonoids and other organic compounds have all been derived from Rhizophora.

"There are several clinical indicators of health and disease including widely used haematological parameters, such as red and white blood cell counts and haemoglobin concentration. Haematological parameters refers to factors related to blood and blood-forming organs" (Waugh and Grant, 2001; Bamishaiye et al., 2009). "These parameters provides vital information on bone marrow function as well as complications occurring in the intravascular space, such as, haemolysis and anemia" (Adeneye *et al.,* 2006). In recent times, peer review research works on the effects of *Rhizophora mangle* on certain physiological functions started surfacing. For example, Obiandu etal (2023) reported on the effects of the extract on some reproductive functions in the male. There is a dearth of scientific data on the effects of the extract on haematological parameters In addition, there are anedoctal reports suggesting that parts of the plants may be used to treat several illnesses in many cultures especially in Africa. This study was carried out with the objective to investigate the effects of the leaves of *Rhizophora mangle* on haematological parameters of male Wistar rats.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Fresh leaves of *Rhizophora mangle* were collected from an estuary location at Eagle Island in Port Harcourt, Rivers State, Nigeria. The leaves were later authenticated in the herbarium unit, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria; and allotted an identification number ((RSU PB 097). The leaves were washed with water to remove dirt, and dried at room temperature ($26^{o}C$) over a period of 3 weeks. They were grinded using a manual engine grinder to obtain 500g of the fine powder. This quantity was soaked in 400ml of ethanol for 48 hours. The solution was filtered to separate the filtrate from the residue. The extract was concentrated under reduced pressure in a heating mantle at 50^{oC}. The yield of the crude extract of *Rhizophora mangle* leaves obtained weighed 127g. The extract was stored in a refrigerator at 4^{oc} until used.

Experimental animals and protocols

Adult male Wistar rats, bred in the experimental animal centre of Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt, Nigeria, weighing between 150–200g at the beginning of experiment were used for the study. The rats were acclimatized for two weeks, and randomly assigned into 4 groups (n = 5). Group 1 served as control and received distilled water. Group 2 and group 3 received 200mg/kg body weight (bw) and 400mg/kg bw of ethanolic leave extract of *Rhizophora mangle* respectively. Group 4 received 600mg/kg bw of extract. The extracts were administered orally, once daily for 30 days. All animals had access to water and feeds *ad libitum.*

This study was conducted in accordance with the National Institutes of Health's Guide for the care and use of laboratory animals [National Institute of Health, USA. 1985].

Blood collection and estimation of haematological parameters

"The animals were anesthesized using chloroform on day 31, after 24 hours of last extract administration. Blood was collected by cardiac puncture and put into labeled sample tubes containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant for use in determination of haematological parameters based on documented methods" [Ochei and Kolhatkar (2007)].

Haematological parameters estimated include, red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC) count platelet counts, mean

corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and white cell differentials.

Statistical analysis.

Statistical tests were done using the Statistical Package for Social Sciences (SPSS) version 23.0 software. Data were analyzed using one-way ANOVA (analysis of variance of means). Comparisons between the groups were made using post hoc LSD test. Differences at the probability level $P < 0.05$ were considered statistically significant. The results were expressed as mean±standard error of mean (Mean±S.E.M) and presented in tables.

RESULTS

The results for the study are presented in tables 1-4

Table 1: Effect of extract on some haematological parameters.

Groups (mg/kg)	Packed cell volume (%)	Haemoglobin (g/dL)	Red blood cell count (10^{12}/L)
Control	38.40±1.89	12.58±0.53	6.42±0.26
200	41.60±1.83	13.98±0.70	7.48±0.34
400	40.80±2.69	13.00±0.88	6.58±0.64
600	41.00±2.14	12.70±0.67	7.02±0.36

Values presented as Mean±SEM. n=5.

Table 2: Effect of extract on WBC and platelet counts

Groups (mg/kg)	White blood cell count (10^{-9}/L)	Platelet count (10^{9}/L)
Control	7.18±0.87	600.00±51.89
200	8.02±1.84	650.80±12.81
400	15.66±5.29	550.80±55.71

600	11.00±0.71	558.00±88.94

Values presented as Mean±SEM. n=5

Table 3: Effect of extract on some haematological indices.

Groups (mg/kg)	MCHC (g/dl)	MCH (fl)	MCV (pg)
Control	32.04±0.34	17.70±1.76	60.10±1.11
200	31.52±0.26	18.42±0.20	58.40±1.02
400	30.86±0.82	19.82±0.85	64.86±4.78
600	31.04±0.02	18.46±0.14	59.68±0.42

Values presented as Mean±SEM. n=5.

Table 4: Effect of extract on white cell differentials

Groups (mg/kg)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes
Control	21.40±3.80	71.80±4.03	1.40±0.24	5.60±0.24
200	20.60±1.91	71.80±2.24	3.00±0.63*****	7.00±0.63
400	33.20±0.92*****	57.60±1.83*****	2.80±0.49	6.60±0.68
600	30.60±0.68*****	59.00±0.45*****	4.00±0.45*****	6.20±0.49

Values presented as Mean±SEM. n=5. ***** Significant at P<0.05 when compared to control.

DISCUSSION

This study was carried out to investigate the effects of the ethanolic extract of *Rhizophora mangle* on the haematological parameters of male Wistar rats. "The assessment of the haematological parameters provides clear understanding of both the physiological and pathological changes in an animal" (Zhou et al., 2009).

In the present study, the packed cell volume was not significantly altered. "The packed cell volume is the proportion of blood occupied by red blood cells, expressed in percentage while the haemoglobin is the iron-containing coloring matter of red blood cells which is responsible for carrying respiratory gases" (Sembulingam A and Sembulingam P., 2016)[a] . "The haemoglobin concentrations as well as the red blood cell counts were not significantly changed as well. The red blood cells are non nucleated formed elements of blood also referred to as erythrocytes and produced from the red bone marrow in adult life" (Sembulingam A and Sembulingam P., 2016)[b]. The extract of *Rhizophora mangle* did not appear to have significantly influenced the growth and differentiation of the red blood cell series in the bone marrow. Most plant extracts that affect red cell production may contain certain factors that has the capacity to stimulate erythropoiesis or have the ability to inhibit bone marrow production of red blood cells. A direct relationship exist between red blood cell, packed cell volume and haemoglobin concentration (Schalm et al.,1975)., hence, a change in one parameter, affects the other. The non significant change in the packed cell volume and haemoglobin concentration in this study may be because of the non significant effect of *Rhizophora mangle* on red blood cell count, because the relationship existing between these parameters is built around the red blood cells.

"The total white blood cell counts were not significantly ($P<0.05$) altered following extract administration. However, the different doses of the extract administered revealed marginal increases in test groups but the total WBC count remained largely unaltered. WBCs are activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms" (Ganong., 2005). The white cells play an important role as the first line of defense against infection and damage to tissues. The non significant effects suggests that the extract may not have ability to boost the immune system.. Immune boosters may be recommended to strengthen the immune system to enable it fight invading organisms and harmonize the body's degenerative changes (Bendich., 1993 ; Almamary., 2002). "The platelet count was not altered after administration of extracts of *Rhizophora mangle.*. The non significant changes indicate that the extract may not interfere with blood clotting function and hemostasis. Platelets play essential roles in the maintenance of normal homeostasis and platelet aggregation; release of thromboxane A_2, platelet factor 4, and beta-thromboglobulin; and expression of glycogen 1b and glycogen IIb/IIIa receptors are indicators of platelet function" [Giles et al.,(1994); Jakubowski et al., (1983)].

"There were no significant changes in the blood indices including MCHC, MCH and MCV. The normal MCHC values could indicate normal blood osmoregulation, normal plasma osmolarity or normal haemoglobin synthesis. A significant increase in neutrophils was observed in the groups that was administered 400mg/kg and 600mg/kg doses of the extract while a significant decrease was seen in lymphocytes. Neutrophils are the first line of defense against invading microorganisms" (Ganong 2005). The significant drop in lymphocytes could have a detrimental effect on the body's defense system. Eosinophil and monocyte counts showed no appreciable changes. The significant changes in some of the white cell differential counts indicates that the extract of *Rhizophora mangle* may contain certain constituents that alter the balance between the differential white cell line..

CONCLUSION

The extract of *Rhizophora mangle* did not affect RBC count, PCV and Hb concentration in male Wistar rats and so did not affect the function of erythropoietic mechanisms of the bone marrow. The effects on the white blood cell suggest that the extract may not also affect leucopoiesis and immunomodulatory functions in the animal body.

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