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EFFECT OF *Pterocarpus erinaceus* STEM BARK EXTRACTS ON LIVER IN MALARIA INDUCED ANAEMIC MICE

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ABSTRACT
This study is aimed at the effect of *Pterocarpus erinaceus* stem bark extracts on liver function makers in malaria induced anaemic mice. The mice were divided into six groups of seven mice each. Mice were induced with *Plasmodium bergei* of 1 x 10^7 infected erythrocytes. The result shows that there is significant (P>0.05) increase in AST (from 37.43 to 9.57), ALT (from 32.21 to 6.86), total proteins, albumin, bilirubin, glucose (from 36.51 to 5.11), and lipid peroxidation (from 35.70 to 13.70) in malaria control compared to normal control. The group IV to VI were treated with 250 and 500 mg/kg Body weight of both aqueous and ethanolic extracts of *Pterocarpus erinaceus* stem bark respectively for three days shows significant (P>0.05) decreased in all the parameters tested when compared to malaria control group, but there was dose dependent. The aqueous and ethanolic *Pterocarpus erinaceus* stem bark extracts have shown to contract the liver injury cause by *Plasmodium bergei.*

KEYWORDS: *Plasmodium bergei, Pterocarpus erinaceus, Liver function test.*

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INTRODUCTION
Anaemia is a condition of red blood cells insufficient to meet the body's physiological demands. According to the World Health Organization (WHO), the threshold haemoglobin (Hb) level for anaemia is less than 120 g/l for non-pregnant women and 110 g/l for pregnant women aged 15 years and above (WHO, 2011). Anaemia is a global public health problem, with major consequences for human health as well as adverse impacts on social and economic development (WHO 2011). The WHO estimates two billion anaemic persons and anaemia is responsible for one million deaths a year, and about three quarters of cases occur in Africa and Southeast Asia (Osungbade and Oladunjoye, 2012). The global prevalence of anaemia in pregnant and non-pregnant women was 38% and 29%, respectively, in 2011, translated to about half a million anaemic non-pregnant women and 32 million anaemic pregnant women. Worldwide, 50% of anaemia cases are caused by iron deficiency, and other important causes, including infections, nutritional deficiencies, and genetic conditions (WHO, 2012 and Sengtavanh *et al.*, 2021).

Plants have formed the basis of traditional medicine system which has been used for thousands of years. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat or to diagnose and prevent illnesses or maintain wellbeing (WHO, 2003 and Gabriel and Onigbanjo, 2010). *Pterocarpus erinaceus* is a deciduous legume tree of African savannahs and dry forests famous for producing one of the finest woods in its native region. It also produces leafy fodder high in protein, which makes an excellent animal feed crucial for the survival of livestock during dry season (Hutchinson et. al. 1958). It has several common names including vene in French. It is called palissandre in Senegal, Kino in Gambia, Bani or Banuhi in Fulfude (Burkina Faso), Gwani, Ngueni in Bambara, Tolo in Djerma, Bu Natombu in Gourmantche, Noega, Noeka, Pempelaga in More, Ban in Serer, Ven or Yirik In Wolof (Sandrine, 2006) Madubiya in Northern Nigeria and Osun dudu in Southwest Nigeria.

The foliage of *Pterocarpus erinaceus is* a nutritious fodder for farm animals and Mali has an active market for this which is in high demand by sheep farmers for fodder (Hutchinson *et.al.*, 1958). Medicinal uses of *Pterocarpus* plant include the use of the leaves as a febrifuge, the bark for tooth and mouth troubles, and bark resin as astringent for severe diarrhoea and dysentery. The grated root is mixed with tobacco and smoked in a pipe as a cough remedy. It has also been found useful in the treatment of fever (Sandrine, 2006). The aim of this study is to determine the effect of

Pterocarpus erinaceus stem bark on liver function in malaria induced anaemia.

MATERIAL AND METHODS

Experimental animals

Wister albino mice (18-22g) and Wistar albino rats (80-100g) of both sexes obtained from Animal Facility Centre, National Veterinary Research Institute Vom, Jos, Nigeria were used for the study. They were housed in polypropylene cages, and given standard laboratory diet and water *ad libitum* and maintained under laboratory conditions of temperature (22 ± 1^0C) and 12h light and dark cycle. The guide for the care and use of Laboratory Animals, 1996 of the Institute of Laboratory Animal Research (ILAR) Commission on Life Science, National Research Council was duly followed.

Collection of plant material

The stem bark of *Pterocarpus erinaceus* was collected in the month of January, 2021 Konkol village in Maiha Local Government Area of Adamawa state, Nigeria. The plant was taxonomically identified and authenticated in Plant Science Department of Modibbo Adama University of Technology, Yola.

Malaria parasite

The malaria parasite (*Plasmodium berghei berghei)* was obtained from the National Institute of Medical Research, Yaba, Nigeria.

METHODS

Extract Preparation

The stem bark was air-dried for 30 days. It was then reduced to powdered form by grinding n pestle and mortar. One hundred and sixty grams (l60g) of the powdered stem bark was cold macerated in 1000ml of absolute ethanol for 24 hours with constant shaking and filtered using Whatmans filter paper No. 1. It was then concentrated to dryness on water bath at 40'C and the crude extract were kept in a desiccator.

To the aqueous extract, one hundred and sixty grams (160g) of the powdered stem bark was cold macerated in 100Oml of distilled water for 24 hours with constant shaking and filtered using Whatmans filter paper No.1. It was then concentrated to dryness on water bath at 40'C and the crude extract were kept in a desiccator. When required, a known quantity of the extract was taken, dissolved in a krown volume of distilled water to obtain the desired concentration.

Parasite Inoculation

This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting 0.2 ml of infected erythrocytes with Plasmodium berghei from donor mice with 3.0 ml of phosphate buffer saline to give standard inoculums of 0.1 x 10^7. Each mouse was inoculated intraperitoneally on day with 0.2 ml of infected blood containing 0.I x10^7 *P. berghei* parasitized red blood cells. After seventy two hours mice were treated for anaemic condition for four days once daily.

Experimental designed for malaria induced anaemia

Forty two adult albino mice of both sexes weighing 22 ± 0.5g were used in this study. The rats were randomly divided into six groups of even mice per group. Each group group were fed and treated as follows:

Group 1: Served as normal. They received neither inoculated with the parasites nor extracts. They received only normal feeds.

Group 2: Served as malaria control. They were inoculated with 0.2ml of 1 x 10^7 infested erythrocytes containing *Plasmodium berghei* and giving normal feed.

Group 3: They were inoculated with 0.2ml of 1 x 10^7 infected erythrocytes containing *Plasmodium berghei* and administration of 250 mg/kg body weight of aqueous extract of *Pterocarcus erinaceus* stem bark begins on the fourth day for four days.

Group 4: They were inoculated with 0.2ml of 1x10^7 infected erythrocytes containing *Plasmodium berghei* and administration of 500 mg/kg body weight of aqueous extract of *Pterocarcus erinaceus* stem bark begins on the fourth day for four days.

Group 5: They were inoculated with 0.2ml of 1x10^7 infected erythrocytes containing *Plasmodium berghei* and administration of 250 mg/kg body weight of ethanolic extract of *Pterocarcus erinaceus* stem bark begins on the fourth day for four days.

Group 6: They were inoculated with 0.2ml of 1x10^7 infected erythrocytes containing *Plasmodium berghei* and administration of 500 mg/kg body weight of ethanolic extract of *Plasmodium erinaceus* stem bark begins on the fourth day for four days.

Twenty four hours after administration of the extracts, the mice were sacrificed and blood samples were collected using cardiac puncture. The blood samples were put into EDTA containers for haematological analysis. Other blood samples were put into clean container and allowed to stand for 30 minutes to clot and centrifuge at 2500 rpm for 10 minutes and serum was collected for biochemical analysis.

Acute Toxicity Study

Acute oral toxicity was performed using the up- and down-procedure of the Organisation for Economic Co-operation and Development. This procedure involves the limit and main test, which was also used for the selection of a starting dose as well as determination of LD50 of the testing material (OECD, 2004).

Biochemical Analysis

Serum total protein was determined using the biuret method (Doumas, 1975). Albumin was determined using the Bromocresol Green method (Spencer and Price, 1977). Transaminase activity (aspartate aminotransferase (AST) and alanine, aminotransferase (ALT)) was determined using the Reitman and Frankel (1957)

method. Glucose was determined using Glucose oxidase methods and the method of thibarbituric acid was used in determining of Lipid peroxidation.

Statistical Analysis

Data were expressed as mean ± standard error of mean for the given number of observations. The results were analysed using student's t-test. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCURSION

The acute toxicity study indicated that the extract caused no mortality within the first 24 hours as well as for the following 14 days. Physical and behavioural observations of the experimental mice also revealed no visible signs of overt toxicity like lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhoea and the like. This suggests that LD50 of the *Pterocarpus erinaceus* stem bark extract is greater than 5 g/kg.

The result shows significant increase in AST and ALT in malaria control group compared to normal control (table 1). An obvious sign of hepatic injury is the leaking of cellular enzymes such as ALT, and AST into plasma due to the disturbance caused in the transport functions of hepatocytes. ALT is more specific to the liver, and it is a better parameter for analyzing hepatic injury. High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver (Darbar *et al.,* 2011). Treatment with 250 and 500 mg/kg body weight of both aqueous and ethanolic extracts of *Pterocarpus erinaceus* stem bark shows significant reduction in serum enzymes. May be the extracts contracted the deleterious effects of plasmodium bergei in the liver due to the presence of tannins and saponins bioactive components of the extracts.

The alteration in serum bilirubin levels is a biochemical indicator of the changes in morphological integrity of hepatobiliary tract, as a sign of liver damage (Raphael *et al.,* 2020). Bilirubin is a product of haeme within the reticuloendothelia system; its elevation in the blood stream can be adduced to over production, increased hemolysis, decreased conjugation or impaired bilirubin transport. This explains the high bilirubin levels in the malaria control rats compared to the normal controls. Bilirubin is an index that is used to assess the normal functioning of the liver instead of the extent of hepatocellular injury (Sasidharan *et al.,* 2010). The groups treated with *Pterocarpus erinaceus* stem bark extracts showed a significant ($P < 0.05$) decrease of bilirubin levels at the doses of 250 and 500 mg/kg body weight. The decrease in bilirubin level may be attributed to the presence of saponins capable of contracting or protecting the hepatocytes membrane (You and Crabb, 2004).

Liver synthesised the several proteins found in blood (Berr *et al.,* 2001). Administration of extract restored the serum total protein levels around the normal values after the decrease observed in malaria control as shown in table 2. Tannins present in the extracts may be responsible by stimulating the gene for proteins biosynthesis (Amang *et al.,* 2020).

Table 1: Effects of *Pterocarcus erinaceus* stem bark on serum enzyme markers in malaria-induced anaemia.

Treatment	AST (u/I)	ALT (u/I)
Normal	9.57±1.02	6.86±1.06
Malaria control	37.43±1.43	32.21±1.05
Anaemia + 250 mg/kg bw E.E.	25.86±1.14*	18.71±0.68*
Anaemia + 250 mg/kg bw A.E	23.57±1.04*	18.71±0.68*
Anaemia + 500 mg/kg bw E.E	17.29±0.89*	13.79±0.71*
Anaemia + 500 mg/kg bw A.E	15.57±0.78*	12.71±0.46*

Values are mean ± SEM, n=7
*Significantly different as compared to phenylhydrazine control group P<0.05
E.E = Ethanolic extract
A.E = Aqueous extract
AST (Aspartate Aminotransferase)
ALT (Alanine Aminotransferase)

Table 2: Effect of Pterocarpus erinaceus stem bark on serum liver function biochemical markers in malaria-induced anaemia

Treatment	T. Proteins (g/dl)	Albumin (g/dl)	T. Bilirubin (mg/dl)	D. Bilirubin (mg/dl)	Glucose (mmol/l)	Lipid Peroxidatio (nmol/l)
Normal	8.50±0.56	1.39±0.11	0.17±0.02	0.15±0.02	5.11±0.98	13.70±1.14
Malaria	37.74±0.85	16.04±2.44	1.21±0.04	1.21±0.04	36.51±1.18	35.46±1.61
Anaemia+250mg/kg bw E.E	17.51±1.63*	1.78±0.19*	0.49±0.02*	0.42±0.01*	9.82±1.24*	16.91±1.27*
Anaemia+250mg/kg bw A.E	16.37±1.46*	6.17±0.24*	0.54±0.08*	0.43±0.03*	20.05±1.48*	24.98±1.68*
Anaemia+500mg/kg bw E.E	12.46±1.35*	1.54±0.10*	0.43±0.02*	0.39±0.00*	8.32±0.89*	16.92±2.46*
Anaemia+500mg/kg bw A.E	12.84±0.51*	2.19±0.28*	0.17±0.03*	0.28±0.01*	16.50±1.34*	7.14±1.01*

Values are mean ± SEM, n=7

*Significantly different as compared to phenylhydrazine control group P<0.05
E.E = Ethanolic extract,
A.E = Aqueous extract.

CONCLUSIONS

This study shows that *Pterocarpus erinaceus* stem bark extract decreased serum levels of (ALT and AST) transaminases, bilirubin, Glucose, and lipid peroxidation. The hepatic effects observed could be due to the presence of phytochemicals presence such as tannins, flavonoids, alkaloids and saponins.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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