

Effect of the Ethanolic Leaf Extract of *Ficus exasperata* on Biochemical Indices of Albino Mice Experimentally Infected with *Plasmodium berghei* (NK 65)

Lebari B. Gboeloh

Abstract—*Ficus exasperata* is a plant used in the traditional management of malaria in south-south Nigeria. An investigation into the effects of the ethanolic extract of the leaf of the plant on some biochemical indices in albino mice infected with *Plasmodium berghei* (NK 65) was conducted. 48 mice with weight range of 13-23 g were grouped into six (A, B, C, D, E, and F). Each group contained 8 mice. Groups A, B, C, D and E were infected with blood containing the parasite. Group F was not infected and served as the normal control. On the 6th day after infection, 4 mice from each group were sacrificed and blood samples are collected for investigation. The remaining mice in each group were treated. Mice in Groups A, B and C were administered orally with 200, 300 and 500 mg/kg body weight of *Ficus exasperata* respectively for six days. Group D was not treated while Group F was given distilled water. Group E was treated with 5 mg/kg body weight of chloroquine. On the 6th day post treatment, these mice were sacrificed and blood samples were collected for biochemical analysis. The results indicated that on the 6th day post inoculation, the levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) in all the mice infected with the parasite were significantly ($p < 0.05$) elevated. However, on the 6th day post administration of extract, the increased levels of AST, ALP and ALT were significantly ($p < 0.05$) reduced in groups administered with 300 and 500 mg/kg body weight of the extract compared with groups D and F. The reduction in the levels of these enzymes is an indication that *F. exasperata* have no hepatotoxic effect on the mice at the dose levels administered.

Keywords—*Ficus exasperata*, albino mice, *Plasmodium berghei*, biochemical parameters.

I. INTRODUCTION

MALARIA is a common parasitic disease in Nigeria and other countries in sub Saharan Africa [1]. Although, the governments of various African countries made serious commitment to the control of malaria during the Roll Back Malaria Conference held in Abuja, Nigeria in 2000, the disease is still a dangerous public health concern [2].

In Africa, various plants are used for the management of different diseases because of their efficacy against bacterial, fungal and parasitic diseases [3]-[5]. Record indicated that there are more than 400,000 species of flowering plants in Africa that have therapeutic properties [6], [4]. These plants play important role in animal, individual and community

health. For instance, Fulani herdsmen in Nigeria were known to use herbs for the treatment of animal parasitic diseases before the arrival of modern medicine [7].

Ficus exasperata is one of the indigenous plants with antiplasmodial activity especially against *Plasmodium berghei* [8]. In view of this, it becomes pertinent to investigate the effect of the leaf extract of the plant on biochemical parameters.

II. MATERIALS AND METHODS

A. Plant Leaf

Fresh leaf sample of *Ficus exasperata* was collected from Maakuru forest in Gbene Nyobe Beerri community, Khana local Government Area of Rivers State, Nigeria. The plant was authenticated by a botanist using taxonomic keys.

B. Leaf Preparation into Powder Sample

The leaf sample was washed with clean tap water and air dried at room temperature for five days. It was later blended into powder sample with an electric blender.

C. Preparation of Ethanolic Leaf Extract

The ethanolic extract of the leaf sample was made using the methods of [9].

D. Acquisition of Mice and Parasite

Three *Plasmodium berghei* parasitized mice and 48 healthy mice with weight range of 13-23 g used in this experiment were purchased from the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria and transported to the research laboratory, Ignatius Ajuru University of Education, Port Harcourt. The mice were conditioned and allowed to adapt to the laboratory environment for 7 days. They were fed with feed produced by Brand Cereals and Oil Limited, Jos, Nigeria using the method of [9]. The mice were handled according to the guidelines for the care and use of laboratory animals [10].

E. Inoculation of Mice with Parasites

The mice exhibited symptoms of malaria on the 5th and 6th day post inoculation and they were sacrificed on the 6th day post inoculation. The mice were sedated using cotton wool soaked in chloroform. Syringes fitted with sterile needles were used to obtain blood from the mice by cardiac puncture. The

L.B Gboeloh is with the Department of Biology, Ignatius Ajuru University of Education, P.M.B 5047, Port Harcourt, Rivers State, Nigeria (phone: +2347030335178; e-mail: lgboeloh@yahoo.com).

blood was homogenized in physiological saline in the ratio of 1 ml of blood to 10 ml of physiological saline.

F. Experimental Design

48 healthy mice were divided into 6 groups of 8 mice per group (A, B, C, D and F) according to their weight. Groups A, B, C, D and E were inoculated with 0.3 ml of the blood-containing parasite six days before treatment intraperitoneally and on the 6th day post inoculation, Groups A, B and C were treated with 200, 300 and 500 mg/kg body weight (b.w) of the ethanolic leaf extract of *F. exasperata* respectively for six days. Group D was not treated and Group E was administered with 5 g/kg b.w of chloroquine for the same period. Group F was neither infected nor treated but fed on distilled water. Four mice from each group were sacrificed on the 6th day post infection and blood samples were collected for investigation. Also, the remaining 4 mice in each group were sacrificed on the 6th day post administration of extract. Blood from the sacrificed mice were obtained from the heart and stored in labelled specimen bottles for investigation of changes in biochemical indices.

G. Biochemical Analysis

Using a variable volume micropipette, 0.5 ml of the blood samples were collected into centrifuge tubes and centrifuged at 3,000 rpm for 10 minutes to recover the serum from the whole blood. With the aid of sterile syringes equipped with needles, the sera were collected into specimen bottles. The biochemical parameters investigated included: AST, ALT, ALP, cholesterol, triglyceride, albumin and total protein.

H. Determination of AST, ALT, ALP, Cholesterol and Triglyceride (TG) Using Teco Diagnostic Reagent

AST and ALT were estimated using the UV-Kinetic method [11] and measurement is made using a reference procedure recommended by [12]. ALP was determined by measuring the rate of hydrolysis of various phosphate esters using the method of Kinetic assay [13] as modified by [14]. Serum cholesterol level in the mice was determined by a method adopted by [15]. Using a precision variable-volume micropipette, 0.01 ml of serum from experimental animal was collected into sterile plain tubes to which was added 1.5 ml of reconstituted reagent. The reagent was incubated in a water bath at 37 °C for 10 minutes. The content was then transferred into the tubes contained in SP-3000 nano Optima UV visible

scanning spectrophotometer and the absorbance was measured.

I. Data Analysis

Data analysis was done using SPSS version 17.0 at statistical difference of $P < 0.05$. Values were expressed as Mean \pm SEM (Standard error of mean) and differences between groups are determined using one-way ANOVA.

J. Ethical Consideration

The Ethics Committee, Ignatius Ajuru University of Education, Port Harcourt, Nigeria gave approval for this study

III. RESULTS

The results indicated that in mice infected with *P. berghei*, there was no significant difference ($p > 0.05$) in the level of AST when treated with 200, 300 and 500 mg/kg bw of the extract (Table I). There was also no significant difference in the activities of ALP and ALT at the dose level of 200 mg/kg b.w compared with Groups D, F and E. However, at dose levels of 300 and 500 mg/kg b.w, a statistically significant ($p < 0.05$) reduction in the levels of ALP and ALT were observed when compared with Group D (Table I).

The effect of the extract on cholesterol and TG levels of infected mice is summarized in Table II. The results showed that there was an initial non-significant increase in the mean value of the level of cholesterol of mice treated with 200 mg/kg b.w of *F. exasperata* when compared with Group D. However, the observed increase was reversed at the dose levels of 300 mg/kg and 500 mg/kg b.w of the extract. The reduction in cholesterol level was significant ($p < 0.05$) when compared with Groups E and F.

There was also an initial increase in the level of TG in mice that received 200 mg/kg b.w of *F. exasperata* when compared with Groups D, F and E. However, at the dose levels of 300 and 500 mg/kg b.w, a significant reduction in the level of TG was observed compared with Groups D, F and E (Table II).

The levels of albumin and Total Protein (TP) of mice administered with 200 mg/kg b.w of the extract showed no significant difference compared with Groups D, F and E (Table III). However, in mice that received 300 and 500 mg/kg b.w of the extract, a numerical but non-significant elevation in the levels of albumin and TP was observed when compared with Groups D and E (Table III).

TABLE I
EFFECT OF ETHANOLIC LEAF EXTRACT OF *F. EXASPERATA* ON BIOCHEMICAL INDICES OF MICE INFECTED WITH *PLASMODIUM BERGHEI*

Group	Extract	Dosage (mg/kg)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
A	<i>F. exasperata</i>	200	121.38 \pm 37.96 ^b	214.37 \pm 37.96 ^b	52.6 \pm 8.46 ^b
B	<i>F. exasperata</i>	300	87.48 \pm 72.69 ^c	248.85 \pm 84.34 ^b	47.29 \pm 39.6 ^c
C	<i>F. exasperata</i>	500	74.38 \pm 33 ^c	179.89 \pm 50.55 ^b	47.74 \pm 11.46 ^c
D _(Control)	-	-	212.14 \pm 12.37	214.81 \pm 135.22	169.73 \pm 232.53
E _(Control)	Chloroquine	5	152 \pm 36.09	200.99 \pm 97.27	24.75 \pm 15.11
F _(Control)	Distilled water		159.1 \pm 16.11	199.7 \pm 5.3	20.33 \pm 5.5

Data are expressed as mean \pm SEM (n=4). Values with superscript a showed a significant difference ($p < 0.05$) when compared with Group F, values with superscript b showed no significant difference between groups while values with superscript c showed significant difference when compared with Group D and values with superscript d showed significant difference when compared with Group E.

TABLE II
EFFECT OF ETHANOLIC LEAF EXTRACT OF *F. EXASPERATA* ON CHOLESTEROL AND TG OF MICE INFECTED WITH *PLASMODIUM BERGHEI*

Group	Extract	Dosage (mg/kg)	Cholesterol	TG
A	<i>F. exasperata</i>	200	118.29±35.62 ^{ad}	282.04±103.34 ^{acd}
B	<i>F. exasperata</i>	300	62.86±19.19 ^{ad}	96.12±74.99 ^{ad}
C	<i>F. exasperata</i>	500	58.00± 6.56 ^{ad}	58.37±37.1 ^{ad}
D _(Control)	-	-	72.86±26.71	129.39±15.51
E _(Control)	Chloroquine	5	206.93±38.29	135.31±38.45
F _(Control)	Distilled water		199.22±6.96	163.42±24.81

Data are expressed as mean ± SEM (n=4). Values with superscript a showed a significant difference (p<0.05) when compared with Group F, values with superscript b showed no significant difference between groups while values with superscript c showed significant difference when compared with Group D and values with superscript d showed significant difference when compared with Group E.

TABLE III
EFFECT OF ETHANOLIC LEAF EXTRACT OF *F. EXASPERATA* ON ALBUMIN AND TP OF MICE INFECTED WITH *PLASMODIUM BERGHEI*

Group	Extract	Dosage (mg/kg)	Albumin (g/dl)	TP (g/dl)
A	<i>F. exasperata</i>	200	3.38±0.35 ^b	4.74±0.75 ^b
B	<i>F. exasperata</i>	300	4.55±0.79 ^{cd}	6.26±0.76 ^{cd}
C	<i>F. exasperata</i>	500	4.26±0.75 ^{cd}	7.91±0.47 ^{ad}
D _(Control)	-	-	3.73±0.33	4.69±0.67
E _(Control)	Chloroquine	5	3.26±0.36	4.58±0.71
F _(Control)	Distilled water		3.25±0.34	4.59±0.73

Data are expressed as mean ± SEM (n=4). Values with superscript a showed a significant difference (p<0.05) when compared with Group F, values with superscript b showed no significant difference between groups while values with superscript c showed significant difference when compared with Group D and values with superscript d showed significant difference when compared with Group E.

IV. DISCUSSION

The assessment of the levels of many enzymes in the body of animals have tremendously assisted in the determination of disease conditions and determination of the level of toxicity or otherwise of medicinal plants and other therapeutic products [16], [17]. For instance, ALP is used as an indicator enzyme for the assessment of the integrity of endoplasmic reticulum and cellular membrane [18] and it is used to assess the integrity of the plasma membrane [19], [17]. The determination of the activity of aminotransferase enzymes can also provide valuable confirmatory or suggestive values of the effects of extracts on the liver [20].

Although interspecies differences in the pharmacokinetic parameters in humans and animals make it difficult to translate some adverse effects of herbal products from animals to humans [21], animal models are experimentally used to determine the safety of herbal products.

The results of the biochemical investigation of the mice used in this study revealed that there was elevation in the levels of ALP, AST and ALT of *P. berghei* parasitized and untreated mice in Group D compared to normal control (Group F). This suggested pathologic effect of malaria on liver functions and according to [22], significant elevation in the activities of serum enzymes indicates hepatotoxic injury while similar reduction in concentration is an indicator for liver impairment. The observed pathology in the liver may be as a result of the functional role of the liver and its position as a major organ involved in the early developmental stage of *Plasmodium*. This could result in changes in the morphology and physiology of hepatocytes in the host [23]. The results

recorded here is in agreement with earlier studies [24], [25], [17]. The researchers recorded increase in the levels of ALT, AST and ALP in *P. berghei* infected mice. Reference [26] also recorded similar result in *P. berghei* infected rats.

In this study, the elevated levels of ALT and ALP recorded in *P. berghei* infected mice were significantly (p<0.05) reduced by the administration of the extract, an indication that there was improvement in hepatic functions of treated mice. Results obtained in this study are slightly different from the report of [27]. Reference [27] recorded a significant elevation in the level of ALP in animals treated with 150 and 300 mg/kg b.w of aqueous leaf extract of *Bryophyllum pinnatum*. This suggests that the proper functioning of the liver may have been impaired by the extract [28]. Reference [17] also recorded an elevation in liver AST, ALP and AST after 14 days of treatment of mice infected with *P. berghei* with 200 mg/kg body weight of methanolic extract of *Carica papaya* and *Alstonia boonai* separately. The result is also contrary to the findings of [29] who recorded increased serum urea and sodium levels in albino rats treated with 500 mg/kg b.w of ethanolic leaf extract of *F. exasperata*.

This study recorded a significant reduction in the levels of ALP and ALT (from 212.14±12.37 in Group D to 87.48±72.69 and 74.38±33 in Groups B and C respectively) at dose levels of 300 and 500 mg/kg body weight when compared with infected and untreated mice (Group D). At the dose levels of 200, 300 and 500 mg/kg body weight, there was no significant difference in the level of AST in treated mice (Groups A, B and C) when compared with Groups E and F. This suggested that the extracts did not have any hepatotoxic effect or renal impairment in mice. AST is a major marker of liver injury. AST and other enzymes (lactate dehydrogenase and creatine kinase) are markers of skeletal and myocardial muscles [30], [31]. When myocardial cells containing AST are damaged due to insufficient oxygen supply or glucose, the cell membrane becomes permeable or ruptures causing the leakage of these enzymes [32] [33], so a non-significant difference in the level of AST in treated animals when compared with Group F suggested no leakage of liver enzymes due to hepatic injury, thus confirming the muscular-protective activity of the leaf of *F. exasperata*. This result agreed with the study conducted by [33] on the cardio protective effects of polyherbal formulation in wistar rats. The result is however contrary to the reports of [34], [35] who recorded no significant difference in the levels of ALP and ALT in mice

treated with extract of *A. sativum*. ALT is a cytoplasmic enzyme found in high concentration in the liver [35] and an elevation in the level of this enzyme in the blood serum indicate hepatocellular injury [37], [38], [26].

Again, this study recorded a gradual decrease in serum cholesterol (CH) and TG levels with increased concentration of the extract. CH is a principal sterol in animal tissue and occurs in cell membrane [39], [40]. The initial elevation in the levels of CH and TG in mice treated at the dose level of 200 mg/kg body weight were significantly ($p < 0.05$) reduced at the dose levels of 300 and 500 mg/kg body weight when compared to Groups D, E and F.

The observed reduction in the levels of CH and glyceride by the extract used in this experiment is an indication that the leaf of the plant may contain saponin. Saponin is a known anti-nutritional factor which reduces the uptake of certain nutrients especially CH at the gut through intraluminal physiochemical interactions. Similar results were recorded by [41] using the aqueous and ethanolic extract of *Blighia sapida* in rats, and [42] using *Glycyrrhiza glabra* (licorice) root extracts in mice and [24] using the combined extracts of *F. exasperata*, *A. vogelii* and *V. album* in mice.

The levels of TP and albumin of mice administered with 200 mg/kg b.w had no significant value but a numerical difference was noticed in mice treated with 300 and 500 mg/kg b.w. This could be as a result of an increase in certain enzymes and proteins earlier released because of compromise in the integrity of cell membrane caused by the malaria parasite [43]. Similar result was recorded by [44] in rats treated with extract of *Viscum album*.

V.CONCLUSION

The results recorded in this study indicated that the ethanolic extract of the leaf of *F. exasperata*, at the dose levels administered, have no hepatotoxic effect on the mice. This may explain and supports the traditional use of the plant for management of malaria by the people of south-south Nigeria.

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