Biochemical, Toxicological, and Histological Changes associated with the use of *Croton zambesicus* as Anti-Malarial Decoction.

O.A. Akinloye[1*]; E.A. Balogun[2]; O.M.O. Idowu[3]; H. Njoku[1]; and R.N Ugbaja[1]

[1]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.
[2]Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.
[3]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria.

E-mail: oaakin@yahoo.com*
Telephone: +2348030824063

ABSTRACT

Malaria, a disease of antiquity, has proved to be a formidable deterrent to the cultural and social-economic progress of man in tropical and sub-tropical zones of the world. Recently, one of the major problems in malaria control is drug resistance. Therefore, this study was designed to elucidate the potentials of *Croton zambesicus* leaf extract in the treatment of malaria as well as its biochemical and histopathological effects as an antiplasmodial agent.

Treatments were administered orally. The mice were infected with $1x10^7$ of *Plasmodium berghei* intraperitoneally. They were sacrificed twenty-four hours after the last administration. Liver function enzymes activities namely: Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) were determined. Lipid peroxidation, cholesterol, triacyglycerol levels and histology of liver and kidney were investigated. Results revealed that the percentage parasitemia of infected mice treated with chloroquine decreased significantly (p<0.05) compared to the control. Infected chloroquine treated mice had increased percentage chemosuppression (92.4%) compared with infected extract treated groups. Ethanolic extract had increased percentage inhibition (19.63%) compared with aqueous extract (17.36%).

Infected untreated mice had increased levels of plasma ALT, AST and ALP compared to infected treated groups. ALT and AST activities of infected ethanolic extract treated mice increased significantly (p<0.05) compared with infected aqueous extract treated groups while infected aqueous extract treated mice had slight increase in ALP activity. Cholesterol of infected aqueous extract treated and chloroquine treated groups increased significantly (p<0.05) while that of ethanolic extract treated group decreased significantly (p<0.05) compared with control. Triacyglycerol level of infected 200mg/kg b.wt aqueous and 200mg/kg b.wt ethanolic extract treated groups decreased significantly (p<0.05) compared to the groups treated with 100mg/kg b.wt dose.

Infected aqueous extract treated groups had significant (p<0.05) increase in malonialdehyde concentration. There was a significant decrease in Packed Cell Volume (PCV) of infected extract treated groups while chloroquine treated and uninfected extract treated had significantly (p<0.05) increase PCV. The hepatocyte of the extracts treated groups showed diffused mild vacuolar degeneration, though with no observable pathological effect on the kidney. The results suggest that both aqueous and ethanolic leaf extracts (200mg/kg b.wt) of *Croton zambesicus* exhibited antimalarial activity comparable to chloroquine, coupled with alteration of some biochemical parameters and histological changes in some vital organs of biotransformation in mice.

(Keywords: biochemical, toxicological, histological, *Croton zambesicus*, antimalaria)

INTRODUCTION

Malaria is a global disease prevalent in the tropics caused by plasmodium species. In Nigeria, malaria is endemic throughout the country. World Health Organization has estimated malaria mortality rate for children under five in Nigeria at 729 per 100,000 (WHO, 2005). Resistance to drug by both the

mosquitoes and the parasite is a growing obstacle in the battle against malaria. Combination therapy has been shown to increase the efficacy of combining drugs (Toure and Oduola, 2004).

Recently, African governments have taken the bold step to fight the proliferation of the disease through some initiative programs such as Global Roll Back Malaria Programme, Malaria in Pregnancy East and South African coalition for prevention and control (MIPESA), National Control Programme. Resistance of the malaria parasites to some commonly used antimalarial drugs has necessitated intensified research in the area of development of new antimalarial drugs especially from medicinal plants. Over 1,200 plants species from 160 families have been reported to be used in the treatment of malarial or fever (Willcox and Bodeker, 2004), but there is a dearth of information on their level of toxicity.

Croton zambesicus belong to the family *Euphorbiaceae,* genus *Croton* and it is commonly known as Ajekofole in Yoruba, Koriba in Hausa. *Croton zambescicus* is a shrub of about 10-16m high, often branching low with a spreading crown and characteristics hanging leaves, which are slivery in their abaxial surfaces.

Ethonobotanically, the leaf decoction is used as antihypertensive and antimicrobial against urinary infection (Adjanohoun *et al*., 1989) and also as an anti-diabetic and malarial remedy (Okokon *et al*., 2005). The leaf alkaloidal fraction has been reported to possess a wide spectrum of antibacterial property (Arbonnier, 2004). The ethanolic leaf and root extracts have been reported to possess antiplasmodial, antipyretic as well as antiulcer activities (Okokon *et al*., 2005).

Herbal medicines are widely used to treat malaria and is often more available than modern medicine, but there is scarcity of information on how safe these herbal medicine are. One of such plant commonly used against malaria is *Croton zambesicus*. This study was therefore aimed at investigating some biochemical, antiplasmodial / chemosuppression, toxicological and histopathological changes that may be associated with the use of *Croton zambesicus* as an antiplasmodial agent in mice.

MATERIALS AND METHODS

Experimental Animal

Forty four adult swiss albino mice with an average weight of 23.35g were obtained from the animal breeding unit of the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. The mice were housed in plastic cages, allowed to acclimatize for two weeks and maintained under standard conditions with free access to rat pellets and water *ad-libitum.*

Parasites

A Chloroquine-sensitive strain of *Plasmodium berghei* was obtained from the Institute for Advanced Medical Research and Training (IAMRAT) College of Medicine, University of Ibadan, Ibadan, Nigeria.

Plant Materials

Fresh leaves of *Croton zambesicus* were harvested in Ilisan, Ikenne Local Government Area, Ogun-State, Nigeria and were authenticated by Dr. D.O Aworinde a Plant Taxonomist in the Department of Biological Science, Federal University of Agriculture, Abeokuta, Nigeria.

Plant Extracts Preparation

Fresh leaves of the plant were air dried for 2 weeks at room temperature. The air dried plant materials was ground to powder with a motor powdered mill. Cold maturation traditional method was used in different solvents (namely water and ethanol) to obtain the crude extract.

Animal Grouping and Extract Administration

The animals were randomly divided into eleven groups: I, II, III, IV, V, VI, VII, VIII, IX, X and XI of four mice in each. Animals in groups I, VII, VIII, IX and X were not inoculated with parasite while those in groups II, III, IV, V, VI and XI were inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *P. berghei* parasitized red blood cells.

After inoculation, the mice were kept for five days to allow for establishment of infection, parasitaemia was well established on the 5th day by screening for malaria parasites (Ryley and Peters 1970).

The mice were treated thus:

Group I: (uninfected mice): received an appropriate volume of distilled water

Group II: (infected mice): received an appropriate volume of water

Group III: (infected mice): received the aqueous extract of 100mg/kg body weight daily.

Group IV: (infected mice): received the ethanolic extract of 100mg/kg body weight daily

Group V: (infected mice): received the aqueous extract of 200mg/kg body weight daily

Group VI: (infected mice): received the ethanolic extract of 200mg/kg body weight daily.

Group VII: (uninfected mice): received the aqueous extract of 100mg/kg body weight daily.

Group VIII: (uninfected mice): received the ethanolic extract of 100mg/kg body weight daily.

Group IX: (uninfected mice): received the aqueous extract of 200mg/kg body weight daily.

Group X: (uninfected mice): received the ethanolic extract of 200mg/kg body weight daily.

Group XI: (infected mice): received chloroquine solution of 10mg/kg body weight daily for three days.

Administration of the extracts was carried out for 10 days.

Estimation of Parasitaemia in Experimental Mice

The parasitaemia was estimated by examination of well-stained thin blood films of blood collected from the tail end of the mice.

Percentage chemosuppression of parasitaemia by the various treatments was calculated by subtracting the average percentage parasitaemia in the treated group from the average percentage parasitaemia in the control group and the value obtained was expressed as a percentage of the average percentage parasitaemia in control group.

Sample Collection and Analyses

The blood samples was collected from each mice by cardiac puncture into heparinized tubes, centrifuged at 3000 rpm for 15 minute and the clear plasma supernatant stored at -4^{o}C until needed for analysis.

Determination of the Effect of Extract on Some Biochemical Parameters of Mice

The various plasma samples collected after treatment of the animals were analyzed according to standard methods for effect of the extract on various biochemical parameters of mice such as cholesterol, triacylglyceride, aspartate aminotransferases (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), Lipid peroxidation, Total antioxidant potential of the plant.

Lipid Peroxidation Determination

The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS) using malondialdehyde as standard, following the method described by Buege *et. al*. (1978)

Alkaline Phosphatase Determination

Alkaline phosphatase (ALP) activity was estimated by spectrophotometrically using p-nitrophenyl phosphate as substrate according to the method of Englehardt *et al.,* (1970).

Aspartate and Alanine Aminotransferase Determination

Plasma aspartate aminotransferase (AST) and alanine aminotransferase were estimated using Randox reagent kit according to the methods of Reitman and Frankel (1957) and Schmidt and Schmidt (1963).

Cholesterol and Triglyceride Determination

Cholesterol and triglyceride levels were determined using the Randox Assay Kits

Total Antioxidant Potential Determination

The radical scavenging activity of the plant extract against 2,2-Diphenyl-1-picrylhydrazyl radical was determined spectrophotometrically at 517nm according to the method of Ayoub, *et.al*., (2010).

Effect of Extracts on Histology of Some Organs: The liver and kidney excised from the mice were fixed in 10% formaldehyde were processed, sectioned and stained with Heamatoxylin and Eosin (H & E) following the standard procedures.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by Duncan Multiple Range Test. Statistical significance difference was considered at $p < 0.05$.

RESULTS

Effect of Treatments on Parasitemia Load

Table 1 shows that the parasite infected untreated group had their parasitaemia level increased with days of infection. The infected treated groups with the standard drug (chloroquine) had a rapid decrease in parasitaemia in the course of treatments compared with infected untreated group (positive control). This shows that there was a significant difference when compared with the infected untreated group ($p< 0.05$). There was a decrease, although not significantly different, in the parasitemia load of groups treated with aqueous extracts and ethanolic extracts.

Effect of Treatments on the Body Weight

The results in Table 2 shows that the parasite infected untreated mice had a decrease in body weight, which is significant when compared with negative control. The infected mice treated with aqueous and ethanolic extract showed a slight increase in body weight, but not significant compared with negative control.

Table 1: Effect of Treatments on Parasitaemia Load of Mice.

Treatment	Day 8	Day 11	Day 15
Infected untreated	3.40 ± 1.71^{b}	4.57 ± 1.01^{b}	6.30 ± 1.56^{b}
Infected aqueous extract treated (100mg/kg)	1.53 ± 0.47^{a}	1.03 ± 0.21^{a}	0.77 ± 0.21^{a}
Infected Ethanolic Extract treated (100mg/kg)	1.13 ± 0.29^{a}	0.87 ± 0.15^{a}	0.63 ± 0.06^{a}
Infected aqueous extract treated (200mg/kg)	1.08 ± 0.22^{a}	0.87 ± 0.15^{a}	0.67 ± 0.15^{a}
Infected ethanolic extract treated (200mg/kg)	1.43 ± 0.51^{a}	1.00 ± 0.20^{a}	0.75 ± 0.21^{a}
Infected chloroquine treated (10mg/kg)	1.20 ± 0.29^{a}	0.80 ± 0.23^{a}	0.48 ± 0.09^{a}

Each value represent the mean ±S.D of n= 4
Mean values within the same column having same superscript are not significantly different at $p > 0.05$

Table 2: Effects of Treatments on the Mean Body Weight of Mice.

Group	Mean weight before treatment (mg/kg)	D8	D11	Mean weight after completion of treatment(mg/kg)
1	23.36±0.81^{a}	22.12±0.43^{ab}	22.92±1.25^{b}	23.63±0.09^{b}
2	23.22±1.76^{a}	21.70±0.70^{a}	19.81±0.26^{a}	19.63±0.53^{a}
3	23.73±1.21^{a}	23.43±0.95^{abc}	23.33±1.12^{b}	24.13±0.53^{b}
4	23.29±1.30^{a}	23.03±1.19^{abc}	23.42±1.12^{b}	23.57±0.94^{b}
5	24.03±0.73^{a}	23.38±1.23^{abc}	23.88±1.16^{b}	24.28±1.24^{b}
6	22.78±1.60^{a}	23.32±1.45^{abc}	23.38±0.31^{b}	22.88±1.10^{b}
7	24.01±1.15^{a}	23.78±0.99^{bc}	23.86±0.98^{b}	23.61±1.25^{b}
8	22.74±1.88^{a}	23.56±0.98^{bc}	24.09±0.52^{b}	24.43±0.18^{b}
9	23.27±1.30^{a}	24.28±0.93^{c}	24.17±0.44^{b}	24.21±0.13^{b}
10	22.74±1.31^{a}	23.78±0.49^{bc}	24.45±0.68^{b}	24.40±0.89^{b}
11	23.63±0.83^{a}	23.51±1.18^{bc}	24.50±1.01^{b}	24.32±1.11^{b}

Values are meant **±** S.D n = 4. Values within the same column having different superscripts are significantly different at p < 0.05

Group 1: Negative control

2: Infected untreated mice

3: Infected mice treated with 100mg/kg aqueous extract

4: Infected mice treated with 100mg/kg ethanolic extract

5: Infected mice treated with 200mg/kg aqueous extract

6: Infected mice treated with 200mg/kg ethanolic extract

7: Uninfected mice treated with 100mg/kg aqueous extract

8: Uninfected mice treated with 100mg/kg ethanolic extract

9: Uninfected mice treated with 200mg/kg aqueous extract

10: Uninfected mice treated with 200mg/kg ethanolic extract.

11: Infected mice treated with 10mg/kg chloroquine solution.

At higher dose, the aqueous and ethanolic extract treated infected mice showed no significant difference when both were compared. There was slight increase in body weight of infected mice treated with chloroquine, but not significant compared with negative control and the extract treated groups. The uninfected mice treated with aqueous and ethanolic extract showed an increase in body weight but not significant when compared to control.

Effect of Treatment on Packed Cell Volume

Infected mice treated with standard drug had an increase in PCV, but not significant (p> 0.05) compared with the negative control PCV. All the infected mice treated with aqueous extracts and ethanolic extracts had a decrease in their PCV values while the uninfected mice treated with these extracts had an increased PCV. There was a significant difference when infected mice treated with high dose of aqueous and ethanolic extracts after completion of treatment were compared with the standard drug (chloroquine) (Table 3).

Table 3: The Effect of Treatment on the Packed Cell Volume of Mice.

Group	Initial Mean PCV	D8	D11	Final Mean PCV
1	55.00±5.00^{a}	64.00±3.61^{c}	65.00±2.65^{e}	69.00±1.41^{c}
2	54.67±8.74^{a}	52.00±7.94^{a}	49.67±4.51^{a}	50.00±2.83^{a}
3	62.00±2.94^{a}	59.67±2.52^{abc}	57.67±2.52^{bcd}	57.50±2.12^{abc}
4	62.00±2.94^{a}	54.00±3.65^{ab}	52.00±4.58^{ab}	56.00±5.29^{ab}
5	64.25±5.32^{a}	57.00±4.76^{abc}	56.67±4.16^{bc}	55.00±9.89^{ab}
6	60.25±5.74^{a}	57.67±2.08^{abc}	55.50±2.12^{abc}	51.00±8.48^{a}
7	61.00±2.71^{a}	64.75±4.57^{c}	63.25±3.30^{de}	63.50±6.25^{bc}
8	58.50±6.14^{a}	60.00±3.74^{abc}	60.67±2.08^{cde}	64.50±2.12^{bc}
9	64.00±4.32^{a}	62.25±3.40^{bc}	60.67±4.04^{cde}	66.00±4.58^{bc}
10	63.75±8.54^{a}	60.00±8.83^{abc}	60.00±3.00^{cde}	66.33±4.73^{bc}
11.	61.75±6.13^{a}	61.00±2.94^{bc}	61.50±3.00^{cde}	67.67±3.78^{c}

Values are mean ± S.D n = 4
Values within a column having different superscript are significantly different at p< 0.05

Effect of Treatments on Lipid Profile Parameters

There was reduction in the level of cholesterol of the infected untreated group compared with the negative control. The infected aqueous extract (100mg/Kg) treated group had increased level of cholesterol, but at high dose (200mg/Kg), there was a decrease in the cholesterol level.

The cholesterol levels in all the treated groups (except groups IV and IX) were found to be higher than that of negative control. The infected ethanolic extract treated (100mg/Kg) group had the highest cholesterol value followed by infected chloroquine treated group. There was a significant ($p<0.05$) difference in the cholesterol levels of infected aqueous and infected ethanolic treated (100mg/Kg) group.

There was a significant ($p<0.05$) reduction in triglycerides level of infected untreated group compared with the control. The uninfected ethanolic treated (200mg/Kg) group had the highest triglyceride level, followed by the infected ethanolic extract treated (200mg/Kg) group while the infected untreated group had the least.

The extent of lipid peroxidation (as measured by the concentration of MDA) was much more pronounced in infected chloroquine treated group while infected aqueous extract treated groups had least value of peroxidation. There was a significant difference in the values of MDA among uninfected aqueous extract treated group and ethanolic extract treated (100mg/Kg) group.

Effect of Treatments on the Activities of Some Enzymes

There was an increase in Alanine aminotransferase (ALT) in infected untreated group. There was a decrease in ALT in infected aqueous extract treated, which exhibit significant difference compared with infected untreated. More so, the infected ethanolic extract treated had an increase in ALT level when the concentration was increased, there was significant difference compared with infected untreated. Infected chloroquine treated had an increase in ALT level, which was non-significant when compared with infected untreated and infected ethanolic extract treated; but significant compared with infected aqueous treated .

Table 4: Effect of Treatments on Lipid Profiles Parameters.

Treatment	Dose mg/kg	Cholesterol (mg/dl)	Triglyceride (mg/dl)	MDA (µm/mg)
Negative control	-	65.86±0.91^{c}	88.95±1.42^{b}	4.26±0.45^{e}
Infected Untreated	-	52.64±1.82^{ab}	45.48±8.53^{a}	2.61±0.33^{bc}
Infected aqueous extract treated	100	93.19±4.72^{de}	88.57±13.32^{b}	1.31±0.06^{a}
Infected ethanolic extract treated	100	44.76±0.64^{a}	113.38±3.76^{c}	2.25±0.28^{b}
Infected aqueous extract treated	200	71.25±13.26^{c}	57.78±2.12^{a}	3.26±0.06^{d}
Infected ethanolic extract treated	200	68.17±2.00^{c}	109.78±4.17^{bc}	2.35±0.09^{bc}
Uninfected aqueous extract treated	100	85.07±7.01^{d}	100.76±14.23^{bc}	3.95±0.15^{e}
Uninfected ethanolic extract treated	100	132.35±5.62^{g}	57.92±3.02^{a}	4.00±0.04^{e}
Uninfected aqueous extract treated	200	97.43±7.44^{e}	89.82±4.44^{b}	4.19±0.32^{e}
Uninfected ethanolic extract treated	200	59.82±3.12^{bc}	109.86±10.39^{bc}	2.84±0.28^{cd}
Infected chloroquine treated	10	109.97±2.24^{f}	106.79±12.25^{bc}	5.58±0.22^{f}

Values are mean **±** S.D n = 4
Values within a column having different superscripts are significantly different at p< 0.05

More so, the uninfected aqueous and ethanolic extracts treated had increase level of ALT, which showed significant difference compared with control(P< 0.05).

There was an increase in the level of AST in infected untreated mice; but this does not exert any significant difference when compared with 200 mg/kg ethanolic extract of infected treated group. Increase in the concentration of the ethanolic extract causes an increase in the level of AST. The infected aqueous extract treated had an increase in the level of AST at high dose, thus showed significant difference compared with the infected untreated. The infected chloroquine treated group had an increase in the AST level, but did not show any significant difference when compared with infected aqueous and ethanolic extract treated groups. There was a significant increase in AST of uninfected group treated with high dose of ethanolic extract compared with negative control, while uninfected aqueous extract treated at high dose had a decrease AST level which is non-significant compared with control.

There was significant increase in the level of ALP of infected untreated group compared with control. The infected aqueous extract treated at higher dose showed slight increase in ALP, while the ethanolic extract treated had a slight decrease in ALP. Both infected extracts treated did not produce any significant difference when compared.

Infected-chloroquine treated did not produce any significant difference when compared with infected aqueous and ethanolic extracts treated (P< 0.05). There was a marked significant difference when the infected chloroquine treated was compared with infected untreated. Moreso, the uninfected aqueous extract treated had an increase in ALP level at high dose, while uninfected ethanolic extract treated had a decrease level of ALP.

There was significant difference when uninfected aqueous extract treated was compared with the control ,while the uninfected ethanolic extract treated (200mg/kg) showed non-significant difference compared with control.

Effect of Treatments on the Weight of Organs

There was significant difference in the weight of organs (liver and kidney) treated with chloroquine compared with the negative control. The aqueous and ethanolic extract treated groups shows no significant difference when compared with the negative control group.

Table 5: Effect of Treatments on the Activities of Some Enzymes.

Treatment	Dose mg/kg	ALT (U/L)	AST (U/L)	ALP (U/L)
Negative control	-	48.50±2.12^{ab}	35.50±0.71^{a}	6.21±0.98^{a}
Infected Untreated (positive control)	-	80.00±4.24^{e}	87.50±2.12^{e}	28.98±1.95^{d}
Infected aqueous extract treated	100	44.50±2.12^{a}	56.50±2.12^{b}	6.90±0.00^{a}
Infected ethanolic extract treated	100	56.67±5.03^{bc}	44.33±3.06^{a}	9.66±3.65^{ab}
Infected aqueous extract treated	200	45.50±2.12^{ab}	74.00±2.82^{cd}	7.59±0.98^{a}
Infected ethanolic extract treated	200	65.50±2.12^{cd}	78.50±14.84^{de}	7.59±2.93^{a}
Uninfected aqueous extract treated	100	64.00±2.00^{cd}	57.67±4.62^{b}	6.90±1.38^{a}
Uninfected ethanolic extract treated	100	65.00±5.66^{cd}	62.50±4.95^{bc}	15.87±2.93^{c}
Uninfected aqueous extract treated	200	78.50±9.19^{e}	37.50±0.71^{a}	12.42±1.95^{bc}
Uninfected ethanolic extract treated	200	74.33±5.03^{de}	81.00±5.57^{de}	10.13±0.78^{ab}
Infected chloroquine treated	10	75.33±9.29^{de}	81.00±8.54^{de}	7.36±0.79^{a}

Values are mean **±** S. D n = 4.Mean values with different superscript are significantly different at (P< 0.05)

Table 6: Effect of Treatments on the Weight of Some Organs (Liver, Kidney, Heart).

Treatment	Dose mg/kg	Liver	Kidney	Heart
Negative control	-	1.31 ± 0.24^{b}	0.47 ± 0.21^{b}	0.10 ± 0.03^{ab}
Infected Untreated	-	1.06 ± 0.38^{ab}	0.41 ± 0.26^{ab}	0.11 ± 0.02^{b}
Infected aqueous extract treated	100	1.49 ± 0.37^{b}	0.31 ± 0.88^{ab}	0.12 ± 0.02^{b}
Infected ethanolic extract treated	100	1.38 ± 0.15^{b}	0.29 ± 0.06^{ab}	0.09 ± 0.00^{ab}
Infected aqueous extract treated	200	1.19 ± 0.38^{ab}	0.22 ± 0.06a	0.10 ± 0.02^{ab}
Infected ethanolic extract treated	200	1.43 ± 0.26^{b}	0.29 ± 0.04^{ab}	0.10 ± 0.02^{ab}
Uninfected aqueous extract treated	100	1.28 ± 0.21^{b}	0.37 ± 0.05^{ab}	0.12 ± 0.02^{b}
Uninfected ethanolic extract treated	100	1.21 ± 0.08^{ab}	0.35 ± 0.04^{ab}	0.11 ± 0.00^{b}
Uninfected aqueous extract treated	200	1.37 ± 0.21^{b}	0.34 ± 0.04^{ab}	0.10 ± 0.01^{ab}
Uninfected ethanolic extract treated	200	1.36 ± 0.32^{b}	0.40 ± 0.15^{ab}	0.11 ± 0.02^{b}
Infected chloroquine treated	10	0.84 ± 0.16^{a}	0.23 ± 0.04^{a}	0.07 ± 0.01^{a}

Values are mean **±** S.D n = 4

Effect of Treatment on the Histology of the Organs

Group 1 (negative control)

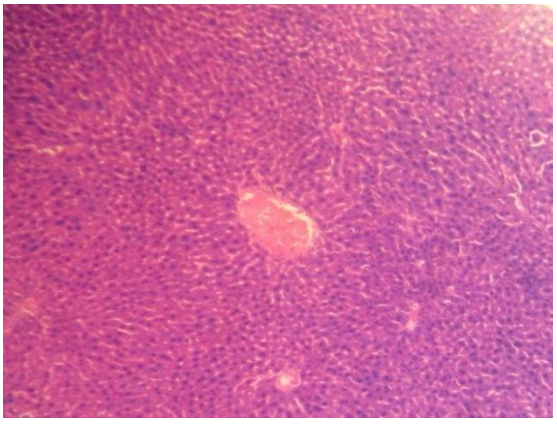

Plate 1

Liver: No visible lesion H & E X200

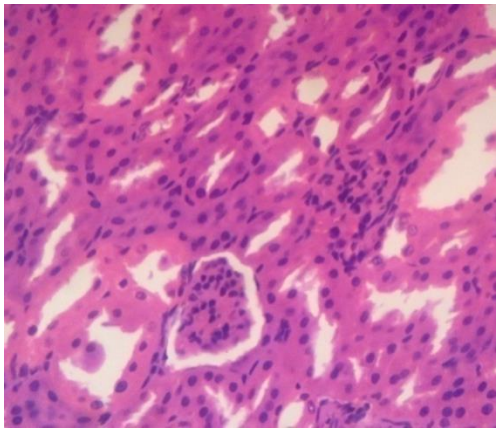

Plate 2

Kidney: No visible lesion. H & E X300

Group 2: Infected untreated

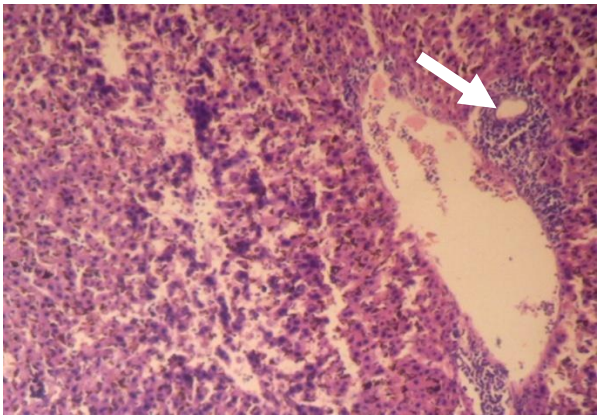

Plate 3

Liver: Section showing Severe diffuse kupffer cells proliferation which contained hemosiderin and hemozoin. The pigments were also found in hepatocytes and fibroblast. Multifocal areas of hepatic necrosis. There was diffuse moderate sinusoidal and periportal mononuclear cells infiltration (Arrow)

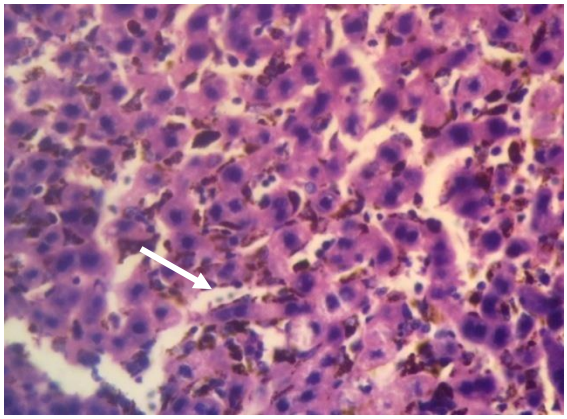

Plate 4

Kidney: Section showing Severe tubular degeneration and necrosis with tubular dilation (Arrow) H &E X 300

Group 3: 100mg/kg aqueous extract

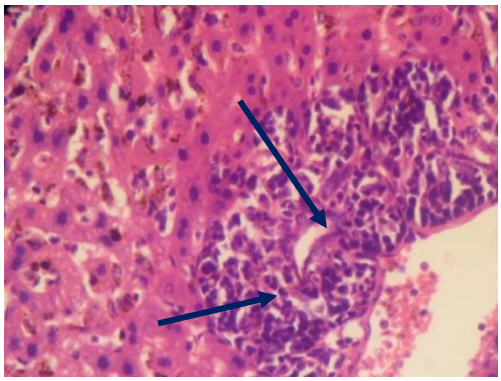

Plate 5

Liver: moderate diffuse kupffer cells proliferation which contained hemosiderin and hemozoin (malaria pigment), pigment were also observed in hepatocytes and fibroblasts. Diffuse moderate sinusoidal and periportal mononuclear cells infiltration diffuse mild vacuolar degeneration of hepatocytes with moderate sinusoidal dilation. H &E X 300

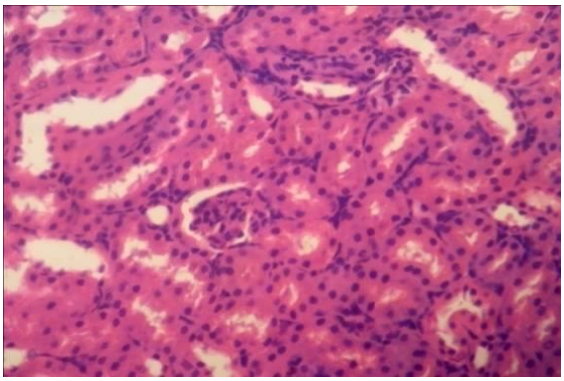

Plate 6

Kidney: No visible lesion H &E X 300

Group 4: 100mg/kg ethanolic extract

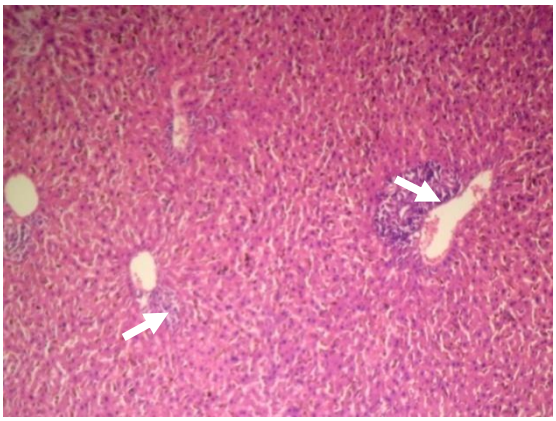

Plate 7

Liver: moderate diffuse kupffer cells proliferation which contained hemosiderin and hemozoin (malaria pigment), pigment were also observed in hepatocytes and fibroblasts. Diffuse moderate sinusoidal and periportal mononuclear cells infiltration diffuse mild vacuolar degeneration of hepatocytes with moderate sinusoidal dilation. Diffuse hepatic atrophy H &E X 200

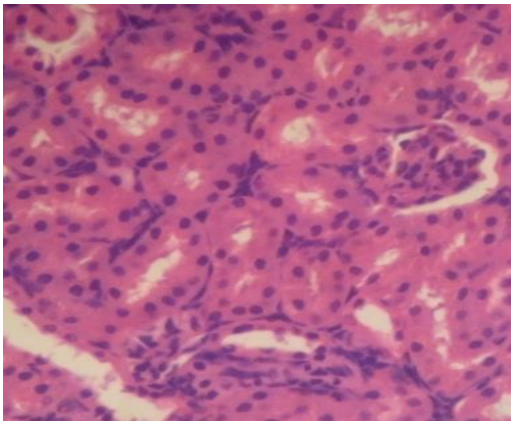

Plate 8

Kidney: No visible lesion H &E X 300

Group 5: 200mg/kg aqueous extract

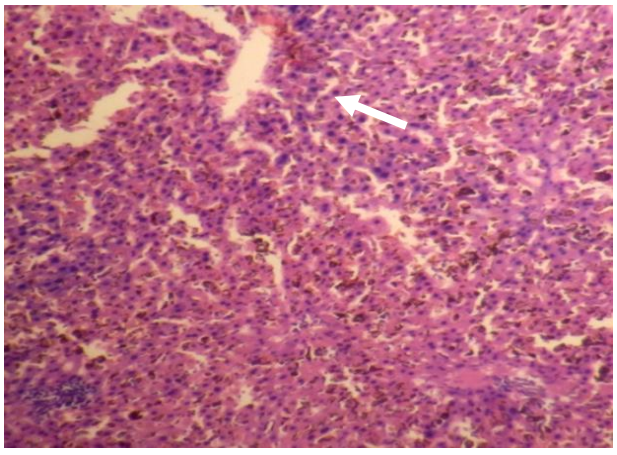

Plate 9

Liver: moderate diffuse kupffer cells proliferation which contained hemosiderin and hemozoin (malaria pigment), pigment were also observed in hepatocytes and fibroblasts. Diffuse moderate sinusoidal and periportal mononuclear cells infiltration diffuse mild vacuolar degeneration of hepatocytes with moderate sinusoidal dilation. H &E X 200

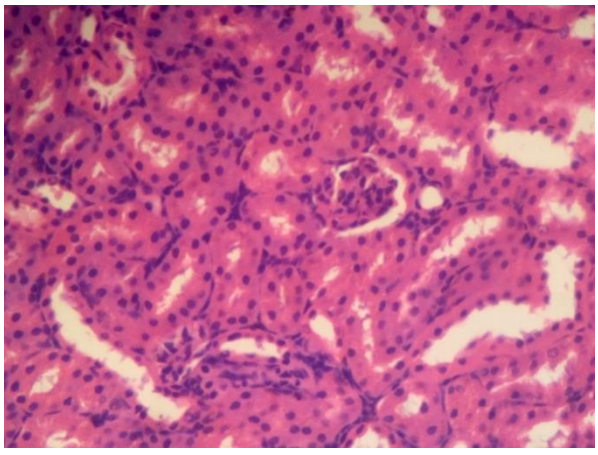

Plate 10

Kidney: Mild tubular degeneration and necrosis H &E X 300

Group 6: 200mg/kg ethanolic extract

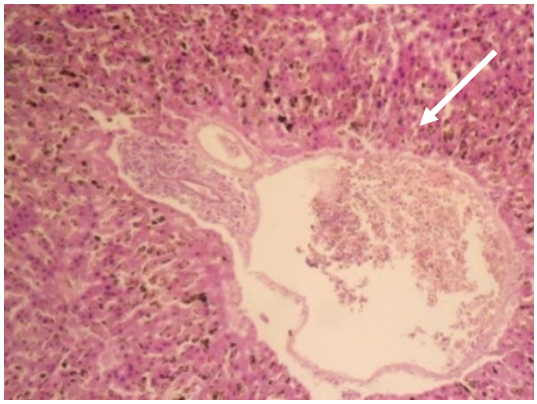

Plate 11

Liver: moderate diffuse kupffer cells proliferation which contained hemosiderin and hemozoin (malaria pigment), pigment were also observed in hepatocytes and fibroblasts. Diffuse moderate sinusoidal and periportal mononuclear cells infiltration diffuse mild vacuolar degeneration of hepatocytes with moderate sinusoidal dilation. H &E X 200

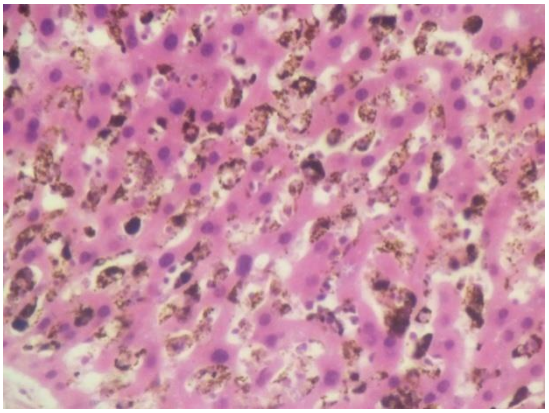

Plate 12

Kidney: Mild tubular degeneration and necrosis with tubular dilation H &E X 300

Group 7: 100mg/kg aqueous extract

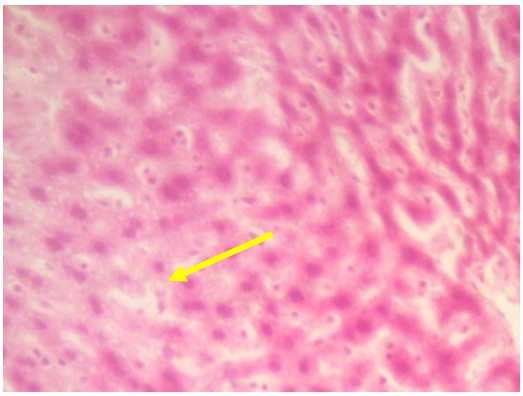

Plate 13

Liver: moderate diffuse vacuolar degeneration of the hepatocytes with necrosis. Hepatic atrophy.H & E X200

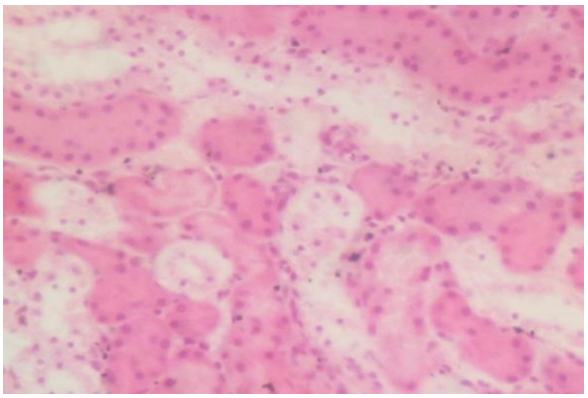

Plate 14

Kidney: moderate multiple foci of tubular degeneration and necrosis

Group 8: 100mg/kg ethanolic extract

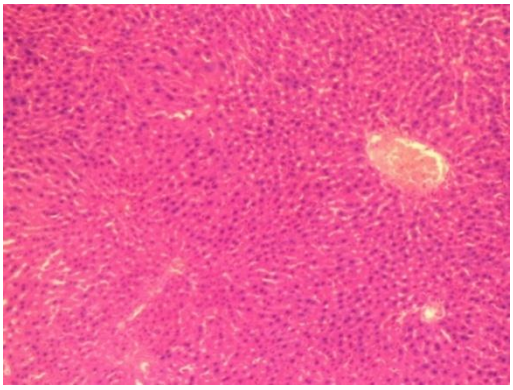

Plate 15

Liver: No visible lesion

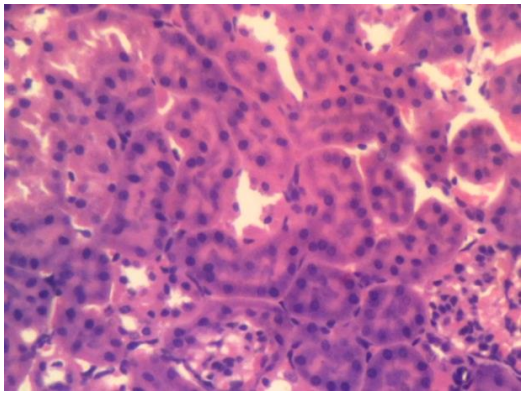

Plate 16

Kidney: No visible lesion

Group 9: 200mg/kg aqueous extract

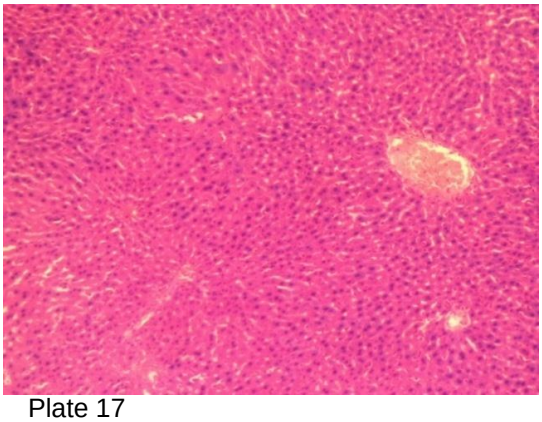

Plate 17

Liver: No visible lesion

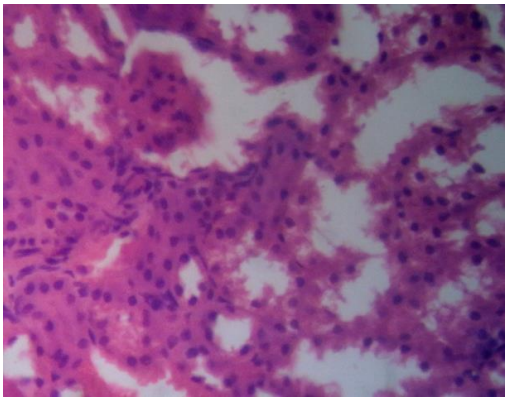

Plate 18

Kidney: mild tubular degeneration and necrosis with tubular dilation H &E X 300

Group 10: 200mg/kg ethanolic extract

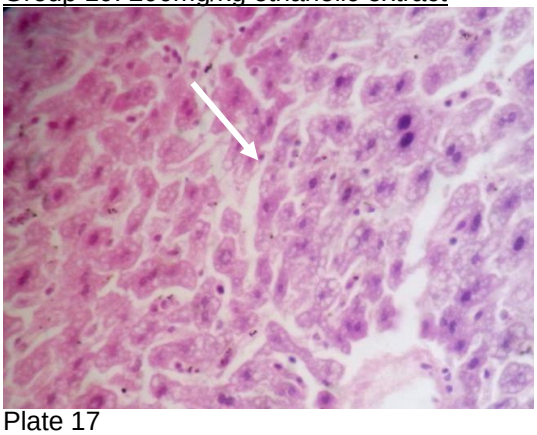

Plate 17

Liver: diffuse moderate hepatic vacuolar degeneration with severe hepatic necrosis. H & E X300

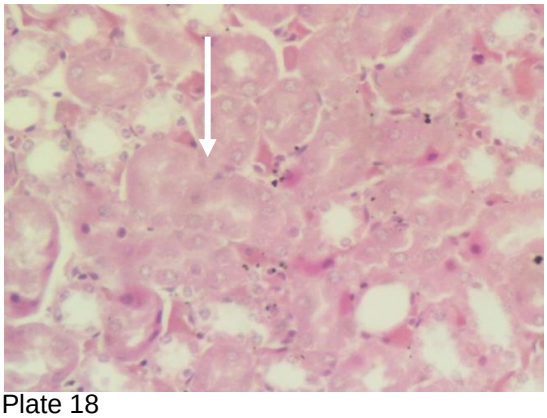

Plate 18

Kidney: diffuse severe tubular degeneration and necrosis. H & E X300

Group 11: CQ

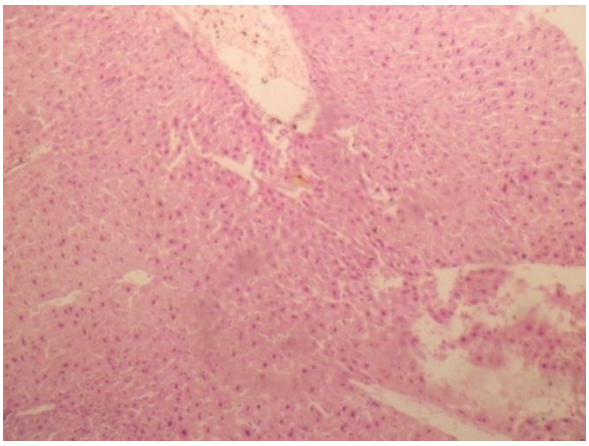

Plate 19

Liver: Focal areas of mononuclear cells aggregates within the sinusoids. Diffuse mild hepatic vacuolations.

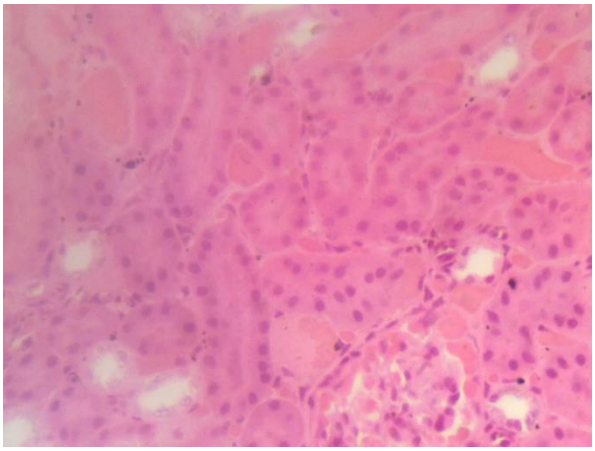

Plate 20

Kidney: No visible lesion

DISCUSSION

Malaria, a tropical disease caused by protozoan parasites of the genus plasmodium is one of the most important infectious diseases in the world. Due to the increasing incidences of resistance to anti-malarial agents there is a need to develop more effective new anti-malarial drugs that are inexpensive and available to people. For instance, resistance of plasmodium to chloroquine has been reported to be responsible for the spread of malaria some areas, as well as recurrence of malaria in areas where the disease has been eradicated. In this respect, traditional medicine, particularly plant based anti-malarial products are more preferred simply not only because of their wide availability but also due to easier means/ways by which they can be administered.

In this study, biochemical and histopathological changes that may be associated with the use of aqueous and ethanolic leaf extract of *Croton zambesicus* as an antimalarial agent were investigated. The antiplasmodial potential of the extracts at all doses (as measured by the levels of parasitaemia) did not show much significant difference when compared with the standard drug. The infected chloroquine-treated group had a rapid decrease in parasitaemia such that it suppresses malaria to a non-detectable level.

The body weights of the mice were found to be affected by extracts treatment in that there was an increase in body weight of infected extracts treated mice and chloroquine-treated mice, but not significant when compared with control. The extracts was observed to cause an increase in body weight of the uninfected treated mice both at lower and higher doses but not significant when compared with control. The study by Okokon, *et al*., (2010) and Ofusori (2007) showed that there was a comparable increase in body weight of the *Croton zambesicus* ethanolic root extract treated rats. This probably suggest that the extract does not interfere with growth processes and may have promoted growth by stimulating the synthesis of body protein.

The infected mice treated with aqueous and ethanolic extracts showed a decrease in PCV, which was significant when compared to the control. This may be ascribed to hemolytic activity of the extract leading to the observed reduction. There was an increase in the PCV of uninfected mice, but showed no significant difference when compared with the control. This is in agreement with the report of Okokon*, et al*.,

(2004) that the ethanolic roots extract of *C. zambesicus* caused a progressive increase in PCV of the treated mice. However, a significant difference was observed when PCV of infected mice treated with aqueous and ethanolic extracts were compared with those treated with standard drug.

Significant increase in the level of plasma cholesterol was observed at low dosage of aqueous extract. This may be attributed to increase in the concentration of acetyl CoA (via β-oxidation of fatty acids), such that acetyl CoA serves as substrate for the biosynthesis of cholesterol. When the concentration of the extract was increased there was decrease in the level of cholesterol in the infected treated mice.

Possible reasons for this could be attributed to the presence of some phytochemical compounds such as saponins and flavonoids in the plant extracts which helps to lower cholesterol level by binding with excess cholesterol thereby preventing its reabsorption leading to the increased excretion of cholesterol. Infected untreated mice had a reduced level of cholesterol compared with normal control. This may be ascribed to the congestion of liver cell with parasitized red cells in sinusoids and centrilobular veins, causing swollen parenchymatous and kupffer cells which ultimately leads to lipid metabolism derangement.

Administration of aqueous and ethanolic extracts at 200 mg/kg into infected treated mice caused a decrease in the level of triglyceride. There was significant difference in the triglyceride level of infected aqueous extract treated mice compared to the ethanolic extract treated group.

Cholesterol and triacylglycerol are measured as a way to determine possible risk of developing diseases involving the arteries especially those of the heart. Therefore, since infected mice treated with chloroquine and high dose of ethanolic extract exhibited an increased cholesterol levels, it therefore meant that consumption of this plant extract should be moderated so as not to predispose an individual to possible risk of atherosclerosis.

This study demonstrates that the aqueous extract treated groups had a decrease in the MDA concentration at lower doses, but at higher concentrations, the MDA levels were increased. The ethanolic leaf extract of *Croton zambesicus* caused a decrease in the concentration of MDA (an index of lipid peroxidation), although showed no significant difference compared with infected untreated.

There was an increase in MDA concentration in infected chloroquine treated, Moreso, the uninfected ethanolic extract at higher doses caused a decrease in MDA concentration, while the uninfected aqueous treated had an increase in the MDA level. The reduction in the concentration of MDA in the infected treated group implies that the extract possess free radical scavenging properties and this could be attributed to the presence flavonoid. This is also in agreement with the report of Mira *et. al.,* (2000) that flavonoid possess the antioxidant activity which could be responsible for the reduction in MDA. More so, Ngadjui *et al* (2002) and Okokon *et al.,* (2005), in their studies on *Croton zambesicus* reported that the flavonoid in *Croton zambesicus* has antioxidant activity which acts in synergy with natural antioxidant. Flavonoids have been associated with antimicrobial effects in various studies using plant extracts (Akpantah, *et.al.,* 2006; Nweze *et al.,* 2004; Abo *et al.,* 1999; Corthout *et al.,* 1991).

There was a contrasting observation in the ALT level of aqueous and ethanolic extracts in the infected treated mice. High dose of aqueous extract caused a decreased ALT level while the ethanolic extract caused an increased ALT level. The aqueous extract treated and the ethanolic extract treated showed a significant difference when compared with the infected untreated. Chloroquine-treated group had an increase in ALT level, which is not significant when compared with infected ethanolic-treated and infected untreated; but significant when compared with aqueous extract treated group. The uninfected aqueous and ethanolic extracts treated group had an increase in the ALT level, which shows significant difference compared with control.

There was a significant increase in the level of AST of infected mice treated with both aqueous and ethanolic extracts at 200 mg/kg. The aqueous extract treated exhibit significant difference compared with infected untreated. Infected chloroquine-treated group had an increase in AST level, but non-significant when compared with both extracts of infected treated groups. There was a significant increase in AST level of uninfected group administered with

higher dose of ethanolic extract while a decrease in the aqueous extract treated compared to control.

The ethanolic extract administered caused slight reduction in the level of ALP of mice treated with the higher doses (200 mg/kg) of the extract. The increases observed in AST, ALT, and ALP activities in the extract treated groups may probably suggest that the extracts have caused leakage of the enzymes into the blood via altered membrane permeability. Cellular damage arising from plant extracts administration can result in the leakage of marker enzymes to the extracellular fluid. More so, the decreased level of AST, ALT, and ALP observed may be ascribed to increased synthesis of plasma membrane proteins during repairs of the damage caused by the extract. The study carried out by Okokon (2010) showed that there was an increase in the level of ALT and ALP of the ethanolic root extract treated rats while there was no significant effect on the level of AST of the extract treated rats.

Liver enzymes such as ALT, AST, and ALP are marker enzymes for liver function and integrity (Jens and Hanne, 2002; Adaramaoye *et al.,* 2008, Ajayi *et al.,* 2009). It has been severally reported that liver enzymes are liberated into the blood whenever liver cells are damaged and enzymes activity in the plasma is increased (Edwards *et al.,* 1995, Effraim *et al.,* 2000). Elevation of these liver enzymes is also associated with cell necrosis of many tissues especially the liver (Adedapo *et al.,* 2004).

The fact that the activities of AST and ALT were increased after treatment with the extracts indicated that the plant extracts has necrotic effect on the liver. ALT is a hepato-specific enzymes that is principally found in the cytoplasm in rats (Benjamin 1978; Ringer and Dabich, 1979) and a specific marker for hepatic injury. The increase in ALP was only with the lowest dose of the ethanolic extract and could reflect damage to the tissue in which it is localized. The significant increase in plasma ALT and AST activity that was observed in most of the treated groups could be an evidence of hepatotoxicity caused by the extract.

There are many medicinal plants used in the treatment of liver diseases, there are also quite some reports of liver injury after intake of herbals including those advertised for the treatment of liver diseases (Wurochekke *et al.,* 2008). Wurochekke *et al.,* (2008) also reported that herbs that contain pyrrolizidine alkaloids, atractylis gummifera and senna alkaloids will cause liver damage.

However, histopathological study of the liver showed diffuse mild vacuolar degeneration of hepatocytes, and hepatic necrosis with the administration of the extracts. Moreso, histopathological study of the kidney revealed some pathological lesion.

These findings are not in consistent with that reported on the leaf by Ofusori *et al.,* (2008) in which no histological defect was observed in the liver of rats treated with (5mg/kg and 10mg/kg) of the leaf extract of *Croton zambesicus*.

CONCLUSION

This research work has clearly demonstrated that administration of both aqueous and ethanolic leaf extracts of *Croton zambesicus* justify the usage of this plant as parasitic remedy and in the same vein alters biochemical parameters such as cholesterol, ALT, AST and ALP in mice leading to alteration of biochemical activity in the mice. The extracts seem to exert hepatotoxic effect though with little or no observable pathological lesion in the kidney of the tested dosage.

We therefore suggest that caution should be exercised while taking *Croton zambesicus* extract as antimalarial decoction.

REFERENCES

1. Abo, K.A., O.A. Ogunleye., and J.S. Ashidi.1999. “Antimicrobial Potential of *Spondias mombin*, *Croton zambesicus*, and *Zygotritonia crocea”*. *Phytother. Res*. 13:494-47.

2. Adjanohoun, E.J., V. Adajakideje, and S. de Souza. 1989. “Contribution auXE tudes Ethobotanque et floristiques en Re Publique Populaire du Be nin”. Vol. 1. Agence de cooperation cutturelle et Technique, Paris. 245.

3. Adaramoye, O.A., D.O. Osaimoje, M.A. Akinsanya, C.M. Nneji, M.A. Fafunso, and O.G. Ademowo. 2008. “Changes in Antioxidant Status and Biochemical Indices After Acute Administration of Artermether, Artermeter-lumefantrine and Halofantrine in Rats”. *Basic Clin. Pharmacol. Toxicol*. 102:412-418.

4. Ajayi, G.O., T,T, Adeniyi, and D.O. Babayemi. 2009. “Hepatoprotective and Some Haematological Effects of *Allium sativum* and Vitamin C in Lead Exposed Whister Rats”. *Int. J. Med. Sci.* 1(3):064-067.

5. Akpantah, A.O., O.A. Olabiyi, A.A. Oremosu, C.C. Noronha, and A.O. Okanlawon. 2006. “Assessment of the Antioxidant Potential of Alcoholic Extract of *Garcinia kola* seed in Alcohol Induced Testicular Oxidative Stress in Spague-Dawley Rat”. *Nig. J. Hlth Biomed. Sci*. 5(1):37-42.

6. Arbonnier, M. 2004. *Trees, Shrubs and Liana of West African Dry Zones*. CIRAD, Margraf Pub. GMBH: Germany. 292-293.

7. Ayoub, R.M., Q. Zahid, J.W. Syed Ali Raza, and M. Arshad. 2010. “Quantitative Determination of Antioxidant Potential of Artermisia Persia”.19(1):23-30.

8. Benjamin, M.N.1978. *Outline of Veterinary Clinical Pathology*. University Press, lowa. 229-232.

9. Buege, J.A. and S.D. Aust.1978. “Microsomal Lipid Peroxidation”. *Methods in Enzymology*. 52:302-305.

10. Corthout, J., L.A. Pieters, M. Claeys, D.A. Vanden Berghe, and A.J. Vlietinck. 1991. “Antiviral Ellagitannins from *Spondias mombin*. *Phytochem*. 30(4):1129-1130.

11. Edwards, C.R., I.A. Bouchier, C. Haslet, and E.R. Chilvers. 1995. *Davidson’s Principles and Practice of Medicine, 17th edn*. Churhill Livingstone: London, UK. .24-36.

12. Effraim, K.D., H.A. Salami, and T.S. Osewa. 2000. “The Effect of Aqueous Leaf Extract of *Ocimum gratissium* on Haematological and Biochemical Parameters in Rabbits”. *Afr. J. Biomed. Res*. 3:175-179.

13. Jens, J.J. and H. Hanne. 2002. “A Review on Liver Function Test”. *The Danish Hepatitis C*. http://home3.inet.tele.dk/omni/hemochromatosis_iron.htm

14. Mira, L., M. Fernandez, M. Santos, R. Rocha, M. Florencio, and K. Jennings. 2000. “Interaction of Flavonoids with Iron and Copper Ions. A Mechanism for their Antioxidant Activity”. *Free Radical Research*.36:1199-1208.

15. Ngadjui, B.T., B.M. Abeghaz, F. Keumedjio, G.N. Folefoe, and G.W. Kapche. 2002. “Diterpenoids from the Stem Bark of *Croton zambesicus”. Phytochemistry*. 60:345-349.

16. Nweze, E.I., J.I. Okafor, and O. Njoku. 2004. “Antimicrobial Activities of Methanolic Extract of *Trume guineesis* (Schumm and Thorn) and *Morinda lucinda* (Benth) used in Nigeria Herbal Medicinal Practice”. *J. Biol. Res. Biotechnol.* 2(1):34-46.

17. Ofusori, D.A., O.P. Oluwayinka, A.E. Adelakun, S.T. Keji, K.A. Oluyemi, O.A. Adesanya, K.O. Ayeigbe, and A.O. Ayoka. 2007. “Evaluation of the Effect of Ethanolic Extract of *Croton zambesicus* on the Testes of Swiss Albino Mice”. *Afr. J. of Biotech.* 6(21): 2434-2438.

18. Ofusori, D.A., A.E. Adelakun, A.O. Ayoka, J.A. Ibeh, A.O. Adeeyo, O.A. Adesanya, and K.O. Ajeigbe. 2008. “Histological Investigation of the Effect of *Croton zambesicus* on the Liver of Swiss Albino Mice”. *The Internet Journal of Gastroenterology*. 7(1):15-20.

19. Okokon, J.E., P.A. Nwafor, and M.D. Ekpo. 2010. “Subchronic Toxicity Studies of the Ethanolic Root Extract of *Croton zambesicus”. Pak. J. Pharm. Sci.* 23(2):160-169.

20. Okokon, J.E., K.C. Iyadi, and C.O. Effiong. 2004. “Effect of Subchronic Administration of Ethanolic Leaf Extract of *Croton Zambesicus* on Hematological Parameters of Rats”. *Nig. J. Physio .Sci*. 19(1-2):10-13.

21. Okokon, J.E., K.C. Ofodum, K.K. Ajibesin, B. Danladi, and K.S. Gammaneil. 2005. “Pharmacological Screening and Evaluation of Anti-Plasmodical Activity of *Croton zambesicus* against Plasmodium Berghei-Berghei infection in Mice”. *Ind. J. Pharmacol*. 35:243-246.

22. Reitman, S. and S. Frankel. 1957. “A Colorimetric Method for the Determination of Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases”. *Am. J. Clin. Pathol*. 28:56-66.

23. Ringer, D.H. and L. Dabieh. 1979. “Haematology and Clinical Biochemistry”. In: *The Laboratory Rats*. Vol.1 edited by H.J. Baker, J.R. Lindsey, and S.H. Weisbroth. Academic Press: London, U.K. 05-116.

24. Ryley, J.F. and W, Peters. 1970. “The Antimalarial Activity of Some Quinolone Esters”. *Am. Trop. Med. Parasito*l. 84:209.

25. World Health Organization (WHO). 2010. *Guidelines for the treatment of malaria. Second edition*. ISBN 9789241547925.

26. World Health Organization (WHO). 2005. *World Malaria Report*.

27. Willcox, M.L. and G. Bodexer. 2004. “Traditional Herbal Medicines for Malaria”. *Bri. Med. J.* 329: 1156 – 1159.

28. Wurochekke, A.U., A.E. Anthony, and W. Obidah. 2008. “Biochemical Effects on the Liver and Kidney of Rats Administered Aqueous Etem Bark Extract of *Xemenia americana*”. *Afr. J. Biotech.* 7(16): 2777-2780.

ABOUT THE AUTHORS

Dr Akinloye is an Associate Professor of Biochemistry at the Federal University of Agriculture, Abeokuta, Nigeria while **Professor Balogun** is a Professor of Biochemistry at the University of Ilorin, Nigeria. **Dr Idowu** is an Associate Professor of Animal Nutrition at the Federal University of Agriculture, Nigeria. **Mr Njoku** ia a Graduate student of Dr Akinloye while **Dr. Ugbaja** is the Co-supervisior of Mr. Njoku.

SUGGESTED CITATION

Akinloye, O.A., E.A. Balogun, O.M.O. Idowu, H. Njoku, and R.N. Ugbaja. 2015. “Biochemical, Toxicological, and Histological Changes associated with the use of *Croton zambesicus* as Anti-Malarial Decoction”. *Pacific Journal of Science and Technology*. 16(2):198-215.