International Journal of Pathogen Research

7(3): 22-37, 2021; Article no.IJPR.70956
ISSN: 2582-3876

Hematological and Histopathological Profiles of Swiss Albino Mice Infected with *Plasmodium berghei* upon Treatment with Cooked *Tetracarpidium conophorum* Seed Ethanol Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Author DE designed the study; author OTF managed the literature searches, conducted the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DE and OTF managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2021/v7i330185
Editor(s):
(1) Prof. John Yahya I. Elshimali, Charles R. Drew University of Medicine and Science, USA.
Reviewers:
(1) Maria Zelinda Romano, Università degli Studi della Campania "Luigi Vanvitelli", Italy.
(2) Ahmed Mahmoud Mostafa Soliman, Egypt.
Complete Peer review History: https://www.sdiarticle4.com/review-history/70956

Original Research Article

Received 10 May 2021
Accepted 16 July 2021
Published 21 July 2021

ABSTRACT

Aim: This study was conducted to verify the hematological and histopathological outcome of cooked ethanol seed extract of *Tetracarpidium conophorum* in swiss albino mice infected with *Plasmodium berghei* (NK65).
Study Design: Descriptive study.
Methodology: Standard methods were conducted to determine the phyto-chemical profile of cooked *T. conophorum* seed ethanol extract, hematological, histopathological indices and biochemical assay. Mice were assembled into five groups (1, 2, 3, 4 and 5) of seven each. The mice in group 4 were treated with a customary antimalarial drug (chloroquine as positive control) at a dose of 5 mg/kg body weight, while mice in groups 3, 2 and 1 was administered with increasing dosages (200, 400, 800 mg/kg body weight) of seed extracts for four consecutive days respectively.
Results: The qualitative phytochemical screening of *T. conophorum* seed extract revealed the

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presence of bioactive components such as saponin, tannins, flavonoid, terpenoid. Saponin (16.82 mg/100 g) was the highest occurring phytochemical followed by terpenoid (10.39 mg/100 g), glycoside (5.9 mg/100 g), tannin (2.8 mg/100 g) and flavonoid (1.17 mg/100 g), while steroid only had a trace presence. Hematological parameters revealed mice in all groups do not possess significantly different mean corpuscular haemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). Mice in group 5 (infected and not treated) demonstrated lowest values of hemoglobin, platelet, and packed cell volume with a significantly lower % eosin. There was considerable decrease in the levels of alanine transaminase and aspartate transaminase in group 5 compared to mice in groups 1, 2, and 3.

Conclusion: Ameliorative effects of seed extract were observed on the liver and kidney of mice at dose levels (200 mg/kg) used, but the 400 mg/kg restored the kidney but had adverse effect on the liver. This study therefore shows that cooked *T. conophorum* is generally less effective and could not be employed for treatment of malaria.

Keywords: Phytochemical; hematological parameters; antimalarial drug; biochemical assay; Tetracarpidium conophorum; Plasmodium berghei.

1. INTRODUCTION

Malaria is a parasitic disease caused by protozoan parasite from the genus *Plasmodium* with female *Anopheles* mosquito acting as the biological vector. Na-Bangchang and Congpuong, [1] stated that, malaria is widespread in tropical and subtropical regions. Despite recent advances in our understanding of the biology and pathogenesis of the *Plasmodium* parasite, this highly infectious disease has been one of the greatest causes of human illness and death. According to World Health Organization (WHO) [2], four species of *Plasmodium* are known to infect human, namely *P. ovale, P. vivax, P. malariae* and *P. falciparum*. Undoubtedly, malaria is still considered as the most serious tropical disease troubling mankind throughout the world with the greatest burden in sub-Saharan African countries. Berendt et al. [3] reported four *Plasmodium* species (*Plasmodium berghei*, *Plasmodium chabaudi*, *Plasmodium vinckei* and *Plasmodium yoellii*) that have been described in African murine rodents and occur geographically in Central Africa. Plasmodium berghei is transmitted by the bite of mosquito (*Anopheles dureni*).

Drulhe et al. [4] opined that experimental animal models have been used in understanding of human disease pathogenesis and development of new drug compounds and vaccines due to the impossibility of some research procedures on humans for practical or ethical reasons. Basi et al. [5] also stated that malaria models have been developed in monkey, rats and mice to understand certain aspects of *Plasmodium* pathogenicity in humans [5]. The many species and strains of *Plasmodium* available and the very large selection of inbreed and outbreed laboratory rodents has created a great number of mouse-parasite combinations that can be used experimentally.

Srinivasan and Viraraghavan, [6] reported that African walnut *Tetracarpidium conophorum* (also called *Plukenetia conophora*) belong to the family of Euphorbiaceae and is found in South-East and South-West Nigeria and Cameroon. A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloids. Similarly, Okerulu and Ani, [7] reported that *Tetracapidium conophorum* can be cooked, roasted or sun dried and the roasted seeds could be ground like melon seeds. *Tetracarpidium conophorum* seed is highly medicinal and has been reported to be effective in the treatment of malaria [8]. The seeds are rich in flavonoid, tannins, alkaloid, protein, carbohydrate, fat and oils, vitamins and minerals which enhance its antimicrobial activities [9]. Hence, the essence of this study is to assess the effects of cooked *Tetracarpidium conophorum* seed ethanol extract on hematological and histopathological profile in Swiss albino mice infected with *Plasmodium berghei.*

2. METHODOLOGY

2.1 Collection of Samples

Tetracarpidium conophorum (African walnut) was purchased at Owena market, Osun State and authenticated at the department of Crop, Soil and Pest Management at the School of Agriculture

and Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Preparation of the Extracts

Seeds were processed for extraction using the method of Santhi and Sengottuvel [10]. The seeds were cooked and separated from the hull, washed and dried at room temperature (27±^{0}C) for a month before pulverizing into a coarse powder with mortar and pestle then stored in an air tight bottle prior to analysis. Five hundred grams (500 g) of the grounded powder was soaked into 700 ml of 75% ethanol, stirred and left for 72 hours. The mixture was filtered using a millipore filter (pore size 0.7 um) and concentrated in a rotary evaporator at 40 ^{o}C. The concentrate was heat dried over a water bath to obtain a solvent free extract and was refrigerated at 4^{o}C.

2.3 Phytochemical Screening of Cooked *Tetracarpidium conophorum* Seed

Qualitative (alkaloids, saponin, tannin, flavonoids, terpenoids, anthraquinone, phlobatannin and cardiac glycoside) and quantitative (tannin, flavonoids, saponin, terpenoids and glycosides) phytochemical analysis was carried out according to standard procedures described by Rehab et al. [11] to determine the bioactive constituents of ethanol extract of cooked *T. conophorum* seed.

2.4 *In-vivo* and *In-vitro* Assay of *Tetracarpidium conophorum* Seed Ethanol Extract on Experimental mice

2.4.1 Collection and grouping of experimental mice

Albino mice was experimented in conformity with international, national and institutional guidelines for care and use of laboratory animals in Biomedical Research by Canadian council of animal care and United State National Institute of Health described by Ogundolie et al. [12] Fifty mice of weights between 15-22g were obtained from the animal house at Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria and were transferred to Federal University of Technology Akure (FUTA). Mice were housed in plastic cages with wood dust as beddings. They were fed with pellets and water ad libitum and acclimatize for 7 days at room temperature (29-30^{o}C) before the commencement of the experiment. A total of 35 mice was randomly divided into five groups A (negative control), B (positive control), C (normal control), D, E and F) of three mice per group for the antimalarial activity and 12 mice was randomly grouped into four groups of three mice per group for acute toxicity test.

2.4.2 Acute toxicity test

The acute toxicity test of *Tetracarpidium conophorum* was carried out using modified Lorkes (1983) method as described by Yerbanga et al. [13]. The extract was prepared by dissolving 2.0g, 3.0g, 4.5g and 5.0g into 5ml volumes of distilled water separately to produce concentrations of 200mg/ml, 400mg/ml and 800mg/ml respectively which was then administered orally to each mouse in the groups. The mice were observed visually daily throughout the experiment for behavioral changes which include decreased activities, licking of paw, body weakness, sleeping and mortality.

2.4.3 Collection of parasites

Chloroquine-sensitive strain of malaria parasite (*Plasmodium berghei* NK 65) in a donor mouse was obtained from Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The Parasite was kept alive by inoculating 0.2mls of it into a healthy mouse (infected mouse) through intra-peritoneal route. By cardiac puncture, 0.2mls of the parasite was withdrawn from the infected mouse and serially diluted with sterile 4.8mls of normal saline to obtain 1x 10^7 *Plasmodium berghei* infected erythrocyte. Mice in groups A, B, D, and E was given 0.2mls of the parasite after the parasitemia level of the infected mouse which has been ascertained to be high (35.8% parasitemia level). Group C (normal control) was not infected. The mice were visually observed for behavioral changes (decreased activities, loss of appetite) Yerbanga et al. [13].

2.4.4 Determination of body weight and temperature of mice

According to Dada and Muhammed, [14] The body weight of each mouse in all groups was measured before and after acute toxicity test at different doses, using sensitive digital weighing balance (Weight milk Water). Temperatures of

mice was measured using a BIOSEB (BIO9882) rectal thermometer with a probe inserted approximately 1.5cm past the anal sphincter into the colon of hand-held mice.

2.5 Administration of Extract and Drug for Parasitemia Determination

After 3 hours of infection with *Plasmodium berghei*, different concentrations (200, 400, 800 mg/kg body weight) of the extract were respectively prepared and administered orally as treatment doses to mice in groups A, B, D, and E. Group 2 mice (positive control) received 0.2 mls of 5 mg/kg of chloroquine, mice in group C (normal control) received 0.2 ml of normal saline, mice in group 1 (negative control) were not treated. The treatment was administered for four consecutive days [12]. On day five, parasitemia level of the mice (except group C) was determined by collecting a drop of blood on a microscope slide from each mouse by venesection of the tail. Thin blood smear was made and allowed to dry at room temperature. It was fixed with methanol before staining with 10% Giemsa stain for 10 minutes. The slides were allowed to air-dry and examined and counted under microscope at X100 magnification (oil immersion). The parasitemia was determined by counting minimum of three fields per slide with 100 RBC per field. The percentage suppression and parasitaemia was then determined.

$$\text{Parasitemia} = \frac{\text{Number of parasitized RBC} \times 100}{\text{Total Number of RBC examined}}$$

$$\text{Average \% chemo} - \text{suppression} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in treatment} \times 100}{\text{Parasitemia in negative control}}$$

2.6 Hematological Analysis

This was carried out to know the effect of leaf extract and *P. berghei* on the hematological parameters: Red blood cells (RBC), White blood cells (WBC), Platelet, Packed Cell Volume (PCV), haemoglobin concentration (HB), Mean Cell hemoglobin Concentration (MCHC), Mean Cell Corpuscular Volume (MCV), Mean Cell hemoglobin (MCH), Lymphocyte, Neutrophil, Monocyte and Eosinophil were assessed. On the fifth day, the mice were subjected to euthanasia under chloroform, dissected and blood was collected through cardiac puncture in an ethylene diamine tetraacetic acid (EDTA) bottles and the blood parameters were analyzed using Abacus 380 hematology analyzer (Tecil, Barcelona, Spain).

2.7 Biochemical Assays

Blood was collected from mice in a lithium heparin bottle through cardiac puncture. The alanine transaminase (ALT) and aspartate transaminase (AST) was determined with automated refloton machine using the recommended test strips (Woodley Trial Solution, New York, US).

2.8 Histopathological Studies

The method of Ogundolie et al. [15] was employed to carry out histopathological analysis in order to determine the effect of the ethanol extract of cooked *Tetracarpidium conophorum* on the parasite on organs of the mice with minor alterations.

2.9 Statistical Analysis

One way analysis of variance (ANOVA) was used to analyze data obtained from this study. Significant difference between means ($p \leq 0.05$) was considered with Duncan's New Multiple Range Test (DNMRT) using statistical package for social science (SPSS).

3. RESULTS

Table 1. Qualitative bioactive components of cooked *Tetracarpidium conophorum* seed extract

Bioactive Components	Inferences
Saponin	+
Tannin	+
Phlobatannin	-
Flavonoid	+
Steroid	-
Terpenoid	+
Alkaloid	-
Anthraquinone	-

Legend: + = Present and - = Absent

3.1 Hematological Analysis of Mice Blood

The hematological profile shows that compared with the control group (that is group 5), rats in all groups do not possess significantly different Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV). For rats in group 1, compared with the control group (that is group 5), they possessed significantly higher Packed Cell Volume (PCV), Hemoglobin (Hb),

Red Blood Cells (RBC) and a significantly lower % Eosin. Their White Blood Cells (WBC), Lymph, % mono were lower but the differences were not significant while their Platelets, % nurtro, Mean Corpuscular Volume (MCV) were higher but not significantly. The rats in group 2 possessed significantly higher Packed Cell Volume (PCV), Hb, Platelet, % nurtro compared with the control group (that is group 5) however, they had significantly lower WBC and %Eosin. Their RBC and %mono were lower but the differences were not significant while their Lymph, % nurtro were higher but not significantly different. For the rats in group 3, compared with the control group (group 5), they possessed significantly higher Packed Cell Volume (PCV), Hb, RBC, Platelet, % nurtro and %mono and possessed significantly lower WBC and %Eosin. There were lower but the differences were not significant while their Lymph, % nurtro were higher but not significantly. The rats in group 4 possessed significantly higher Hb, RBC, Platelet, % nurtro compared with the control group (that is group 5) however, they had significantly lower WBC. Their RBC and % mono and % Eosin were lower but the differences were not significant while their PCV, Lymph, % nurtro were higher but not significantly different as shown in Table 5.

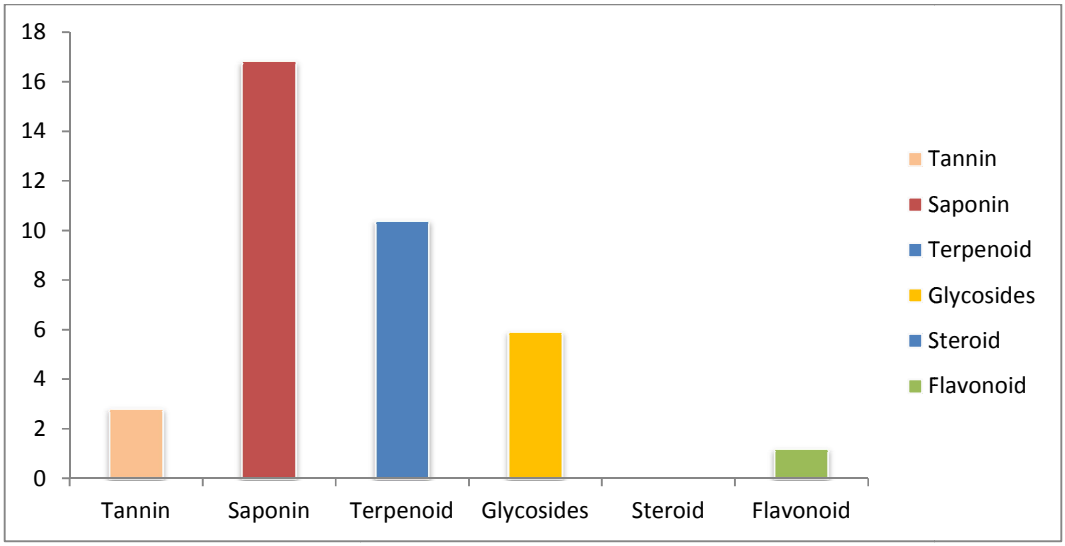

Fig. 1 Quantitative constituents of ethanol seed extract of cooked *Tetracarpidium conophorum*. Bars are presented as Mean ±S.D of replicates (n=3)

Table 2. Acute toxicity of cooked *Tetracarpidium conophorum* seed extract

Groups	Dosage (mg/kg)	Mortality	Mortality (%)	Signs of Toxicity
1	800	0/4	0	Nil
2	400	0/4	0	Nil
3	200	0/4	0	Nil
4	Chloroquine	0/4	0	Nil

Table 3. Result of analysis of rats' weight

	Weight of Mice (g)				
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Before Treatment	19.71 ±0.74	19.71±0.92	18.14±0.59^{de}	18.00±0.44^{bcd}	19.71±0.60
1	17.42±1.49^{a}	21.00±1.69^{a}	20.42±0.75^{ad}	20.14±1.26	19.00±0.49
2	17.17±1.60	21.17±2.02^{b}	20.29±0.87^{b}	20.71±0.75^{ab}	18.57±1.29
3	18.67±0.67^{a}	21.33±2.06^{c}	19.86±0.91	20.14±0.55^{c}	19.57±0.95
4	16.00±0.00	21.00±2.19	20.42±0.43^{ce}	20.43±0.43^{d}	19.17±1.01
5	14.50±0.50	16.00±1.87^{abc}	17.80±0.37^{abc}	18.71±0.57^{a}	18.17±0.91

Data are presented as Mean ± S.E (n<=7). Values with the same superscript letter(s) along the same column are significantly different (p≤0.05)

Table 4. Result of analysis of infected RBC in mice

Infected RBCs in Mice					
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Before Treatment	73.43±8.82^{abc}	103.57±20.97^{abc}	116.14±11.76^{abcd}	126.71±6.29^{abcde}	85.57±13.89^{ab}
1	72.29±13.90^{de}	123.33±13.82^{aijkl}	106.29±11.26^{efgh}	64.00±13.58^{afghi}	90.57±12.38^{fg}
2	49.33±12.72^{a}	94.83±9.05^{defi}	62.71±11.02^{aeijk}	15.57±2.17^{bfjkl}	102.71±11.48^{af}
3	43.33±15.30	45.50±5.58^{dgj}	38.14±6.87^{bfilm}	3.43±.65^{cgjmn}	92.86±12.16^{cd}
4	21.50±2.50^{bd}	31.50±6.03^{behk}	8.43±2.27^{cgjl}	0.00±.00^{dhkmo}	109.33±12.06^{ce}
5	2.50±2.50^{ce}	7.20±1.43^{cfghl}	2.00±.95^{dhkm}	0.00±.00^{eilno}	118.67±10.8^{bdeg}

Data are presented as Mean ± S.E (n<=7). Values with the same superscript letter(s) along the same column are significantly different (p≤0.05)

3.2 Histopathological Examination Profile

The result of the histo-pathological examination carried out on the liver and kidney of experimental mice is indicated on Plate 1-10. The liver of mice in the negative control, (group 5) was observed to be characterized with small vacuole formation in the parenchyma as shown in Plate 1 compared to the liver of mice administered with 5 mg/kg of chloroquine which showed high level of vacuole formation as shown in Plate 2. The liver of the mice administered with 200 mg/kg of *T. conophorum* seed extract showed the hepatocytes multifocal tubular epithelial coagulation necrosis and inflammation as shown in Plate 3. The liver of mice administered with 400 mg/kg of *T. conophorum* seed extract shows the hepatocytes peri-portal inflammation and hepato-cellular necrosis on the parenchyma tissue of the liver as shown in Plate 4. However, the liver of experimental mice administered the highest dose of the seed extract (800 mg/kg) the hepatocytes showed severe multifocal hepato-cellular coagulation necrosis and inflammation as shown in Plate 5.

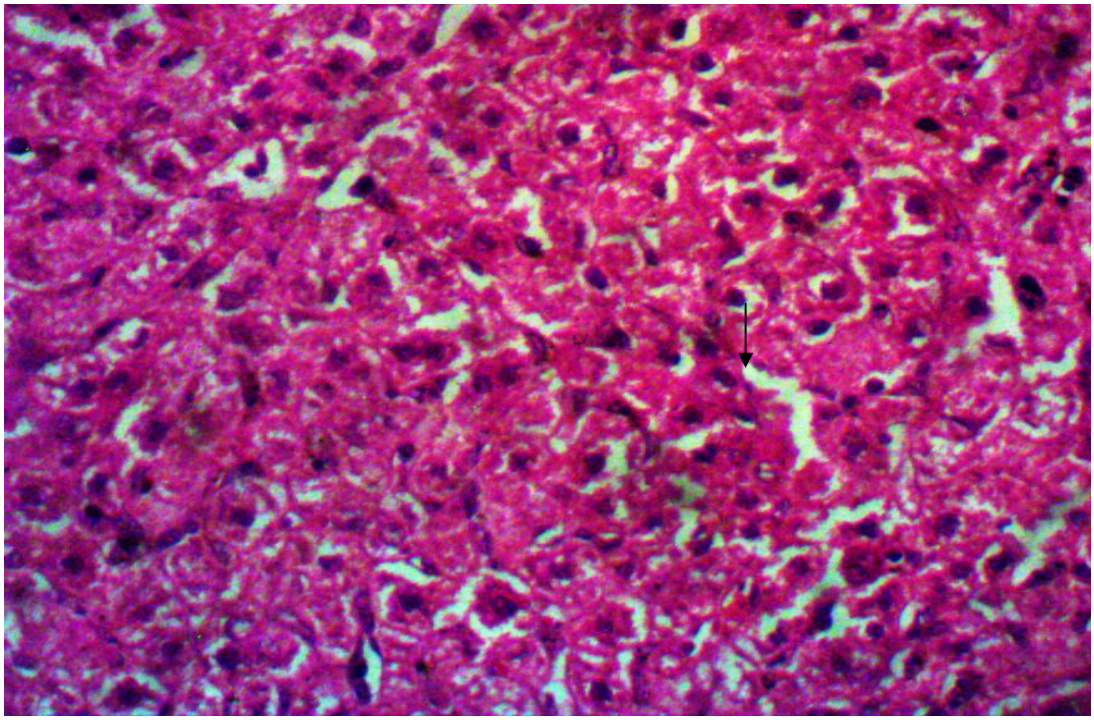

Plate 1. Photomicrograph of liver of albino mice infected with *Plasmodium berghei* untreated (negative control group A) showing change in liver morphology with small vacuole formation in the parenchyma

Table 5. Hematological profile of mice

Group	% PCV	Hb g/dl	RBC X106ml	WBC X103ml	Platelet	% 1ymp	% nurtro	% Mono	% Eosin	MCV	MCHC	MCH
1	24±1.21^{abc}	8.0±0.44^{a}	4.2±0.40^{a}	9106±283.91^{abc}	80000±3120.99^{a}	59±0.82^{ab}	38±1.81^{a}	1±0.00^{ab}	2±0.29^{abc}	57.14±1.18	33.33±1.39	19.04±0.45
2	25±1.15^{de}	8.3±0.66^{b}	4.3±0.59	7050±297.23^{adef}	88000±1583.90^{bc}	66±2.58	48±3.56^{ab}	2±0.21^{a}	0±0.00^{ade}	58.14±1.26	33.2±1.33	19.30±0.45
3	27±0.49^{afg}	9.0±0.62^{c}	4.6±0.19^{b}	6050±408.49^{bdg}	89000±1376.80^{ad}	70±2.52^{a}	48±3.49^{c}	3±0.30^{bcd}	1±0.29^{bdf}	58.70±1.26	33.33±1.29	19.56±0.45
4	30±1.07^{bdf}	10±0.617^{d}	5.2±0.34^{c}	5700±366.59^{ceh}	76000±2274.29^{be}	69±1.57^{b}	39±1.54	2±0.00^{c}	2±0.00^{f}	57.70±1.50	33.33±0.66	19.23±0.33
5	18±0.44^{ceg}	6.1±0.40^{abcd}	3.2±0.24^{abc}	10150±356.0^{fgh}	71000±3725.70^{cde}	63±2.05	34±2.25^{bc}	2±0.00^{d}	3±0.80^{ceg}	56.25±1.50	33.88±0.23	19.06±0.32

Data are presented as Mean ± S.E (n<=7). Values with the same superscript letter(s) along the same column are significantly different (p≤0.05).

Table 6. Biochemical analysis of mice

Group	AST (NL)	ALT (Nil)	Tbl (mg/dl)	BUN Mg/dl	Create Mg/dl	Chol (mg/dl)	Trig (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	215±2.62^{abc}	48±1.77^{abcd}	0.6±0.04^{abc}	14.6±0.31^{abc}	0.7±0.04^{abc}	47±1.77^{ab}	44±1.77^{ab}	14±0.53^{abc}	24.2±0.71^{ab}
2	205±2.46^{def}	30±1.02^{ae}	0.3±0.03^{adef}	12.8±0.36^{ade}	0.5±0.04^{ad}	42±1.02^{cd}	42±1.93^{cd}	12±0.49^{a}	21.6±1.39^{c}
3	184±1.88^{adg}	31±1.40^{bf}	0.2±0.02^{bdgh}	12.2±0.37^{bf}	0.4±0.06^{be}	39±1.40^{acd}	39±1.66^{ef}	12±0.65^{bd}	19.2±0.91^{ade}
4	154±2.80^{begi}	28±1.41^{cg}	0.1±0.02^{cegi}	11.2±0.23^{cdg}	0.4±0.06^{cf}	30±1.02^{bce}	30±1.45^{aceg}	10±0.44^{cde}	14.0±0.95^{bcdf}
5	313±3.16^{cthı}	58±1.20^{detg}	0.7±0.04^{thı}	15.2±0.48^{etg}	0.9±0.09^{deı}	47±1.68^{de}	49±0.84^{bdtg}	13±0.49^{e}	24.2±0.77^{ef}

Key : AST- Aspartate Transaminase; A LT- Alanine Transaminase; Tbl-Total bilirubin ; LDL-Low density lipoprotein ; BUN- Blood Urea Nitrogen; Creat- Creatinine; Chol : Cholesterol; Trig- Triglyceride; HDL - High density lipoprotein

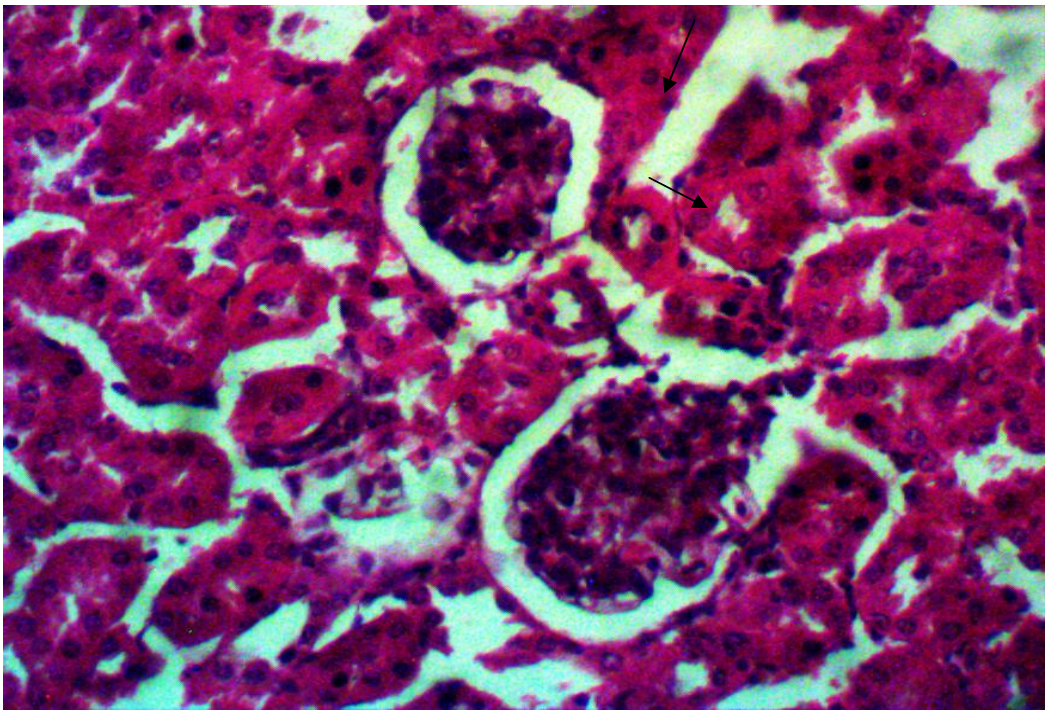

Plate 2. Photomicrograph of liver of albino mice infected with *Plasmodium berghei* (positive control group that is, chloroquine treated) the hepatocytes showed high level of vacuole formation. H&E (×40)

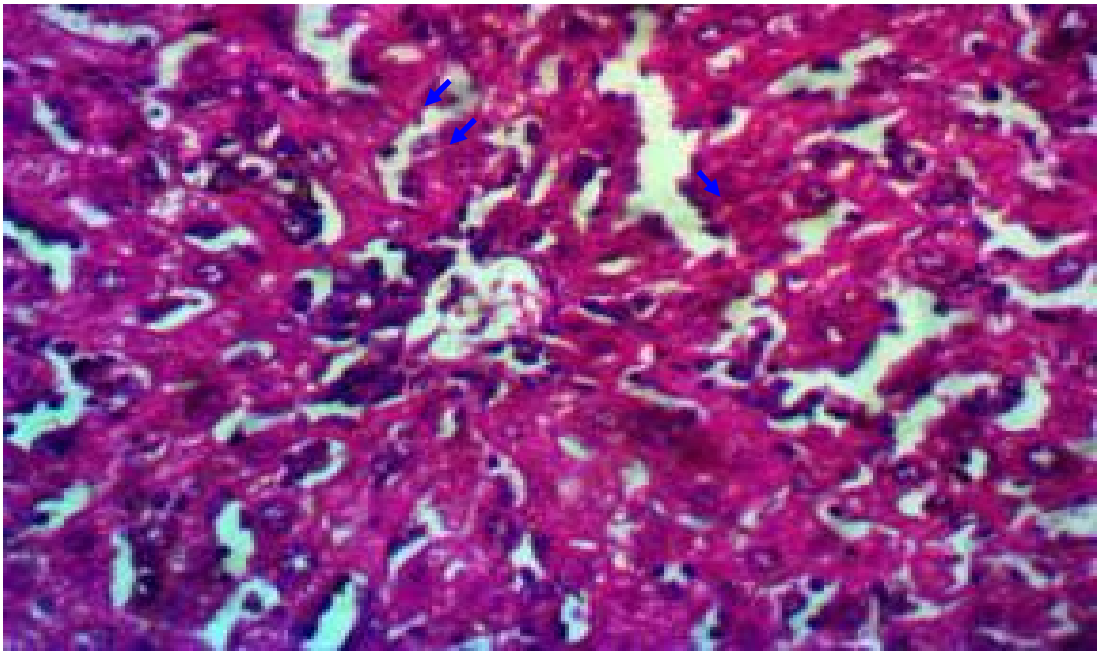

Plate 3. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (200 mg/kg seed extract), the hepatocytes showed severe cord atrophy, multifocal hepatocellular coagulation necrosis and inflammation (arrows). H&E (×40)

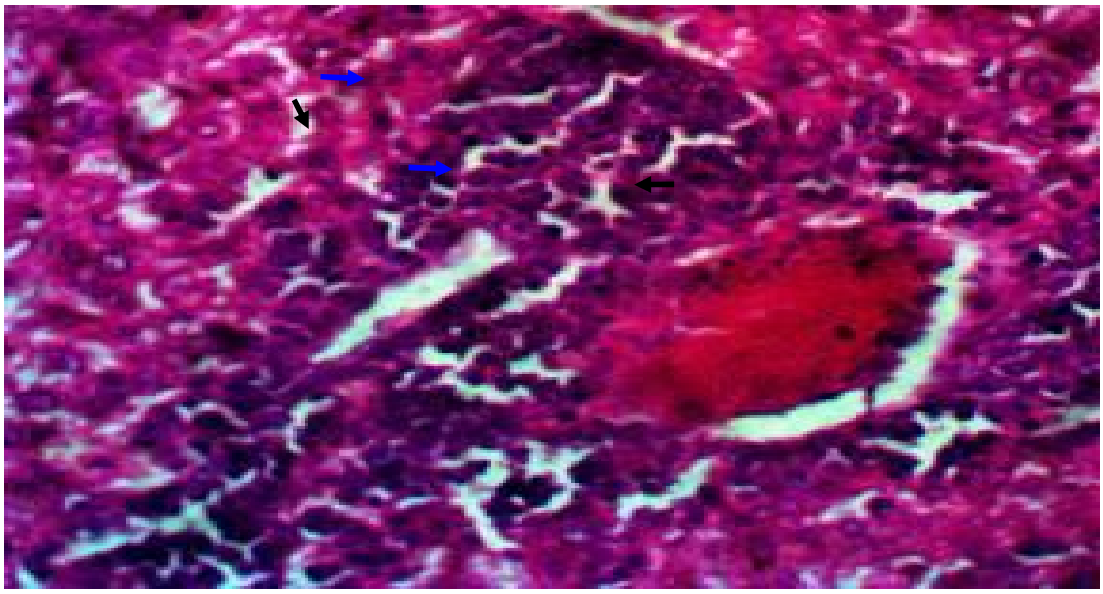

Plate 4. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (400 mg/kg seed extract), the hepatocytes showed periportal inflammation (arrows) and zone 1 hepatocellular necrosis (black arrows). H&E (×40)

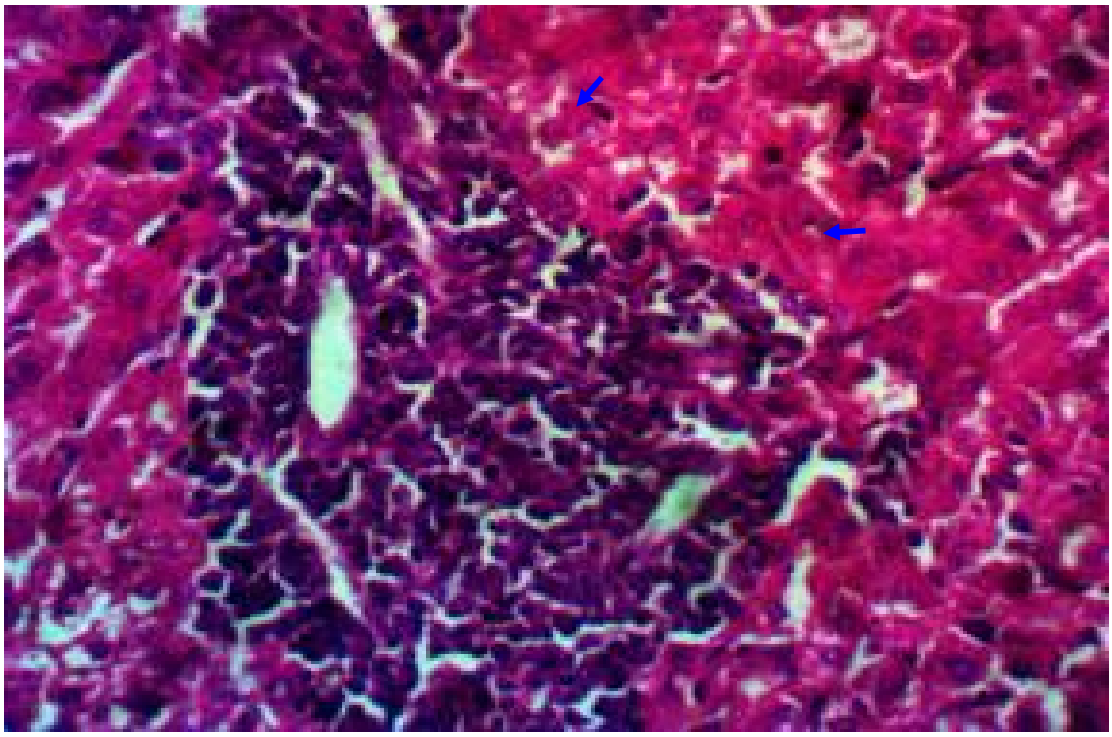

Plate 5. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (800 mg/kg seed extract), the hepatocytes showed severe multifocal hepatocellular coagulation necrosis and inflammation (arrows). H&E (×40)

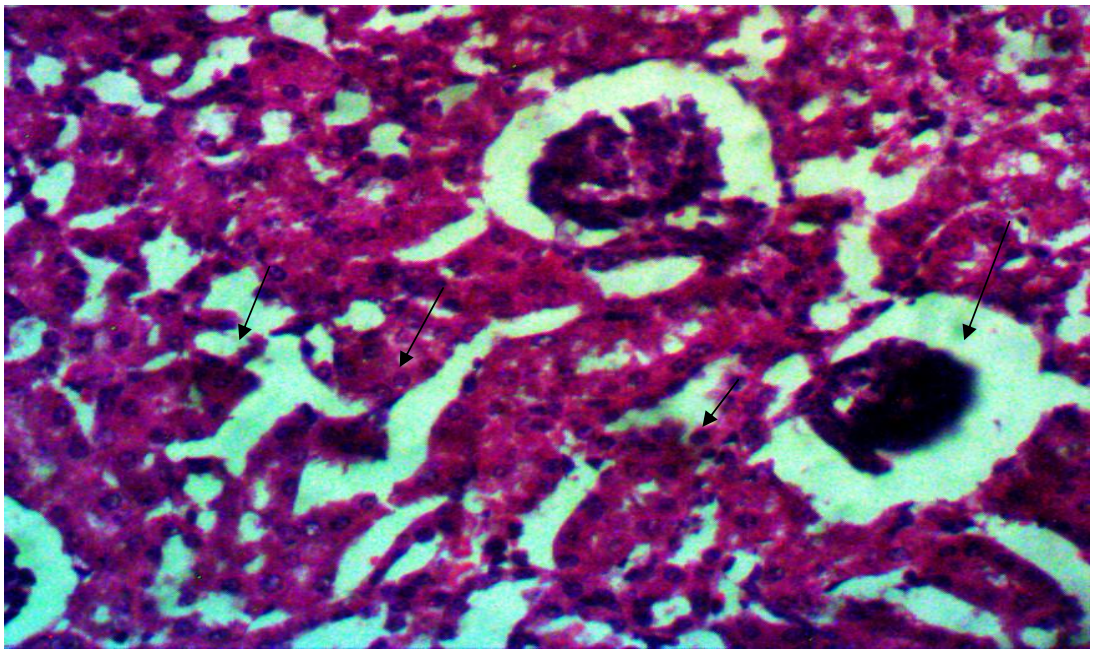

Plate 6. Photomicrograph of kidney of albino mice infected with *Plasmodium berghei* untreated (negative control group 5) it shows glomerular swelling degeneration of distal, proximal tubules and renal parenchymal vacuolation (arrowed)

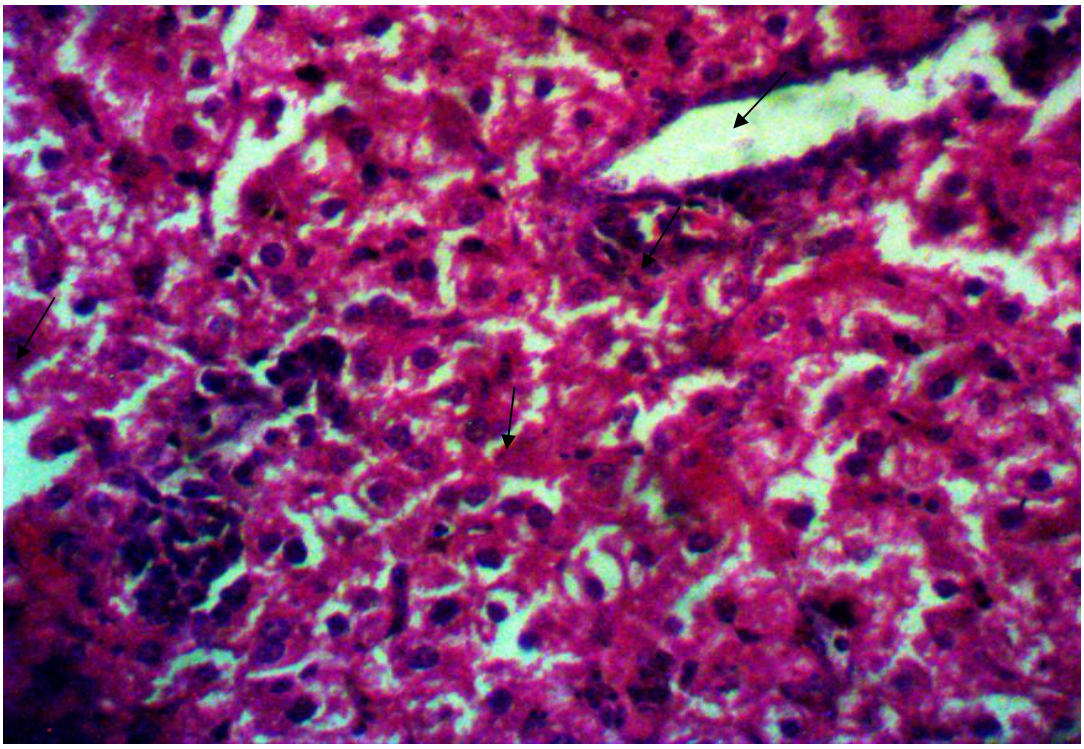

Plate 7. Photomicrograph of kidney of albino mice infected with *Plasmodium berghei* (positive control group that is, chloroquine treated) It shows is vacuole space formation in the cortex with glomerulus

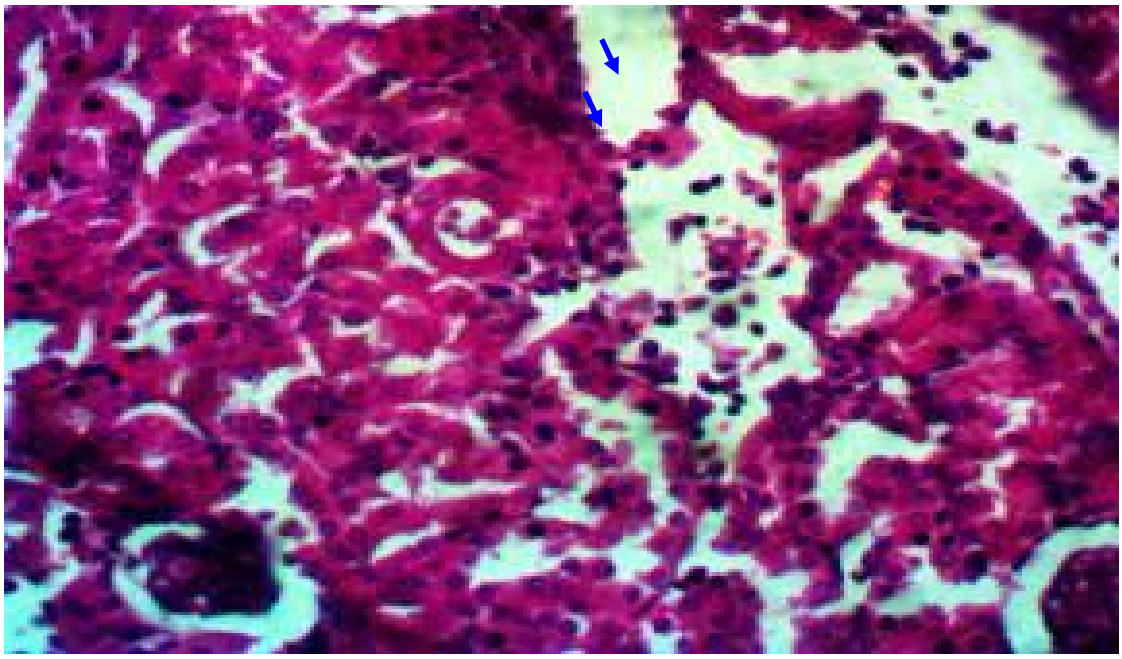

Plate 8. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (200 mg/kg seed extract), the hepatocytes showed multifocal tubular epithelial coagulation necrosis and inflammation (arrows). H&E (×40)

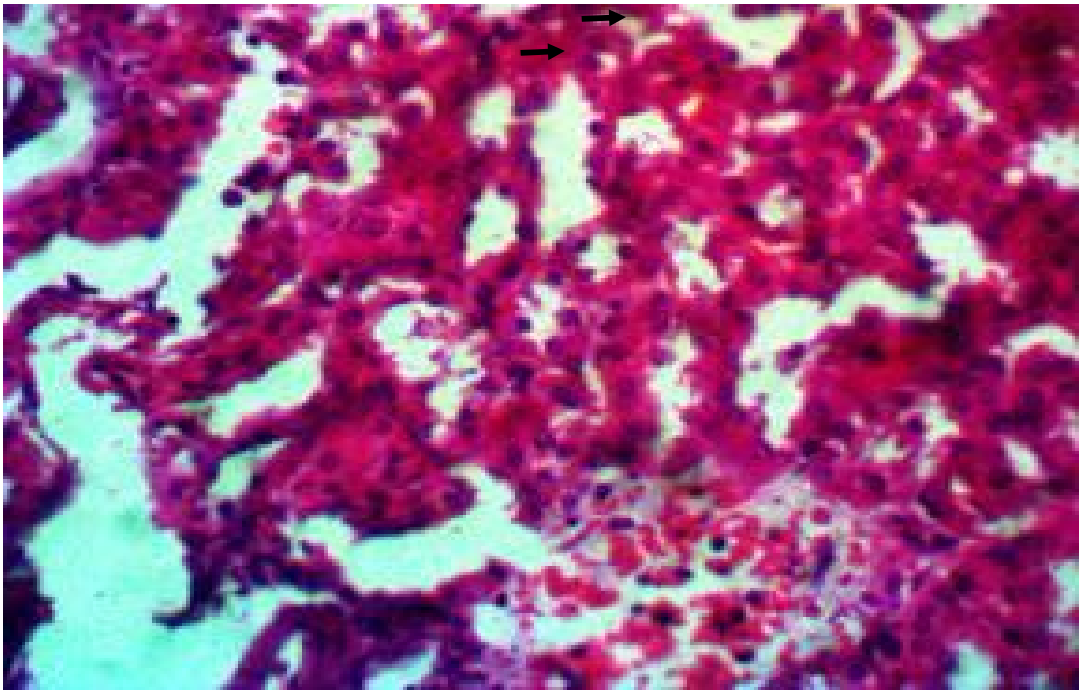

Plate 9. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (400 mg/kg seed extract), the hepatocytes showed diffused tubular atrophy, epithelial coagulation necrosis (arrows) and inflammation. H&E (×40)

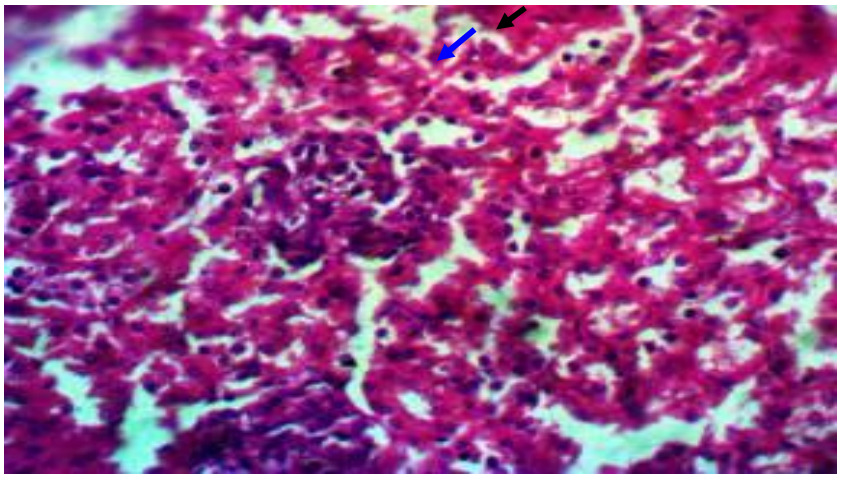

Plate 10. Photomicrograph of a liver section of mice infected with *Plasmodium berghei* and treated with cooked *Tetracarpidium conophorum* (800 mg/kg seed extract), the hepatocytes showed patchy tubular epithelial coagulation necrosis (black arrow) and inflammation (blue arrow). H&E (×40)

4. DISCUSSION

The qualitative phytochemical screening of *Tetracarpidium conophorum* seed extract revealed the presence of bioactive components such as saponin, tannins, flavonoid, terpenoid. Saponins, alkaloids and anthraquinone were not present as shown in Table 1. The quantitative phytochemical screening of *Tetracarpidium conophorum* seed extract revealed high abundance of saponin and terpenoid. Saponin (16.82mg/100g) was the highest occurring phytochemical followed by terpenoid (10.39mg/100g), glycoside (5.9mg/100g), tannin (2.8mg/100g) and flavonoid (1.17mg/100g), while steroid only had a trace present as shown in Fig. 1. The effects of cooked *Tetracarpidium conophorum* seed ethanol extract on hematological and histopathological profile in Swiss albino mice infected with *Plasmodium berghei* was examined in this study. Findings from this study revealed that the qualitative phytochemical screening of the ethanol *T. conophorum* seed extract contain secondary metabolites (alkaloids, oxalate, tannins, saponins, phenols, flavonoids, phytate, terpenoids). This is in agreement with the findings of Edem et al. [9]. Quantitative phytochemical screening also revealed the presence of phytate, oxalate and tannins which is in agreement with Enujiugha and Ayodele, [16] who reported the presence of tannins in raw *T. conophorum* seed ethanol extract. The outcome of the phytochemical profile of African walnut in this study could be owed to a bitter taste usually observed upon drinking water immediately after eating the seeds.

The result of acute toxicity is shown in Table 2. There were no signs of toxicity such as paw licking, sleeping, reduced activity, respiratory distress observed in mice and there were no mortality. The result of the acute toxicity of *Tetracarpidium conophorum* ethanol extract on wistar rats observed in this study is parallel the findings of Hodge and Sterner, [17] who deduced that the acute toxicity test of the seed extract at different concentrations which revealed no mortality rate and behavioural signs of toxicity on all the experimental mice is expected. This indicates that the extract could probably be non-toxic. This is in agreement with Hodge and Sterner, [17] who both reported that based on Hodge and Sterner Toxicity scale, any chemical exhibiting LD50 above 1000 mg/kg is practically non-toxic.

The result of the effect of *Tetracarpidium conophorum* seed extract on body weight of mice is shown in Table 3. The body weight of mice in group 5 (infected and not treated) was reduced from 19.71g on day 0 to 18.17g on day 5 even though there was insignificant increment from day 2 to day 3 (18.57 to 19.57). Similar situation occurs in the weight of mice in group 1 (19.71g to 14.50g wherein insignificant increment was perceived only from day 3 to 4), group 2 (19.71g to 16.00g wherein insignificant increment was perceived only from day 3 to 4). However, group 3 had significantly increase in weight on the first

day of treatment (18. 41g to 20.42g) then the increment and decrement from a day to the next day tends to be insignificant until from day 4 to day 5 when a significant decrement in weight was observed (20.42g to 17.80g). It should also be noted that the reduction in weight between days 0, 1 and 2 were also significant with that of day 5, hence we could conclude a reduction in weight in group 3 also. It is only in group 4 that we observed a significant increase in weight of mice because the reduction in weight observed between day 2 (20.71g) and day 3 (20.14g) and between day 4 (20.43g) and day 5 (18.71g) were not significant. Decrease in mice weight observed on the second day of infection with the *Plasmodium* parasite, could presumably be due to the decrease in food intake and disturbed metabolic functions associated with malaria infection. This corroborates the findings of Bashir et al. [18]. The decrease in weight that was observed in mice treated with different concentration of the seed extract could be due to the presence of oxalate and tannins which has been reported to be anti-nutritional factors in *T. conophorum* as supported by Edem et al. [9]. On the other hand, this decrease in mice weight could have been probably due the reason advanced that tannins reduce the bioavailability of proteins and protein value of foods in parallel with Ford and Hewith, [19]. Malaria, according to Bashir et al. [18] was reported to have induced low temperature in mice. This reason could have probably been responsible for the observed decrease in the temperature of the malaria infected experimental mice in this study. In addition, the decrease in temperature observed in the untreated *Plasmodium berghei*-infected mice could be attributed to the debilitating effects of malaria on the host (mice) which could have brought loss of body heat due to the large surface area to body mass ratio of small animal like mice. This is in agreement with World Malaria Report, [20]. Increase in temperature observed in mice administered with different concentrations (200 mg/kg, 400 mg/kg and 600 mg/kg) of *T. conophorum* seed extract agrees with findings of Ford and Hewith, [19] who reported an increase in temperature of malaria infected mice treated with the extract of *Russelia equisetiformis*.

The result of the effect of *Tetracarpidium conophorum* seed extract on numbers of infected RBC in mice is shown in Table 4. The numbers of infected RBC in mice in group 5 (infected and not treated) tends to increase from ≈86 on day 0 to ≈118 on day 5 even though there were insignificant reduction from day 2 to day 3 (≈102 to ≈92). However, there was significant reduction in the numbers of infected RBCs infected across all groups treated (group 1 to group 4) from the day the rats were infected to the final observation day even though there was significant increment (but negligible) in the numbers of infected RBCs in group 1 between day 1 and day two (≈103 to ≈123). The %PCV was seen to experience some changes after treatment (Table 5). This phenomenon is normal due to the effect of the extract on the cells in the blood especially the Red Blood Cells (RBCs) since the plasmodium parasites are usually localized in these cells and any treatment protocol will involve lysing of the cells and consequently affect the percentage packed cell volume. This condition is usually temporary because when the parasites are cleared from circulation, the cells begin to gradually divide and replenish the blood. The slight fluctuation in the erythrocytes counts (Table 4) is also understandable from the point of parasite lysing infected cells while the change experienced in the leukocytes counts (white blood cells) (Table 5) may be due to the fact that they is responsible for defensive response of the body to disease because they help to boost the immune system of the body and scavenge parasite in the blood. Findings from this study which revealed an increase in the levels of blood parameters (HGB) (haemoglobin), PCV (Packed cell volume, RBC (Red blood cells) in the mice administered different concentrations of the seed extracts (groups 1 to 3) compared with group A mice (infected and not treated) is in contrast with the findings of Apeh et al. [21] who reported decrease in the levels of haemoglobin, Packed cell volume, Red blood cells in mice fed with *T. conophorum* seed extract and attributed it to the presence of cyanide which might have hindered the formation of the haematological indices in mice. Decrease in the values of HGB, RBC and PVC observed in the negative control group is expected and could probably be due to anaemia as reported by Chang and Stevenso, [22].

The result of biochemical analysis shows that for mice in group 1, compared with the control group (group 5), they possessed significantly lower AST, ALT. Their Tbl, BUN, Create, Trig were lower but the differences were not significant while their HDL were higher but not significantly different from that of the control group. For mice in group 2, compared with the control group (i.e. group 5), they possessed significantly lower AST, ALT, TBL, BUN, Create, Trig. Their HDL and LDL were lower but the differences were not

significantly different from that of the control group. For mice in group 3, compared with the control group (i.e. group 5), they possessed significantly lower AST, ALT, TBL, BUN, Create, Chol, Trig and LDL. Only their HDL was lower but not significantly different from that of the control group. All biochemical in all rats in group 4 were significantly lower compared with the biochemical constituents of control group after treatment (i.e. group 5) as shown in Table 6. Increase in the level of AST (Aspartate transaminase) and ALT (Alanine transaminase) observed group A (mice infected and not treated) and increase in ALT observed in group 1 (mice administered with the highest dose of the seed extract) could be attributed to the reason suggested by Ojo et al. [23] that very high level of AST and ALT are usually due to liver disorder or decreased blood flow to the liver caused by malaria infection. However, decrease in the ALT and AST in the mice administered 400 mg/kg (group 2) could be as a result of hepatoprotective and nephroprotective ability of the seed extract and decreased AST level observed in mice administered 800 mg/kg (group 1) concentration of the seed extract could be due to the nephroprotective ability of the extract at that dose. This reason was advanced by Atawodi et al. [24] who, on serum biochemistry of mice administered *Dodonaea angustifolia* reported decrease in the levels of AST and ALT in mice.

The kidney of mice in group A (infected and not treated) was characterized with glomerular swelling tightly filling the Bowman's capsule, degeneration of distal and proximal tubules, renal parenchymal vacuolation as shown in Plate 6. Mice in group B (chloroquine treated group) showed vacuole formation in the cortex with glomerulus filling the Bowman's space as shown in Plate 7. The kidney of mice in group 3 (200 mg/kg body weight of extract) showed multifocal tubular epithelial coagulation necrosis and inflammation as shown in Plate 8. For mice in group 2 (administered with 400mg/kg body weight of the extract) the kidneys showed diffused tubular atrophy, epithelial coagulation necrosis as shown in Plates 9. For mice in group 1 (administered with 800mg/kg weight of the extract) the kidneys showed patchy tubular epithelial coagulation necrosis and inflammation as shown in Plate 10.

The change in liver morphology with high vacuole formation in the parenchyma observed in mice infected and not treated (group 5) is expected and was reported to be due to cellular trauma and morphological change in the tissue architecture, a normal reaction of the liver tissue to infection. This is in agreement with the findings of Innocent et al. [25] who reported change in the morphology of parenchyma tissue of the liver, hyperplasia and high vacuole formation in the liver of mice infected with *Plasmodium berghei.* The Normal morphology of the liver with well-arranged parenchyma tissues of the liver and intact hepatocytes observed in the normal control group (group 4) and group 2 (administered 400mg/kg of seed extract) is expected. It could be due to the restorative effect of the seed extract on the liver. This is in line with the findings of Syed et al. [26] who worked on the hepatoprotective and nephroprotective activity of *Phyllanthus amarus* seed extract reported that the liver of wistar rat show a normal morphology and intact hepatocytes. Hyperchromatic liver cell with fatty infiltration observed in mice administered 800 mg/kg body weight of the extract could be due to the accumulation of fat in the liver of the mice, as *Tetracarpidium conophorum* seed extract has been reported to contain high fat content which could be a health risk factor [27].

The glomerular swelling, degeneration renal parenchymal vacuolation, necrosis in the glomerulus and cellular proliferation appear in mesengial area, which are signs of renal toxicity observed in the kidney of mice infected and not treated (group 5) is expected. This could be due to the free hemoglobin which catalyzes oxidative damage, hypoxia and lactic acidiosis, promoting metabolic acidiosis which is aggravated by the altered renal function that is observed in patients with malaria [28]. This agrees with the findings of Bashir et al [18] who reported vacuolation in the tubules, necrosis in glomerulus of mice infected with *Plasmodium berghei*. The distinct glomerulus, well intact proximal and distal tubules observed in group 2 (treated with 400mg/kg seed extract) and 1 (treated with 800mg/kg seed extract) suggests that the seed extract of *T. conophorum* improved the architecture of the kidney. This agrees with the findings of Ezejindu et al. [29] who reported that the leaf extract of *Moringa oleifera* did not produce adverse effect on the kidney of experimental mice.

5. CONCLUSION

The presence of flavonoid and saponin in the seed extract of *Tetracarpidium conophorum* in this study could have been responsible for the

observed boost of the immune system in the mice. It was observed that non anti-plasmodial activity could probably be attributed to the absence of steroids in the seed extract. The cooked seed in this study has shown to be generally less effective compared to raw seeds utilized in a previous study as reported by Ogundolie and Dada, [15]. Further evaluation needs to be carried out before it could be employed for treatment of malaria. The seed extract at 200mg/kg when administered to the mice was observed to have restorative effects on the liver and kidney of the mice. However, the 400mg/kg restored the kidney but had adverse effect on the liver. Hence, *T. conophorum* seed extract at the dose of 200mg/kg could be considered suitable to treat human malaria infection.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

Authors appreciate the efforts of Dr. Fayehun Lawrence at the crop, soil and pest management Department, Federal University of Technology, Akure in authenticating the *Tetracarpidium conophorum* seeds used in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Na-Bangchang K, Congpuong K. Current malaria status and distribution of drug resistance in East and Southeast Asia with special focus to Thailand. Tohoku J Exp Med. 2016;211:99–113.
2. World Health Organization the world health report: Fighting disease, fostering development. Geneva: World Health Organization. 2016;137.
3. Berendt AR, Tumer CDH, Newbold CL. Cerebral malaria: The sequestration hypothesis. Parasitology Today. 2014;10(10):412-414.
4. Druilhe P, Hagan P, Rook GAW. The importance of models of infection in the studyof disease resistance. Trends Microbiol. 2015;10(10):38-46.
5. Basir R, Fazalul Rahiman SS, Hasballah K, Chong WC, Talib H, Yam MF, Jabbarzare M, Tie TH, Othman FM, Moklas MA, Abdullah M, Ahmad Z. Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria. Iran J Parasitol. 2012;7(4):62-74.
6. Srinivasan A, Viraraghavan T. Removal of oil by walnut shell media. J. Bioresource Technol, 2018;99:8217-8220.
7. Okerulu IO, Ani CJ. The phytochemical analysis and antibacterial screening of extracts of Tetracarpidium Conophorum. J of Chem Soc of Nigeria. 2015;26(1):53-55.
8. Malu SP, Obochi GO, Edem CA, Nyong BE. Effects of methods of extraction on phytochemical constituents and antibacterial properties of Tetracarpidium conophorum seeds. Global J of Pure and Appl Sci. 2009;15(3):373-376.
9. Edem CA, Dosunmu MI, Bassey FI. Determination of proximate composition, ascorbic acid and heavy metal content of African Walnut (*Tetracarpidium conophorum*). Pak J Nutrition. 2009;8:225-226.
10. Santhi K, Sengottuvel R. Qualitative and quantitative phytochemical analysis of concanensis nimmo. Intl J Curr Microbiol Appl Sci, 2016;5(1):633-640.
11. Rehab MOM, Saad MHA. Study of phytochemical screening and antimicrobial activity of citrus aurantifolia seed extracts. Am of Anal Chem. 2016;7:254-259.
12. Ogundolie OO, Dada EO, Osho IB, Oloruntola DA. Effects of raw ethanol seed extract of tetracarpidium conophorum on heamatological and histopathological parameters in swiss albino mice infected with *Plasmodium berghei*. J. of Appl Life Sci Intl. 2017;12(2):1-14. Article no.JALSI.33244

13. Yerbanga RS, Koama BK, Matsabisa JB, Ouedraogo JB, Ouedrago GA. Evaluaton of the antiplasmodial activity and lethality of the leaf extract of *Cassia alata* L. (Fabaceae). Pak J of Biol Sci. 2016;19:171-178.
14. Dada, Muhammed. Antiplasmodial activity of ethanolic leaf extract of Eucalyptus citriodora in swiss Albino mice infected with plasmodium Berghei NK 65: South-Asian J. of Microbiol. 2018:1(2):1-8, Article no.SAJP.43986
15. Dada EO, Ogundolie OO. *In vivo* antiplasmodial activity of raw ethanol seed extract of tetracarpidium conophorum in Swiss albino mice infected with plasmodium berghei. J. of Adv in Medical and Pharma Sci. 2016;9(2):1-8, Article no.JAMPS.26789.
16. Enujiugha VN, Ayodele-Oni O. Evaluation of oil nutrient and anti-nutrients in lesser known underutilized oilseeds. Intl J Food Sci and Technol. 2003;38:525-528.
17. Hodge A, Sterner B. Toxicity classes: Canadian center for occupational health and safety. 2005;53-56.
18. Basir R, Fazalul Rahiman SS, Hasballah K, Chong WC, Talib H, Yam MF, Jabbarzare M, Tie TH, Othman FM, Moklas MA, Abdullah M, Ahmad Z. Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria. Iran J. Parasitol. 2012;7(4):62-74.
19. Ford JE, Hewitt JW. Protein quality of cereal and pulses. Application of microbiological and other in vitro methods in the evaluation of rice (Oryza sativa), sorghum (Soighum vilgare), barley and field bean (Vicia faba). British J. Nutrition. 2010;33:314-352.
20. World Malaria Report. WHO Press, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland. 2011;1-137.
21. Apeh VO, Agu CV, Ogugua PN, Uzoegwu EG, Anaduaka TE, Agbalu IS. Effect of cooking on proximate, phytochemical constituents and haematological parameters of Tetracarpidium conophorum in mlae albino rats. Euro J of Medicinal Plants. 2014;4(12):1388-1399.
22. Chang KH, Stevenso MM. Malarial anemia: Mechanism and implications of insufficient erythropoiesis during blood-stage malaria. Intl J of Parasitol. 2014;24:1501-16.
23. Ojo OO, Nadro MS, Tella IO. Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatotoxicity. Afri J Biotechnol. 2006;5(9):755-760.
24. Atawodi SE, Nuratu A, Bature EI, Isah MA. Lipid profile, haemoglobin level and antioxidant effect of pre- treatment with Methanol extract of Byrsocarpus coccineus Shum & Thonn. Leaf against Carbon Tetrachloride-Induced injuries to liver, kidney and heart of rats. British Biotechnol J. 2014;4(12).
25. Innocent AE, Inyang AA, Rosemary BB, Enobong IB, Sediongde JU. Erythropoeitic and hepatoprotective potential of ethanol extract of Nauclea latifola in mice infected with Plasmodium berghei. Amer J of Medical Sc and Med. 2014;2(10): 7-12.
26. Syed AB, Iqbal MM, Ibrahim MD. hepatoprotective and nephroprotective activity of Phyllanthus anmarus Schum & Thonn. Seed extract. Ann of Phytomed. 2012;1(2):97-104.
27. Hassan LG, Umar KJ. Nutritive value of balsam apple (*Momordica balsanina* L) leaves. Pak J of Nutrition. 2007;5(6):522–529.
28. Renia L, Howland S, Claser C, Charlotte Gruner A. Cerebral malaria: Mysteries at the blood-brain barrier. Virulence. 2012;3(2):193-201.
29. Ezejindu DN, Udemezue OO, Akingboye AJ. Protective effects of Moringa oleifera leaf extracts on the kidneys of adult wistar rats. Amer J of Eng Res. 2014;3(2):157-16.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/70956