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Effects of Hydro-ethanolic Leaf Extract of *Tithonia diversifolia* on Parasitaemia Level, Serum Metabolites and Histopathology of Organs in Swiss Albino Mice Infected with *Plasmodium berghei NK*65

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAO and EOD designed the study. Author DAO performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EOD and IBO managed the analyses of the study. Authors DAO and OOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed to determine the antiplasmodial, biochemical and histopathological activities of hydroethanolic leaf extract of *Tithonia diversifolia* in Swiss albino mice infected with *P. berghei* NK65. Thirty (30) mice were randomly distributed into six groups of five mice each. Groups A, B and C were infected with *P. berghei* and treated with 0.2 mL of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight of *T. diversifolia* extract respectively. Group D (positive control) was infected with *P. berghei* and treated with 0.2 mL of 5 mg/kg body weight of chloroquine. Group E (negative control) was infected with *P. berghei* and treated with 0.2 mL of normal saline while group F (normal control) was not infected and administered with 0.2 mL of normal saline for four consecutive days. Blood samples and selected organs were collected using standard procedure.

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Biochemical analysis and histopathological evaluation of the organs were also carried out according to the standard methods. The ethanolic leaf extract of *T. diversifolia* produced average parasitaemia level of 1489.37, 1318.25 and 136.75 at doses of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight in groups A, B and C respectively. Group D (positive control) and group E (negative control) having 0 and 3607 parasitaemia respectively. The AST, bilirubin, triglyceride, urea and creatinine were not significantly ($p>0.05$) affected by the treatment. However, significantly higher ($p<0.05$) level of ALT and cholesterol were recorded in group E. Light microscope examination of liver and kidney tissue of mice treated with extract did not show any structural abnormality. This study shows that the hydro ethanolic leaf extract of *T. diversifolia* has antiplasmodial effect and may not exact toxic effect on the internal organs.

Keywords: Histopathological; Tithonia diversifolia; Swiss albino mice; liver; kidney; biochemicals hydroethanolic.

1. INTRODUCTION

Malaria is a life threatening parasitic disease caused by a protozoan parasite of the genus *Plasmodium.* CDC [1] also reported malaria as one of the prevalent communicable diseases that is most dangerous in the world and that the disease contributed to major socio-economic problems which lead to global instability and poverty. It had been reported to be the cause of mortality and morbidity worldwide [2]. According to United Nations Children's Fund [3], malaria is endemic throughout the tropical countries and it is the leading cause of morbidity and mortality in sub-Sahara Africa. Over 80 percent of deaths that occur mostly among children and pregnant women were attributed to *P. falciprum* infection [4].

The use of antimalarial drugs such as chloroquine, amodiaquine, sulphadoxine, pyrimethamine had been a popular means of treating malaria; however, the resistance developed by malaria parasites has been the major problem to the use of these conventional antimalarial drugs [5]. Several factors such as; drug resistance of malaria parasite to affordable antimalarial drugs e.g chloroquine, environmental changes, self medication, the failure of health systems in area of civil strife or ethnic war, and limitation in national health services were reported as major contributors to the spread of malaria [6].

Traditional medicine has remained the most affordable, easily accessible source of treatment in the primary health care system of resource poor communities. Local people have a long history of traditional plant usage for medicinal purposes [7]. Medicinal plant is an important element of indigenous medical systems all over the world. Ethnobotany provides a rich resource for natural drug research and development. In many developing countries about 80% of the populations rely on traditional medicine for the treatment of various ailments including life-threatening ones such as malaria. Currently, there is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost.

Tithonia diversifolia (Hemsely) A.Gray mostly known as Mexican sunflower and it has been considered by several researchers to be a medicinal plant that is widely used in folk medicine to treat various illness such as hepatitis, gastrointestinal disorder, apply on wounds and measles [8,9]. Its antiplasmodial activities has been attributed to many active components present in *T. diversifolia* leaf such as taginins A, B, C in which taginin C is main active component against *Plasmodium* [10]. According to Dada and Oloruntola [11], the qualitative and quantitative phytochemical screening revealed the presence of some bioactive compounds such as alkaloids, tannins, saponins, flavonoids and glycosides which is also in agreements with the findings of Goffin et.al. [10]. There has been relatively low study carried out on the effect of the use of hydro ethanolic leaf extract of *T. diversifolia* on major internal organs of *P. bergehi* infected mice. Therefore, in this present study, the effect of ethanolic leaf extract of *T. diversifolia* on parasitaemia level, biochemical and histopathology of liver and kidney was evaluated.

2. METHODOLOGY

2.1 Preparation of Plant Extract

T. diversifolia leaves were collected from Ikere Ekiti, in Ekiti State, Nigeria. After the

identification and the authentication of the plant leaves by a plant scientist from Crop, Soil & Pest management Department, School of Agricultural & Agricultural Technology, The Federal University of Technology Akure.

These leaves were washed in clean water, drained and chopped into smaller pieces, spread lightly on the polythene under shed to air dry at room temperature for four weeks until it becomes crispy. Then they were pulverized using mortar and pestle. Five hundred grams (500 g) of the pulverized leaf powder was macerated in 4.5litre of 75% ethanol for 72 hours and then filtered using Millipore (pore size 0.7 µm) filter paper. By the use of rotary evaporator at reduced temperature of 40℃, the filtrate was concentrated to recover the extract before it was freeze-dried. The yielded extract was about 30.0 g and stored in an air tight container for the study. Phytochemical analysis of yielded hydro-ethanolic leaf extract of *T. diversifolia* was carried out using standard procedures [12] before the commencement of the experiment.

The acute toxicity test for hydro-ethanolic leaf extract of *T. diversifolia* extract had been reported by Dada and Oloruntola [11] that the highest dosage of 1600mg/kg did not show any behavioral signs of toxicity and mortality, it is therefore considered to be probably safe.

2.2 Experimental Animals

Swiss albino mice of average weight of 20 g±1.5 were obtained from Animal house of Multi-disciplinary Laboratory, Faculty of Health Sciences, OAU, Ile Ife, Nigeria. These mice were acclimatized for 7 days during which they were fed with pelletised feed (growers mash) and water *ad libitum.* Chloroquine sensitive strain of malaria parasite (*P. berghei* NK 65) in a donor mouse was obtained from the Department of Pharmacognosy, Faculty of Pharmacy Obafemi Awolowo University Ile-Ife, Nigeria. All animals were cared for by a veterinarian in accordance with the "Guide for the Care and Use of Laboratory Animals".

The antimalarial activity was determined in this study using the Peter's 4-day suppressive test method [13]. A total of thirty (30) swiss albino mice were put into 6 groups; 5 mice per group (thus 3 test groups and three control group). Group A to E were passaged with 0.2 ml of standard inoculum of *P. berghei* while group F (normal control) was passaged with 0.2 ml of sterile distilled water. Three hours post infection with *P. berghei.* Groups A, B and C were administered 200, 400 and 600 mg/kg body weight of ethanolic leaf extract respectively, group D and E were administered 5 mg/kg body weight of chloroquine phosphate and 0.2 ml of sterile normal saline respectively while group F were administered 0.2 ml of sterile normal saline. All doses were administered orally for 4 consecutive days. On day 5 of the experiment, 2 drops of blood sample were respectively collected onto grease free microscope slide from the mice caudal vein. Thick and thin blood smear were made and allowed to air-dry. The air-dried films were fixed with 75% methanol for 2 minutes, they were then stained with 10% Giemsa stain for 15 minutes. The blood smear samples were rinsed with buffered distilled water pH 7.2. The parasitaemia was determined using the method described by Cheesbrough [14]. Using the oil immersion objective (x100), white blood cells were counted systematically, counting at the same time the number of parasites in ten fields. The counting was done using hand tally counter. Avereage level of parasitaemia for each group was obtained by dividing the sum of parasitaemia in each group by number of mice per group (5 mice). The entire experimental management, handling and care were approved by the Research and Ethics Committee of the Microbiology Department, The Federal University of Technology, Akure, Nigeria.

Parasites density per microlitre (µL) of blood=

$$\frac{\text{Parasites counted X White Blood Cells count}}{200}$$

2.3 Biochemical Assay

Blood samples were collected from mice into a plain bottle by cardiac puncturing, allowed to clott and the serum separated was used for the analysis. The biochemical assay; urea, creatinine, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride and total cholesterol were determined using automated reflotron machine (Roche U_L US Listed Laboratory Equipment 8c79. Germany).

2.4 Histopathological Studies

Prior to organ excision, the chemical method of euthanasia was used for the animals. Ether was used to soak the cotton wool pad, and then it was placed below metal gauze in a desiccator

jar. The animal was placed in the jar one after the other and observed continuously until they became unconscious [15]. For the histopathological study, the standard procedure of Baker and Silverton [16] for sectioning was used. Liver and kidney of each mouse were excised, rinsed in normal saline, blotted dry on tissue paper and were transferred into a universal bottle containing 10% formol saline as preservatives. The fixed organs were washed in running tap water severally to remove oxidizing agents in fixative. The organs were sliced; thereafter the tissues were then transferred into different grade of alcohol (70%, 90% and absolute) for 4 hours each in order to cause tissue dehydration. The tissue were cleared in clearing agent (xylene) for 1½ hour and later transferred into a bath of molten paraffin wax in a mould using warm pair of blunt-nosed forceps. The surface of the mould was blown until a thin film of wax had solidified and later immersed gently into a container of cold water for 30 minutes. The embedded tissues were sectioned by trimming the block using microtome machine. The ribbon produced was transferred gently onto the surface of warm water in a water bath (10℃). When the tissue sectioning was fully expanded, a prepared clean, grease-free slide was dipped obliquely into the water as close to the section as possible and allowed its surface to touch the edge of the section. Slides were completely removed with attached section and the sections were adjusted to a suitable position on the slides with a mounted needle. The haematoxylin and eosin (H&E) staining method was used to demonstrate the general structure of the tissues. The slides with section were transferred to hot plate (45℃) for 1hour to ensure that the sections were thoroughly dried before staining. Paraffin wax was removed from the slides by immersing the slides in xylene for 30 minutes. Slides were then transferred into absolute alcohol for 30 seconds to remove the xylene. Using blunt-nosed forceps, the slides were transferred into the different descending grades of alcohol (90%, 70%) for 30 seconds each and were finally washed thoroughly in distilled water.

Slides were flooded with solution I (Haematoxylin) for 30 minutes. They were washed thorough in running tap water, and differentiated in solution II (Acid alcohol) until no more colour is seen. The slides were flooded with solution III (Scott's tap water) for 10 minutes and counterstained in solution IV (Eosin) for 2 minutes. Finally, they were washed in running water until the excess eosin was removed. They were dehydrated in ascending grades of alcohol (70%, 90% and absolute) for 30 seconds each, cleared in two changes of xylene for 3 minutes. The slides were mounted using cannada balsam and cover slip.

2.5 Statistical Analysis

Descriptive statistics was used for the level of parasitaemia in the experimental mice. Results obtained from the biochemical assay study were statistically analyzed using one way analysis of variance (ANOVA). Significant differences between the treatment means were determined at 95% confidence level.

3. RESULTS

Fig. 1 shows the antiplasmodia activity of hydro-ethanolic leaf extract of *T. diversifolia* in swiss albino mice infected with *P. berghei.* There were 60.03%, 63.45% and 92.96% reduction of average parasitaemia level in groups of swiss albino mice infected with *P. berghei* and treated with 200 mg/kg (group A), 400 mg/kg (group B) and 600 mg/kg (group C) leaf extract respectively when compared to the group infected with *P. berghei* and treated with 0.2 mL normal saline (group E).

The result of biochemical parameters in mice treated with various doses of the hydro- ethanolic leaf extract of *T. diversifolia* are shown in Table 1. All these parameters: Aspartate amino transferase (AST), bilirubin, triglyceride, urea and creatinine were not significantly ($P>0.05$) affected by the treatment applied except for alanine amino transferase (ALT) and cholesterol. The ALT value obtained from mice in group E (negative control) was higher than the observed value in groups A, B, C, D and F. Similar trend was also observed with cholesterol level, in which the highest value recorded in group E was higher than the observed value in groups A, B, C, D and F.

3.1 Effect of Ethanolic Leaf Extract of *T. diversifolia* on Mice Liver

Liver is an organ that was encapsulated with a thin layer of connective tissue. The hepatocyte contains central nuclei with densely stained chromatin and with slightly eosinophilic stained cytoplasm. Hepatocytes are well arranged polygonally along the sinusoid arraying from the hepatic central vein. The microscopic

examination of the liver sections of the treated groups of mice showed a normal architecture (Plates 1-6). Mice treated with hydro ethanolic leaf extract of T. diversifolia (Plates 1-3) showed no significant changes in their liver as compared with normal control group (Plate 6). Except in negative control (Plate 5) that there was shrunken of the nuclei cells and vacuolar formation.

3.2 Effect of Ethanolic Leaf Extract of *T. diversifolia* on Mice Kidney

The assessment of tissue sections of the kidney showed no damage in both extract treated groups (Plate 7-Plate 9) and control groups Plate 10 and Plate 12 (positive and normal control groups). Microscopic examination indicated that there was no difference observed between the kidney sections of the groups except group 5 (negative control) which shows parenchyma vacuolation, with glomerulus swelling tightly filling the bowman's capsules. All other groups exhibited normal features: Bowman's space, convoluted tubules and glomeruli were clearly seen and normal as compared to the control group F (Plate12).

4. DISCUSSION

The increased parasitaemia level in negative control group observed in this study corroborates the findings of Jude et al. [17] who observed increased parasitaemia in negative control with ethanolic extract of *Aspilia africana.* However, the 100% chemosuppressive effect of chloroquine recorded in this present study showed that the drug is still one of the drugs of choice against malaria parasite. This result agreed with report Oyewole et al. [8] on the antimalarial activity of *T. diversifolia* and chloroquine.

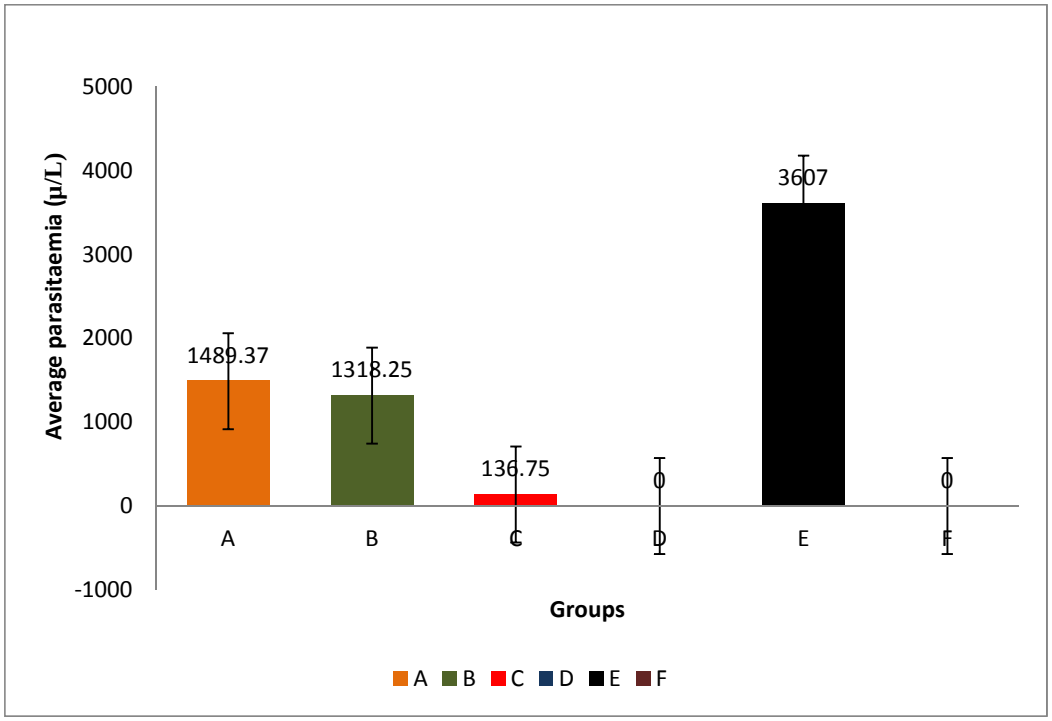


Fig. 1. Antiplasmodial activity of ethanolic leaf extract of *Tithonia diversifolia* in Swiss albino mice

Group A: P. berghei + 200 mg/kg body weight leaf extract;
Group B: P. berghei + 400 mg/kg body weight leaf extract;
Group C: P. berghei + 600 mg/kg body weight leaf extract;
Group D: P. berghei + 5 mg/kg body weight Chloroquine;
Group E: P. berghei + 0.2 mL normal saline,
Group F: 0. 2 mL normal saline

Table 1. Effect of ethanolic leaf extract of *T. diversifolia* on serum metabolites of Swiss albino mice infected with *P. berghei*

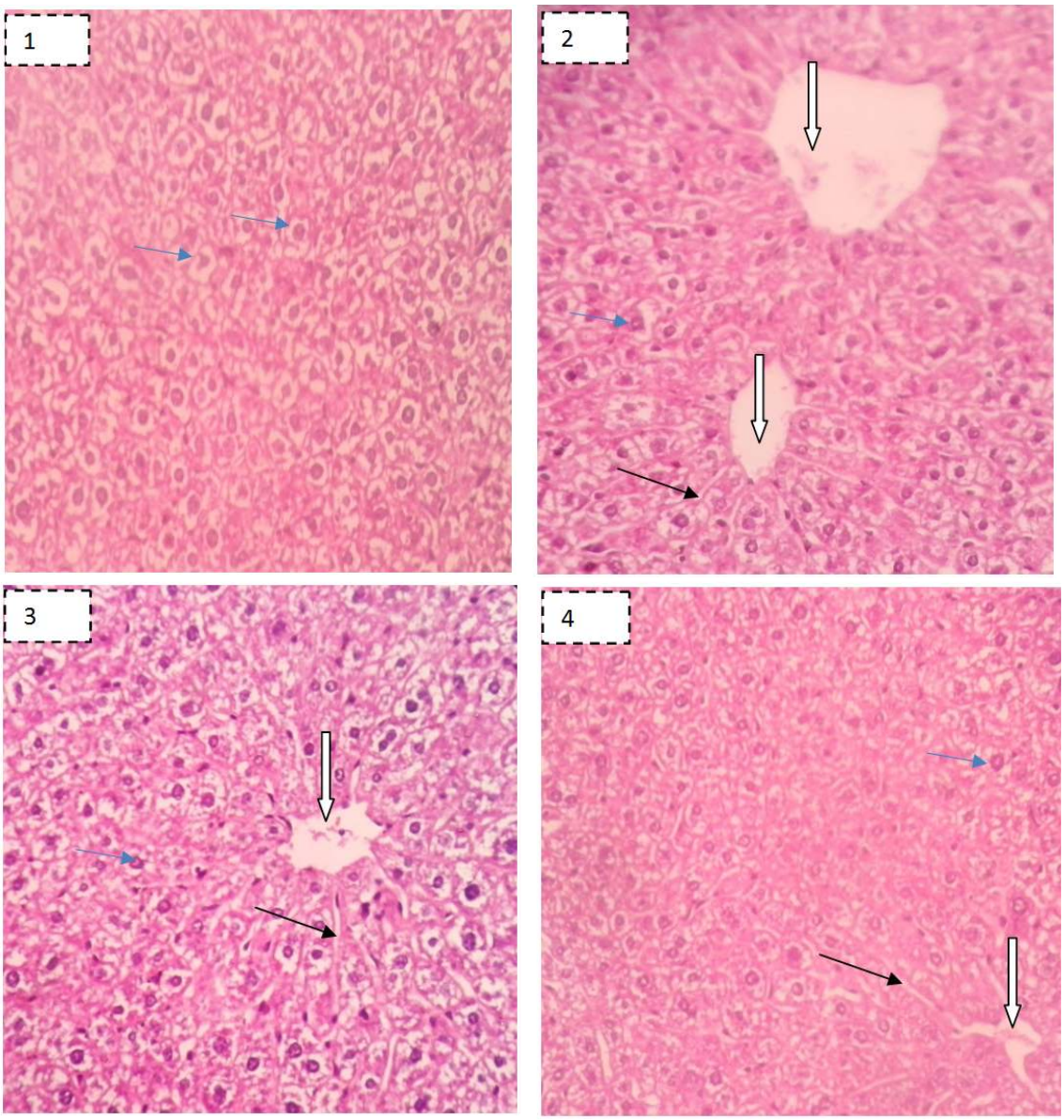
Parameters	Group A	Group B	Group C	Group D	Group E	Group F	P values
AST (µ/l)	226.06±17.87[a]	247.33±14.71[a]	378.66±10.5[a]	271.33±6.97[a]	395.31±15.56[a]	290.00±29.05[a]	0.23
ALT (µ/l)	24.66±1.69[b]	23.53±4.74[b]	26.70±1.42[b]	25.86±2.81[b]	39.90±4.43[a]	23.91±2.85[b]	0.03
Cholesterol (mmol/l)	2.34±0.14[b]	2.40±0.14[b]	2.42±0.13[b]	2.55±0.31[b]	4.44±0.57[a]	2.42±0.07[b]	0.01
Bilirubin (µmol/l)	61.77±31.15[a]	66.70±10.42[a]	70.70±14.37[a]	77.90±7.74[a]	92.73±22.51[a]	74.03±12.44[a]	0.85
Triglyceride (mmol/l)	1.12±0.19[a]	1.14±0.24[a]	1.16±0.24[a]	1.17±0.10[a]	1.30±0.06[a]	1.20±0.06[a]	0.95
Urea (mmol/l)	2.73±1.70[a]	2.79±0.84[a]	3.13±1.12[a]	2.93±0.62[a]	3.05±1.18[a]	4.16±0.61[a]	0.93
Creatinine (µmol/l)	24.10±3.11[a]	22.73±2.81[a]	23.50±3.55[a]	23.01±3.93[a]	22.80±3.81[a]	23.80±3.05[a]	1.00

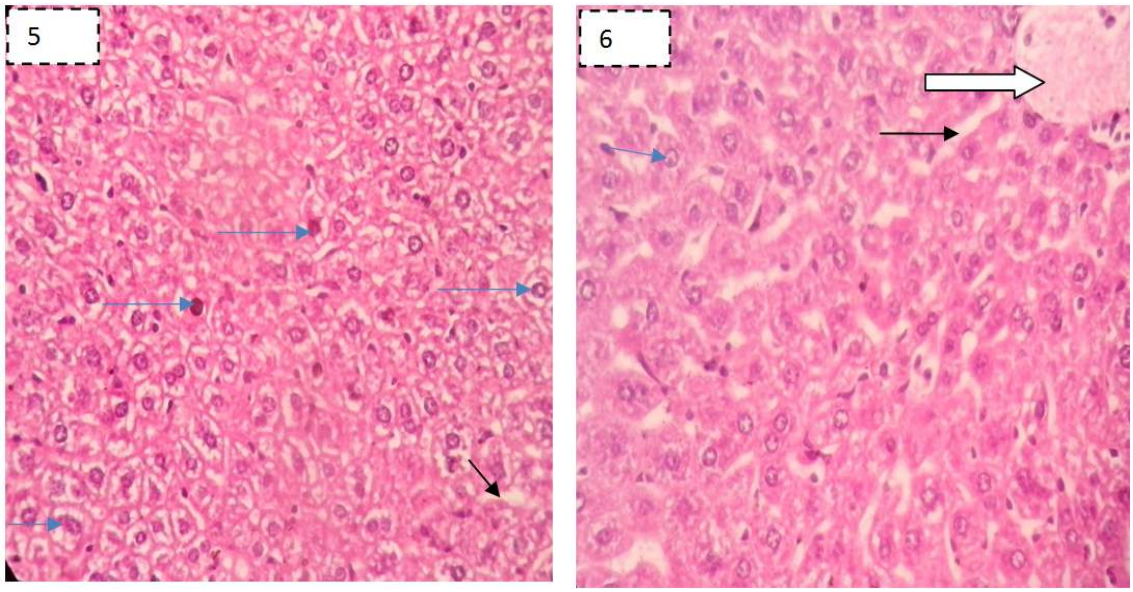
Means with different letters in the same row are significantly (P<0.05) different

Group A: P. berghei + 200 mg/kgbody weight leaf extract; Group B: P. berghei + 400 mg/kgbody weight leaf extract;
Group C: P. berghei + 600 mg/kgbody weight leaf extract; Group D: P. berghei + 5 mg/kgbody weight Chloroquine;
Group E: P. berghei + 0.2 mL normal saline, Group F: 0.2 mL normal saline. AST: Aspartate amino transferase; ALT: Alanine amino transferase

While the reduced parasitaemia in treated groups extract could probably be attributed to the presence of phytoconstituents such as alkaloids and flavonoids in the studied extract. This study is also in agreement with the findings of Abdulrazak et al. [18] and this is because alkaloids play a particular role on malaria treatment because quinine is the first chemical that was identified in alkaloid and is used for malaria treatment.

Serum biochemical analyses were carried out to monitor the effect on the renal and hepatic functions and also on lipid profile. The major activities of liver enzymes (ALT and AST) in the serum or plasma can be used to assess the integrity of the organ indirectly after a certain exposure to chemicals agents. The increases in the serum level of ALT on group E (infected untreated) compare with the control and extract administered groups observed in this study is in line with the findings of Nwodo et.al. [19] who suggested that increase of ALT might be an indication that liver was affected by malaria parasite which amount to leakage of this enzyme from damaged cell by *P. berghei*. The decrease in liver enzymes observed in the hydro ethanolic extract treated groups probably suggests hepatoprotective ability of *T. diversifolia* which is

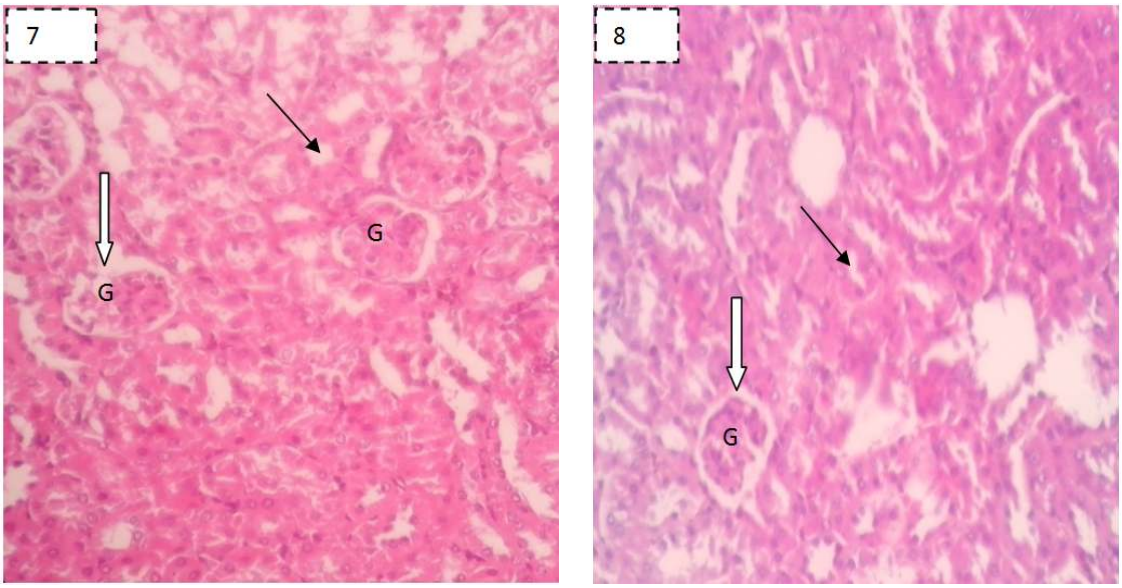


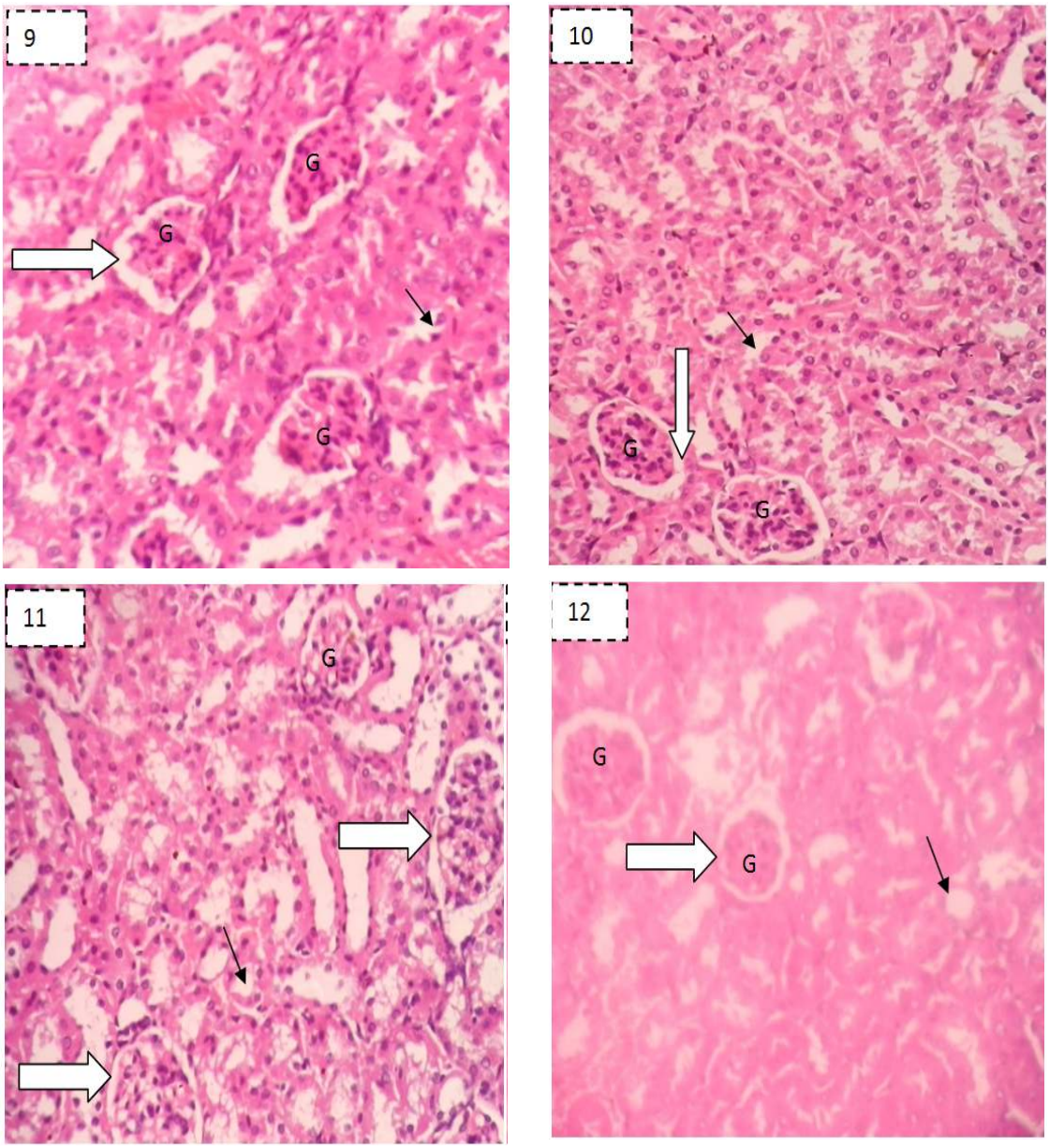


Plates 1-6. Photomicrographs of liver sections (X400) of the mice treated with 200 mg/kg (plate1), 400 mg/kg (plate 2), 600 mg/kg (plate 3) hydro ethanolic leaf extracts of *T. diversifolia* showing no histopathological changes. Plate 4 (positive control group) revealed normal architecture. Plate 5 (negative control group) shows shrunken of the hepatocytes, while Plate 6 (normal control group) shows normal architecture of the liver

Note: white arrow: central vein; black arrow – sinusoid; blue arrow- hepatocytes

in agreement with Osadebe et al. [20] who observed decrease in the ALT and AST and that this may probably be due to the presence of saponin, flavoniods and tannins in the leaf extract which suggest the that leaf possess antioxidant and anti-inflammatory activities that are capable of reducing and protecting organ damage from oxidative stress. Also, it may probably be due to the relatively lower concentration or short term administration of the extract. This observation is in line with the findings of Adebayo et al. [21] who observed no alteration in serum AST and ALT activities at the concentrations used in the study.





Plates 7-12 Photomicrographs of kidney sections (X400) of the mice treated with 200 mg/kg (plate 7), 400 mg/kg (plate 8), 600 mg/kg (plate 9) hydro ethanolic leaf extracts of *T. diversifolia* showing no histopathological changes and the positive control groups (plate 10), normal control (plate 12) shows normal architecture as compared with negative control group (plate11) in which its shows glomeruli swelling tightly filling the Bowman's space with parenchyma vacuolation

Note: White arrow-bowman's space; G - Glomerulus; Black arrow-convoluted tubule

Saponins are known to elicit serum cholesterol lowering activity, thereby reducing the enterohepatic circulation of bile acids which are reported to increase fecal cholesterol excretion [22]. This probably may account for the observed value in groups of mice treated with the hydro ethanolic leaf extract (group A to C). The increase in the total cholesterol in the untreated infected group (group E) compared to other groups is in agreement with Oluba et al. [23] who attributed the increase in total cholesterol with the decreased uptake of cholesterol by the

infected red blood cells in high level of parasiteamia.

The insignificance (P>0.05) in bilirubin level in the plant treated mice (group A-C) compared with group D (chloroquine treated) and group F (normal control) observed in this study probably suggest that the leaf extract does not pose any adverse effect on the bile duct or heamoglobin metabolism pathway. High level of bilirubin in group E in this study may be probably due to the breaking down of the red blood cells (haemolysis) usually occur in malaria infection or obstruction of bile duct this is in line with Siwe et al. [24].

In the present study, treatment with hydro ethanolic leaf extract of *T. diversifolia* did not cause any pathological effect in the liver and kidney of mice in the extract treated groups, this agrees with the findings of Passoni et al [25] and may probably be due to the smaller concentration or short term administration of the extract. The change in liver morphology with high vacuole formation in the parenchyma observed in mice infected and not treated (group E) is expected as it may be due to stress or trauma imposed on the liver tissue in malaria infection.

5. CONCLUSION

In conclusion, the results of this study demonstrate that the hydro ethanolic leaf extract of *T. diversifolia* has antiplasmodial and hepatoprotective activities in mice. Further investigation to determine the exact phytoconstituents that is responsible for these activities and its effect on long term administration is recommended for further studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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