OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2018.58.72

Research Article
Effects of Combined Treatment of *Tithonia diversifolia* and Chloroquine on Selected Organs of Chloroquine Resistant *Plasmodium yeolii* Infected Mice

[1]Omoboyowa Damilola Alex, [2]Nwodo Fred and [1]Joshua Parker Elijah

[1]Department of Chemical Science, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

Abstract

Background and Objective: The influence of orthodox drugs-plants based combination therapy on the organs function remains the basis of toxicity study. This study evaluated the effects of ethanol leaf extract and bio-active fraction of *Tithonia diversifolia* administered singly and in combination with chloroquine on selected organs using chloroquine resistant *Plasmodium yeolii* (*P. yeolii*^{R}) infected mice. **Materials and Methods:** Ninety-six mice were divided into 12 groups of 8 mice each (n = 8). Groups II to XII mice were infected with *P. yeolii*^{R} and treated with varying doses of crude ethanol extract and bio-active fraction of *T. diversifolia* leaf singly and in combination with chloroquine. Liver marker enzymes, kidney function test and organs' histology of the experimental animals were assayed using standard protocol. Data obtained were analyzed using one way analysis of variance, followed by *post hoc* multiple comparison test using SPSS software. **Results:** The infected mice treated with antimalarial drugs (chloroquine and artemether) and varying doses of the crude extract and bioactive fraction (C70:M30) singly and in combination with therapeutic dose of chloroquine showed significant ($p<0.05$) reduction in alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities compared with the infected mice administered 0.2 mL of distilled water. Co-administration of bioactive fraction and chloroquine to parasitized mice showed significant ($p<0.05$) higher total protein concentration compared with the parasitized mice administered 0.2 mL of distilled water. The infected mice administered 0.2 mL of distilled water showed significant ($p<0.05$) increase in total protein, urea and creatinine level compared with the normal control mice. Histological examination of the liver revealed that, combination of low dose of extract with chloroquine showed a mild hepatitis with mild degeneration of the liver cells. However, hemozoin pigment deposition tends to be more in the low dose compared with the high dose of extract treated mice. The examined sections of the kidney showed the normal renal histo-architecture. **Conclusion:** This study showed the therapeutic effects of synergistic treatment of *T. diversifolia* and chloroquine in alleviating hepatotoxicity resulting from chloroquine resistant *P. yeolii.*

Key words: Infected mice, liver, hemozoin, anti malarial drug, renal histo-architecture, *Plasmodium yeolii*, histology

Citation: Omoboyowa Damilola Alex, Nwodo Fred and Joshua Parker Elijah, 2018. Effects of combined treatment of *Tithonia diversifolia* And chloroquine on selected organs of chloroquine resistant *Plasmodium yeolii* infected mice. Asian J. Biol. Sci., 11: 58-72.

Corresponding Author: Omoboyowa Damilola Alex, Department of Chemical Science, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria Tel: +2347032665874

Copyright: © 2018 Omoboyowa Damilola Alex *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria has remained one of the most devastating diseases in tropical and sub-tropical regions of the world despite the global fight against the disease[1]. Malaria in pregnancy increases the infant risk of low birth weight, abortions and still birth in addition to the maternal burdens of anaemia and mortality[2].

Malaria has been reported to be one of the factors responsible for acute renal failure among children in malaria endermic areas[3]. Renal tubular changes have been reported to be associated to *P. falciparum* infection more than glomerular changes and complication may range from minor blood urea, hyper-nareamia, hyperkalemia, low urine specific gravity, metabolic acidosis, tubular necrosis and acute renal failure accompanied by frequent oliguria and hyper catabolism[4].

Liver involvement in severe malarial infection is commonly a significant cause of morbidity and mortality among humans[5]. This could result from invasion of the liver cells by sporozoites during malarial parasite life cycle. The changes caused in the hepatic cell by sporozoites can lead to the leakage of parenchymal and membranous enzymes of the liver into the circulatory system; the severity of malaria infection has been linked with the increase in the level of liver enzymes in the blood[4,6].

Chemotherapy remain the kernel of malaria control and the previously efficacious chloroquine has substantially failed as a prophylactic and therapeutic anti-malarial which have paved way for the use of other anti-malarial drugs including sulphadoxine-pyrimethamine, mefloquine and halofantrine[7]. These drugs are not only expensive than chloroquine but also parade heterogeneous levels of toxicity and may invoke poor compliance in patients[7]. Investigation of anti-malarial potentials of orthodox drugs in combination with plants used in traditional therapy of malaria has been one of the means of searching for new anti-malarial drugs that will liberate human race from the burden of resistant malaria.

Tithonia diversifolia (Hemsl) A gray, is commonly referred to as Mexican sun flower or tree marigold, it is a bushy perennial weed commonly find on the fields and road sides of Nigeria[8]. *Tithonia diversifolia* is administered in several forms; oral decoction of the leaves for the treatment of hepatitis, diabetes, malaria, pain, chemoprevention and anti-helicobacter pylori[9]. Exploitation of the anti-malarial actions of plant extracts in combination with monotherapies like chloroquine with a view to enhance drug efficacy in resistance malaria infection is currently generating scientific interest without consideration of the cytotoxic effects. This study therefore; aimed to assess the effects of ethanol leaf extract and bio-active fraction of *T. diversifolia* administered with chloroquine on pathology of vital organs (liver and kidney) of albino mice infected with chloroquine resistant *Plasmodium yeolii* parasites.

MATERIALS AND METHODS

Animals: Adult male albino mice of 3-4 months old weighing 20-34 g were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 h light and dark cycle maintained on a regular feed and water *ad libitum*. The animal experiments were conducted as per the University of Nigeria Scientific Research Committee in Nigeria. The experimental protocol was approved by the Faculty of Science Animal Ethics committee of University of Nigeria with reference number pg/12/64667.

Preparation and extraction of plant material: The plant material for this study (*Tithonia diversifolia* leaves) was collected randomly from trees within the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State in July, 2015 and authenticated by the plant taxonomist, Mr. Esimekhuai Donatus of the Department of Botany, University of Ibadan, Oyo State, Nigeria. The extraction and fractionation procedure was carried out as reported by Omoboyowa *et al.*[10].

The Fraction Chloroform 70 and methanol 30% (C70: M30) was observed to show the highest significant percentage parasitaemia chemo-suppression in the activity guided study reported in Omoboyowa *et al.*[10]. The bioactive fraction and the crude ethanol extract were combined with chloroquine for this study.

Chemicals/Reagents: All the chemicals used in this study were of analytical grade and supplied by sigma incorporated, USA.

Experimental design: Ninety-six mice were divided into 12 groups of 8 mice each (n = 8) and treated according to the experimental design below:

- **Group I:** Uninfected control given normal saline only
- **Group II:** Infected control given normal saline only
- **Group III:** Infected mice treated with 10 mg kg^{-1} b.wt., of chloroquine
- **Group IV:** Infected mice treated with 5 mg kg^{-1} b.wt., of artemether lumefantrine

- **Group V:** Infected mice treated with 100 mg kg^{-1} b.wt., of crude ethanol extract
- **Group VI:** Infected mice treated with 200 100 mg kg^{-1} b.wt., of crude ethanol extract
- **Group VII:** Infected mice treated with 100 mg kg^{-1} b.wt., of crude ethanol extract in combination with 10 mg kg^{-1} b.wt., of chloroquine
- **Group VIII:** Infected mice treated with 200 mg kg^{-1} b.wt., of crude ethanol extract in combination with 10 mg kg^{-1} b.wt., of chloroquine
- **Group IX:** Infected mice treated with 100 mg kg^{-1} b.wt., of active (C70:M30) fraction
- **Group X:** Infected mice treated with 200 mg kg^{-1} b.wt., of active (C70:M30) fraction
- **Group XI:** Infected mice treated with 100 mg kg^{-1} b.wt., of active (C70:M30) fraction in combination with 10 mg kg^{-1} b.wt., of chloroquine:
- **Group XII:** Infected mice treated with 200 mg kg^{-1} b.wt., of active (C70:M30) fraction in combination with 10 mg kg^{-1} b.wt., of chloroquine

Procurement of parasitaemia: Chloroquine resistant *Plasmodium yeolii* used for this study was obtained from the MR4/ American type culture collection (ATCC), Manassas, USA maintained by serial passage in mice. Preparation of inoculum was from donor mouse with *Plasmodium yeolii* parasitaemia established by microscopic examination of thin blood film under oil immersion at X100 magnification and measured as percentage erythrocytes[7]. Each mouse was infected with standard inoculums of approximately 10^7 parasitized erythrocytes suspension in normal saline (0.2 mL) from donor mouse.

Drugs: The monotherapeutic drug administered for the control and treatment groups were chloroquine (CLARION, Nigeria) and the ACT drug administered was Lokmal® a brand of artemether lumefantrine (EMZOR, Nigeria). These were dissolved in normal saline to obtain fixed dose of 10 and 5 mg kg^{-1}, respectively and used in the assay.

Biochemical assay: The activity of aminotransferases (AST and ALT) was determined by the method of Reitman and Frankel[11]. Plasma alkaline phosphatase activity was assayed spectrophotometrically according to the method described by King and King[12]. The concentration of bilirubin was determined by the method of Malloy and Evelyn[13]. The plasma urea concentration was carried out according to the method outlined by Watt and Chrisp[14], the creatinine concentration was determined according to the method described by Peters[15] and the total protein concentration was evaluated using the method outline by Lowry *et al.*[16] as described in Randox assay kits.

Liver histology: The animals were sacrificed and the abdominal cavity of each rat opened, the liver and kidney were taken out. The organs were fixed in 10% formalin. After complete fixation the blocks was embedded in paraffin and sections cut at 5 µm (micron) which were then stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examinations of the sections were then carried out under a light microscope[17].

Statistical analysis: The data obtained were analyzed using one way analysis of variance. The results were expressed as Mean±SD. *Post hoc* multiple test comparison was used to compare the group means after each treatment with control measurements. Significant differences were obtained at $p \leq 0.05$.

RESULTS

The results of the liver function parameters (AST, ALT, ALP and Total bilirubin) of *P. yeolii*^{R} infected mice treated with ethanol extract and C70:M30 fraction of *T. diversifolia* singly and in combination with chloroquine is shown in Table 1. The *P. yeolii*^{R}-infected mice without treatment showed significant ($p<0.05$) decrease in ALP activities and total bilirubin level and significant ($p<0.05$) increase in ALT activity compared to the normal control mice. The infected mice treated with 10 mg kg^{-1} b.wt., of chloroquine showed non-significant ($p>0.05$) reduction in ALT, ALP activities and significant ($p<0.05$) reduction in ALT compared to the parasitized mice administered 200 mg kg^{-1} b.wt., of C70 M30 fraction in combination with chloroquine.

The results of the selected kidney function parameters (urea, creatinine and total protein) of *P. yeolii*^{R} infected mice treated with ethanol leaf extract and C70:M30 fraction of *T. diversifolia* singly and in combination with 10 mg kg^{-1} b.wt., of chloroquine are shown in Table 2. The *P. yeolii*^{R} infected mice without treatment showed significantly ($p<0.05$) higher urea, creatinine and lower total protein concentration compared to the normal control mice. The parasitized mice treated with 10 mg kg^{-1} b.wt., of chloroquine and

Table 1: Effect of Co-administration of Ethanol leaf Extract of *T. diversifolia* and chloroquine on liver function parameters of mice infected with *Plasmodium yeolii*^{R} (pr1)

Treatments	ALT (iu L^{-1})	AST (iu L^{-1})	ALP (iu L^{-1})	T. Bil(mg dL^{-1})
Normal Control	11.3±1.8	94.3±11.3	55.3±4.7	1.73±0.34^{b}
Plasmodium yeolii^{R} infected without treatment	21.7±6.2^{a}	78.5±4.1	22.4±0.09^{a}	0.60±0.29^{ac}
Plasmodium yeolii^{R} infected + 10 mg kg^{-1} b.wt., of chloroquine	17.2±2.3	109.8±4.6	22.0±0.3^{a}	1.37±0.43
Plasmodium yeolii^{R} infected + 5 mg kg^{-1} b.wt., of artemether	25.2±5.8^{a}	100.3±2.3	17.9±3.4^{a}	0.97±0.49^{a}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract	19.8±2.7	123.4±24.0^{b}	21.3±0.8^{a}	0.23±0.07^{ac}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract	20.7±1.0	78.6±1.0	22.3±0.6^{a}	0.60±0.25^{ac}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract and CQ	27.8±2.1^{ac}	126.2±17.2^{b}	21.2±1.9^{a}	0.17±0.07^{ac}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract and CQ	22.5±5.2^{a}	89.9±13.8	18.1±0.4^{a}	0.23±0.03^{ac}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction	13.5±0.03	86.2±0.3	21.3±1.7^{a}	0.53±0.07^{ac}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction	18.7±1.8	94.7±0.4	23.8±0.3^{a}	0.57±0.26^{ac}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	23.3±2.7^{a}	93.8±2.8	19.5±2.5^{a}	0.43±0.18^{ac}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	14.0±1.5	91.3±2.1	21.4±0.06^{a}	0.27±0.03^{ac}

CQ: Chloroquine, C70 M30: Chloroform 70% Methanol 30%, $^{a}p<0.05$ significant compared to the normal control mice, $^{b}p<0.05$, significant compared to the infected mice administered 0.2 mL of distilled water, $^{c}p<0.05$, significant compared to the infected mice treated with 10 mg kg^{-1} b.wt., of chloroquine

Table 2: Effect of co-administration of ethanol leaf extract of *T. diversifolia* and chloroquine on kidney function parameters of mice infected with *Plasmodium yeolii*^{R} (pr1)

Treatments	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})	*T. protein* (g dL^{-1})
Normal Control	31.70±2.40^{b}	1.13±0.12^{b}	5.72±0.44^{bc}
Plasmodium yeolii^{R} infected without treatment	38.37±1.33^{a}	1.43±0.02^{a}	3.97±0.09^{a}
Plasmodium yeolii^{R} infected + 10 mg kg^{-1} b.wt., of CQ	35.00±1.85^{a}	1.29±0.05^{a}	3.70±0.12^{a}
Plasmodium yeolii^{R} infected + 5 mg kg^{-1} b.wt., of artemether	37.83±1.53^{a}	1.37±0.05^{a}	3.57±0.26^{a}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract	29.93±2.66^{bc}	1.18±0.05^{b}	3.27±0.20^{ab}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract	46.50±0.35^{abc}	1.66±0.02^{abc}	3.60±0.38^{a}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract and CQ	43.63±0.18^{abc}	1.45±0.07^{a}	3.40±0.12^{a}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract and CQ	39.20±0.70^{a}	1.40±0.02^{a}	3.73±0.32^{a}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction	33.57±0.09^{b}	1.25±0.01^{b}	3.70±0.25^{a}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction	34.77±2.74	1.25±0.12^{b}	3.57±0.09^{a}
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	40.53±0.55^{ac}	1.45±0.03^{a}	4.37±0.15^{a}
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	34.80±1.01	1.21±0.03^{b}	4.27±0.09^{a}

CQ: Chloroquine, C70 M30: Chloroform 70% Methanol 30%, $^{a}p<0.05$ significant compared to the normal control mice. $^{b}p<0.05$ significant compared to the infected mice administered 0.2 mL of distilled water, $^{c}p<0.05$, significant compared to the infected mice treated with 10 mg kg^{-1} b.wt., of chloroquine

Table 3: Histopathological effects on the liver

	Pathological effect			
Treatments	Hepatic necrosis	Kupffer cell hyperplasia	Periportal mononuclear cell infiltration	Haemozoin decomposition
Normal control	-	-	-	-
Plasmodium yeolii^{R} infected without Treatment	+++	+++	-	+++
Plasmodium yeolii^{R} infected + 10 mg kg^{-1} b.wt., of CQ	++	++	+	+
Plasmodium yeolii^{R} infected + 5 mg kg^{-1} b.wt., of Artemether	+++	++	++	++
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract	+	+	+	++
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract	+	+	+	++
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of crude extract and CQ	+	+	+	+
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of crude extract	+	+	+++	++
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction	+	+	+	++
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction	+	+	+	++
Plasmodium yeolii^{R} infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	+	+	+	++
Plasmodium yeolii^{R} infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	+	+	+	+

+: Mild, ++: Moderate, +++: Severe, -: Not detected, CQ: Chloroquine, C70 M30: Chloroform 70% Methanol 30%

5 mg kg^{-1} b.wt., of artemether showed non-significant ($p>0.05$) reduction in urea, creatinine and total protein concentration compared to the infected mice without treatment. The mice infected with *P. yeolii*^{R}, treated with 100 mg kg^{-1} b.wt., of C70:M30 fraction in combination with 10 mg kg^{-1} b.wt., of chloroquine showed non-significant ($p>0.05$) higher urea, creatinine and total protein concentration compared to the parasitized mice treated with 10 mg kg^{-1} b.wt., of chloroquine only.

As Table 3 showed the pathological effects of the synergistic treatment on the liver of malaria infected mice. Hepatic necrosis, kupffer cell hyperplasia and haemozoin

Table 4: Histopathological effects on the kidney

Treatments	Pathological effect	
	Tubular nephrosis	Perivascular interstitial mononuclear cell infiltration
Normal control	-	-
Plasmodium yeolii^{R} infected without treatment	+++	+++
Plasmodium yeolii infected + 10 mg kg^{-1} b.wt., of CQ	++	-
Plasmodium yeolii infected + 5 mg kg^{-1} b.wt., of artemether	++	-
Plasmodium yeolii infected + 100 mg kg^{-1} b.wt., of crude extract	+	+
Plasmodium yeolii infected + 200 mg kg^{-1} b.wt., of crude extract	+	+
Plasmodium yeolii infected + 100 mg kg^{-1} b.wt., of crude extract and CQ	++	-
Plasmodium yeolii infected + 200 mg kg^{-1} b.wt., of crude extract and CQ	++	+
Plasmodium yeolii infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction	+	++
Plasmodium yeolii infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction	+	+
Plasmodium yeolii infected + 100 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	++	+
Plasmodium yeolii infected + 200 mg kg^{-1} b.wt., of C70 M30 fraction and CQ	++	+

+: Mild, ++: Moderate, +++: Severe, -: Not detected, CQ: Chloroquine, C70 M30: Chloroform 70% Methanol 30%

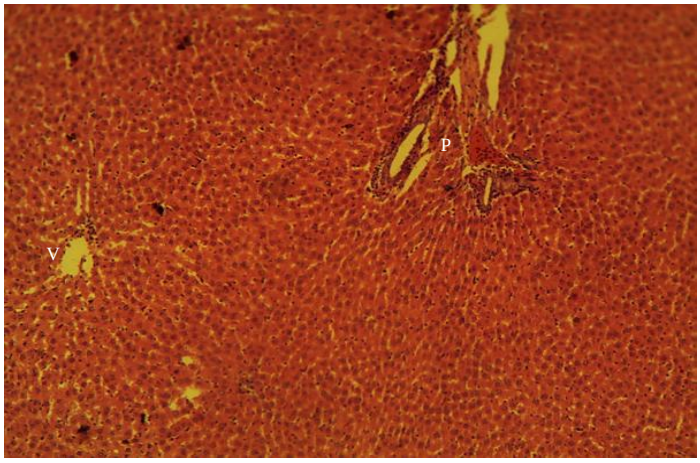

Fig. 1: Section of the liver histo-architecture of the mice in the normal control group that were given distilled water without *P. yeolii* infection. Liver from this group showed normal hepatic histo-architecture, it showed normal hepatocytes arranged in radiating chords around the central veins, diverging towards the portal areas with normal bile ducts, hepatic arteries and hepatic veins. P: Portal area
V: Central vein. H&E X100

decomposition were observed to be severe (+++) in the parasitized mice without treatment compared to mild (+) effects observed in the *P. yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of C70: M30 fraction and therapeutic dose of chloroquine.

The histopathological effects of synergistic treatment of *T. diversifolia* and chloroquine on *P. yeolii*^{R} infected mice. Tubular nephrosis and perivascular interstitial mononuclear cell infiltration were observed to be severe in *P. yeolii*^{R} infected mice without treatment which were moderate and mild respectively in infected mice treated with 200 mg kg^{-1} b.wt., of C70:M30 fraction and therapeutic dose of chloroquine in Table 4.

Liver histology: Histopathological studies revealed some damages caused by the chloroquine resistant malaria parasite and extent of reduction of these damages by the administered drugs, crude extract and bioactive fraction singly and in combination with chloroquine, for example, death of cells of the liver (hepatic necrosis) and deposition of haemozoin in the hepatic cells as shown in Fig. 1-12.

Kidney histology: Histopathological studies revealed minor damages caused by the chloroquine resistant malaria parasite and effect of administered crude extract and bio-active fraction of *Tithonia diversifolia* singly and in combination with chloroquine, on the renal system of *P. yeolii*^{R} infected mice as shown in Fig. 13-23.

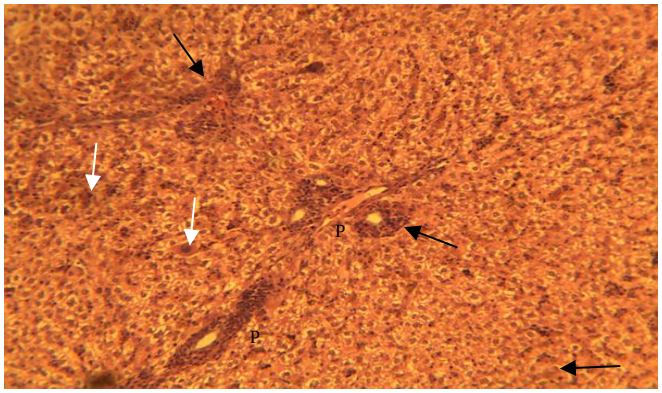

Fig. 2: Section of the liver from mice infected with *Plasmodium yeolii*^{R} without treatment showed severe diffuse hepatocellular vacuolar degeneration with widespread haemozion pigment deposition (white arrow) as well as mild multifocal and periportal infiltration of inflammatory leucocytes (black arrow)
P: Portal area. H&E X100

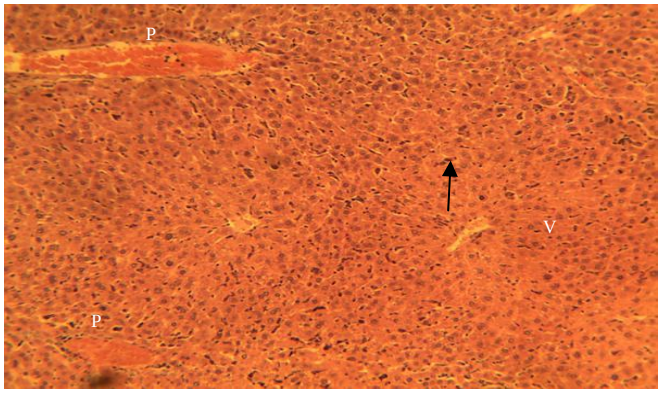

Fig. 3: Section of the liver from *Plasmodium yeolii*^{R} infected mice administered 10 mg kg^{-1} b.wt., of chloroquine showed mild Hemozoin pigment deposition (black arrow). The pigments appears to be most present in the Kupffer cells
P: Portal area, V: Central vein. H&E X100

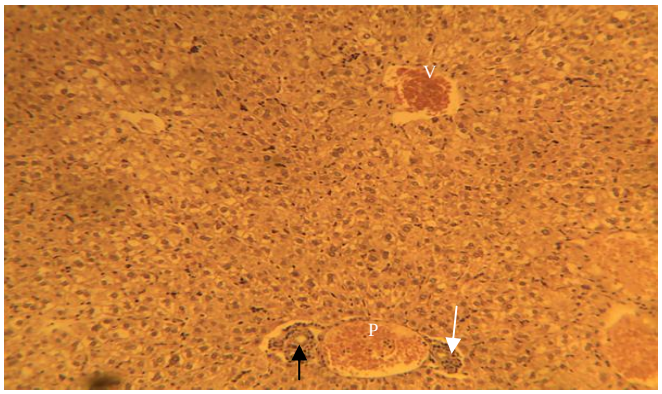

Fig. 4: Section of the liver from *Plasmodium yeolii*^{R} infected mice administered 5 mg kg^{-1} b.wt., of artemether showed severe widespread vacuolar hepatocellular degeneration with slight periportal deposition of inflammatory leucocytes (black arrow). Hemozoin pigments appear to be widespread, in Kupffer cells (white arrow)
V: Central vein, P: Portal area. H&E X100

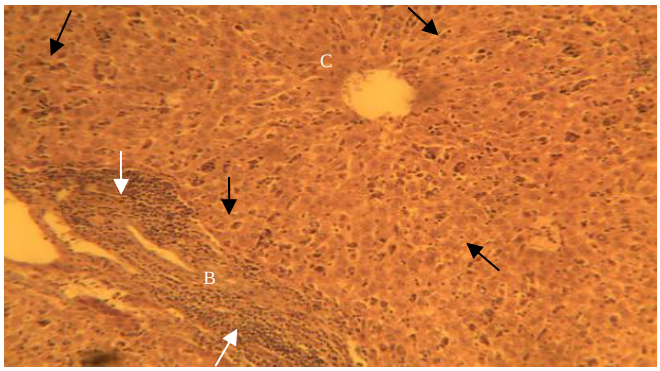

Fig. 5: Photomicrograph of the liver of *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* showing hemozoin pigment deposition in cytoplasm of hepatocytes and Kupffer cells (black arrow) and mild deposition of mononuclear inflammatory leucocytes around the portal triad (white arrow)
B: Bile duct, C: Central vein. H&E X100

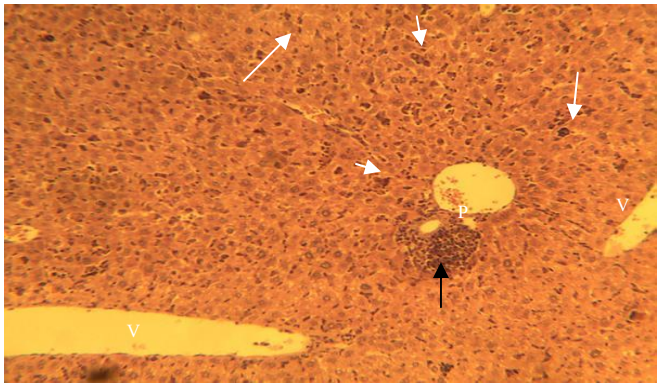

Fig. 6: Examined section of the liver from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* showed mild hepatocellular degeneration and necrosis, widespread hemozoin (malaria pigment) deposition in the cytoplasm of Kupffer cells (white arrow), multifocal aggregates of mononuclear inflammatory cells as well as moderate periportal infiltration of mononuclear infiltrates (black arrow)
P: Portal area, V: Central vein. H&E X100

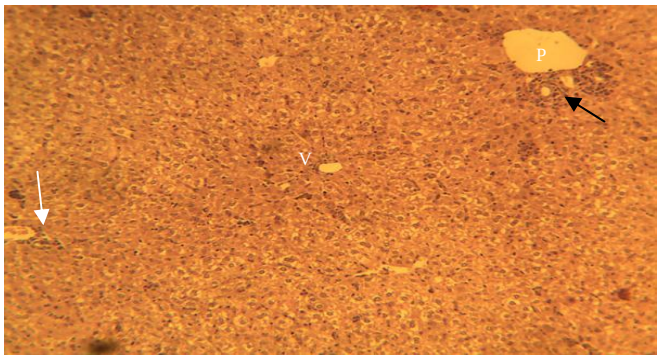

Fig. 7: Section of the liver from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine showed a moderate widespread vacuolar degeneration, mild haemozoin pigment deposition (white arrow) and mild periportal infiltration of inflammatory leucocytes (black arrow)
P: Portal area, V: Central vein. H&E X100

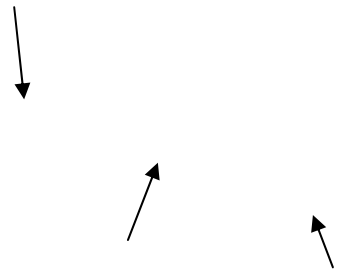

Fig. 8: Examined section of the liver from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine showed mild hepatocellular degeneration and necrosis, widespread haemozoin (malaria pigment) deposition in the cytoplasm of Kupffer cells (white arrow), multifocal aggregates of mononuclear inflammatory cells as well as severe periportal infiltration of mononuclear infiltrates (black arrow)
P: Portal area . H&E X100

Fig. 9: Examined section of the liver from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of C70: M30 fraction of *Tithonia diversifolia* showed mild hepatocellular degeneration and necrosis, widespread haemozoin (malaria pigment) deposition in the cytoplasm of Kupffer cells and mild periportal infiltration of mononuclear infiltrates (black arrow)
P: Portal area, V: Central vein. H&E X100

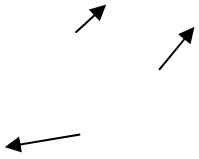

Fig. 10: Examined section of the liver from *Plasmodium yeolii* ^{R} infected mice administered 200 mg kg^{-1} b.wt., of C70: M30 fraction of *Tithonia diversifolia* showed mild hepatocellular degeneration and necrosis, widespread hemozoin (malaria pigment) deposition in the cytoplasm of Kupffer cells (white arrow), multifocal aggregates of mononuclear inflammatory cells as well as periportal infiltration of mononuclear infiltrates (black arrow)
P: Portal area, V: Central vein. H&E X100

Fig. 11: Examined sections of the liver from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of C70: M30 fraction of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine showed mild hepatocellular degeneration and necrosis, widespread hemozoin (malaria pigment) deposition in the cytoplasm of Kupffer cells (white arrow), multifocal aggregates of mononuclear inflammatory cells as well as moderate periportal infiltration of mononuclear infiltrates (black arrow)
P: Portal area. H&E X100

Fig. 12: Sections from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of C70: M30 fraction of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine showed widespread, mild to moderate hepatocellular vacuolar degeneration (black arrow). Mild periportal hepatitis with mild deposition of hemozoin pigments in Kupffer cells (white arrow)
C: Central vein, P: Portal area. H&E X100

Fig. 13: Section of the kidney from mice infected with *Plasmodium yeolii*^{R} without treatment
G: Glomerulus. H&E X400

Fig. 14: Section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 10 mg kg^{-1} b.wt., of chloroquine
G: Glomerulus, H&E X400

Fig. 15: Section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 5 mg kg^{-1} b.wt., of artemether
G: Glomerulus, H&E X400

Fig. 16: Photomicrograph of the kidney of *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia*
G: Glomerulus. H&E X400

Fig. 17: Examined section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia*
G: Glomerulus

Fig. 18: Section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine
G: Glomerulus, H&E X400

Fig. 19: Examined section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of ethanol leaf extract of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine
G: Glomerulus, H&E X400

Fig. 20: Examined section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of chloroform 70: methanol 30 fraction of *Tithonia diversifolia*
G: Glomerulus, H&E X400

Fig. 21: Examined section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of chloroform 70: methanol 30 fraction of *Tithonia diversifolia*
G: Glomerulus, H&E X400

Fig. 22: Examined section of the kidney from *Plasmodium yeolii*^{R} infected mice administered 100 mg kg^{-1} b.wt., of chloroform 70: Methanol 30 fraction of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine
G: Glomerulus, H&E X400

Fig. 23: Section of kidney from *Plasmodium yeolii*^{R} infected mice administered 200 mg kg^{-1} b.wt., of chloroform 70: Methanol 30 fraction of *Tithonia diversifolia* and 10 mg kg^{-1} b.wt., of chloroquine
G: Glomerulus, H&E X400

DISCUSSION

The intake of medicinal plants as alternative medicine has been supported because of their relatively cheap availability. This is coupled with the belief that they could significantly improve human health in terms of prevention and control of various ailment and infections. They also exhibit far less frequent side effects when compared with modern medicine[18].

The result of the liver function parameters of *P. yeolii*^{R} infected mice treated with crude extract and bioactive fraction of *T. diversifolia* singly and in combination with therapeutic dose of chloroquine shown in Table 1 revealed that ALT activity was significantly ($p<0.05$) higher in the infected mice administered 0.2 mL of normal saline compared with normal control mice while the infected mice treated with anti-malarial drugs (chloroquine and artemether) and varying doses of the crude extract and bioactive fraction (C70:M30) of the leaf singly and in combination with therapeutic dose of chloroquine showed reduction in liver enzyme activities compared with the infected mice administered 0.2 mL of normal saline, suggesting that the administration of the crude extract and bioactive fraction singly and in combination with chloroquine interact to ameliorate the hepatic damage caused by activities of the parasite. The result of the liver enzymes is in agreement with report of Martey *et al.*[19]. The result of the total bilirubin revealed significantly ($p<0.05$) higher concentration of the parameter in the mice infected with *P. yeolii*^{R} administered 0.2 mL of normal saline and 10 mg kg^{-1} b.wt., of chloroquine compared with the infected mice treated with varying doses of the crude extract and bioactive fraction singly and in combination with chloroquine. This suggests ameliorative impact of the treatment on hepatobiliary function of the liver. The rapid haemolysis of the red blood cell results in increased plasma bilirubin levels when the hepatic cells cannot excrete the bilirubin as rapidly as it is been formed, there is an increase in the plasma level of bilirubin[20,21]. Obstruction of the bile ducts as it occurs on gallstone or liver damage such as hepatitis may not affect the rate of bilirubin formation but the bilirubin formed cannot pass from the blood into the intestine[21,22].

The mean concentration of urea, creatinine and total protein of chloroquine resistant *P. yeolii* infected mice treated with anti-malarial drugs; crude extract and bioactive fraction of *T. diversifolia* singly and in combination with chloroquine are shown in Table 2. The results revealed that, the infected mice administered 5 mg kg^{-1} b.wt., of artemether, varying doses of the crude extract and bioactive fraction only significantly ($p<0.05$) lower the mean total protein concentration compared with the infected mice administered 0.2 mL of normal saline. Co-administration of bioactive fraction and chloroquine to parasitized mice showed significantly ($p<0.05$) higher total protein concentration compared with the parasitized untreated control mice. The infected mice administered 0.2 mL of normal saline showed significant ($p<0.05$) increase in total protein, urea and creatinine level compared with the normal control mice. The higher value observed in these parameters may be attributed to impairment in renal function associated with *P. yeolii*^{R} infection. These results are consistent with the report of

Ogbadoyi and Gabi[23], who observed increase in these parameters in *P. falciparum* infected patients. High level of proteinuria is characteristics features of renal dysfunction[24]. In healthy kidneys, protein are normally completely filtered from the blood stream and then re-absorbed allowing no protein or small amounts of protein into the urine[25]. Therefore, that the amounts of protein in the plasma of malaria infected mice were significantly ($p<0.05$) higher than the normal control mice is indicative of some level of renal dysfunction which may be attributed to the effect of the malaria infection. The result obtained is consistent with the report of Akanbi[4]. In acute renal failure, plasma urea increases more rapidly than plasma creatinine concentration. Despite all these consideration, plasma urea levels do not reflect the performance of the kidneys as compared to creatinine. This is because; urea production is also affected by dehydration, food intake and tissue catabolism[25]. Thus, an increase in plasma creatinine concentration in the infected mice suggests that the normal functioning of the kidney have been compromised.

The predominant histological changes observed in malarial hepatopathy involve vacuolar (fatty) degeneration, inflammatory cellular infiltration and hemozoin (malarial pigment) deposition in cytoplasm of tissue macrophages (Kupffer cells) and connective tissues. These lesions are used to qualify the severity of the malarial hepatopathy. The *P. yeolii* [R] infected mice treated with 10 mg kg^{-1} b.wt., of chloroquine and 5 mg kg^{-1} b.wt., of artemether showed better liver histology. However, that of chloroquine treated animals appears more normal than the artemether treated mice. It could imply that chloroquine has less hepatotoxic effect compared with Artemether. Treatment of parasitized mice with 100 mg kg^{-1} b.wt., of crude extract shows relatively normal hepatocytes but evidence of severe hepatitis was observed. The liver histology of infected mice without treatment showed hepatocellular damage with severe hepatic necrosis, kupffer cell hyperplasia and haemozoin decomposition. This result is consistent with the hepatotoxicity of malaria parasite reported by Otegbade *et al.*[26]. Hemozoin deposits appear to be higher with the level observed in parasitized mice treated with 200 mg kg^{-1} b.wt., of the crude extract which also showed mild hepatitis. Co-administration of 100 mg kg^{-1} b.wt., of the crude extract and 10 mg kg^{-1} b.wt., of chloroquine showed a mild hepatitis with mild degeneration of the liver cells when combined administration of 10 mg kg^{-1} b.wt., of chloroquine with the 200 mg kg^{-1} b.wt., of crude extract to parasitized mice revealed severe lesions. Its possible implication is that, a combination of the drug and crude extract may be detrimental to the liver. Treatment of parasitized mice with 100 mg kg^{-1} b.wt., of the bioactive fraction showed a better liver histology compared with the result observed when treated with 200 mg kg^{-1} b.wt., of the fraction with respect to inflammation. Treatment of parasitized mice with 100 and 200 mg kg^{-1} b.wt., of the bio-active fraction combined with 10 mg kg^{-1} b.wt., of chloroquine showed mild hepatocellular damage and inflammation. However, haemozoin pigment deposition tends to be more in the co-administration of 100 mg kg^{-1} b.wt., of the fraction with 10 mg kg^{-1} b.wt., of chloroquine compared with administration of 200 mg kg^{-1} b.wt., in combination with 10 mg kg^{-1} b.wt., of chloroquine. The sections of the kidney collected from the animals in all the groups did not show significant change in the renal histo-architecture as shown in Fig. 13-23. This implies that neither the infection, nor the treatments had an effect on the histo-morphology of this organ. Nonetheless, this does not negate the possibility of biochemical lesions in the organ as these may occur without obvious morphological changes depending on the level of intoxication.

CONCLUSION

The present study has demonstrated the protective effect of combine treatment of chloroquine and active fraction of *T. diversifolia* leaf extract to reverse organs' damage initiated by chloroquine resistant *P. yeolii*. Further studies might define the bioactive compounds responsible for this observed therapeutic effect of *T. diversifolia*.

SIGNIFICANCE STATEMENT

This study discover the interaction between the crude extract and bioactive fraction (C70 M30) of *Tithonia diversifolia* leaf administered with therapeutic dose of chloroquine in ameliorating the hepatic and nephrotic damage resulting from activities of chloroquine resistant *Plasmodium yeolii* parasite during malarial infection that can be beneficial for the use of this plant to enhance the efficacy of chloroquine against resistant malaria. This study will help researchers to uncover the critical areas of herbal therapy that many researchers were not able to explore. Thus, a new theory on safety of orthodox drugs-plants combination therapy may be arrived at.

ACKNOWLEDGMENT

The authors wish to acknowledge MR4/American Type Culture Collection for providing the malaria parasite *Plasmodium yeolii* contributed by Wallace Peters and Brian L. Robinson used in the study.

REFERENCES

1. Akpan, E.J., J.E. Okokon and I.C. Etuk, 2012. Antiplasmodial and antipyretic studies on root extracts of *Anthocleista djalonensis* against *Plasmodium berghei*. Asian Pacific J. Trop. Dis., 2: 36-42.
2. Stainers, H.M. and S. Krishna, 2012. Tratment and Prevention of Malaria. Springer, Basel, pp: 1-2.
3. Mockenhaupt, F.P., S. Ehrhardt, J. Burkhardt, S.Y. Bosomtwe and S. Laryea *et al*., 2004. Manifestation and outcome of severe malaria in children in Northern Ghana. Am. J. Trop. Med. Hyg., 71: 167-172.
4. Akanbi, O.M., 2015. The influence of malaria infection on kidney and liver function in children in Akoko area of Ondo state, Nigeria. J. Parasitol. Vector Biol., 7: 163-168.
5. Viriyavejakul, P., V. Khachonsaksumet and C. Punsawad, 2014. Liver changes in severe *Plasmodium falciparum* malaria: Histopathology, apoptosis and nuclear factor kappa B expression. Malaria J., Vol. 13. 10.1186/1475-2875-13-106
6. Onyesom, I. and N. Onyemakonor, 2011. Levels of parasitaemia and changes in some liver enzymes among malarial infected patients in Edo-Delta Region of Nigeria. Curr. Res. J. Biol. Sci., 3: 78-81.
7. Iwalokun, B.A., 2008. Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. Afr. Health Sci., 8: 25-35.
8. Owoyele, V.B., C.O. Wuraola, A.O. Soladoye and S.B. Olaleye, 2004. Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. J. Ethnopharmacol., 90: 317-321.
9. Sanchez-Mendoza, M.E., A. Reyes-Ramírez, L. Cruz-Antonio, L. Martínez-Jiménez, J. Rodríguez-Silverio and J. Arrieta, 2011. Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: Role of nitric oxide, prostaglandins and sulfhydryls. Molecules, 16: 665-674.
10. Omoboyowa, D.A., O.F.C. Nwodo, P.E. Joshua and O.T. Soniran, 2017. Anti-malaria activity of bioassay-guided fractionation of the crude ethanol extract of *Tithonia diversifolia* leaves. Niger. J. Parasitol., 38: 198-204.
11. Reitman, S. and S. Frankel, 1957. Method of alanine and aspartate aminotransferase determination. Am. J. Clin. Pathol., 28: 56-68.
12. King, P.R.N. and F.J. King, 1954. Alkaline phosphatase determination. Clin. Pathol., 7: 322-329.
13. Malloy, H.T. and K.A. Evelyn, 1937. Recommendation on a uniform bilirubin standard: In standard methods of clinical chemistry. J. Biol. Chem., 119: 481-490.
14. Watt, G.W. and J.D. Chrisp, 1954. Spectrophotometric method for determination of urea. Anal. Chem., 26: 452-453.
15. Peters, W., 1998. Drug resistance in malaria parasites of animals and man. Adv. Parasitol., 41: 1-62.
16. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
17. Ogunneye, A.L., D.A. Omoboyowa, O.O. Banjoko and H.O. Adetomiwa, 2014. Effect of sub-chronic lamivudine-quinine co-administration on the liver of normal adult wistar rats. Int. J. Pharm. Sci. Rev. Res., 24: 345-350.
18. Okoko, T. and I.F. Oruambo, 2008. The effect of *Hibiscus sabdariffa* calyx extract on cisplatin-induced tissue damage in rats. Biokemistri, 20: 47-52.
19. Martey, O.N.K., O. Shittah-Bay, J. Owusu and L.K.N. Okine, 2013. The antiplasmodial activity of an herbal antimalarial, AM 207 in *Plasmodium berghei*-infected Balb/c mice: Absence of organ specific toxicity. J. Med. Sci., 13: 537-545.
20. Burtis, C.A. and E.R. Ashwood, 2006. Teitz Fundamentals of Clinical Chemistry. W.B. Saunders, Philadelphia, pp: 231-236.
21. Guyton, A.C. and J.E. Hall, 2006. Resistance of Body to Infection II: Immunity and Allergy. Textbook of Medical Physiology. 11th Edn., Elsevier, UK., pp: 439-456.
22. Ganong, W.F., 2003. Review of Medical Physiological. 20th Edn., Lange Medical Books/Me Graw-Hill Medical Publishing Division USA., pp: 414-417.
23. Ogbadoyi, E.O. and B. Gabi, 2007. Assessment of renal function in malaria patients in Minna, North Central Nigeria. Afr. J. Infect. Dis., 1: 57-64.
24. Rui-Mei, L., A.U. Kara and R. Sinniah, 1998. *In situ* analysis of adhesion molecule expression in kidneys infected with murine malaria. J. Pathol., 185: 219-225.
25. Raphael, E.C. and A.U. Benjamin, 2010. Assessment of renal function of *Plasmodium falciparum* infected children in Owerri, Eastern Nigeria. Res. J. Med. Sci., 4: 208-212.
26. Otegbade, O.O., J.A. Ojo, D.I. Adefokun, O.O. Abiodun, B.N. Thomas and O. Ojurongbe, 2017. Ethanol extract of *Blighia sapida* stem bark show remarkable prophylactic activity in experimental *Plasmodium berghei*-infected mice. Drug Target Insights, Vol. 11. 10.1177/1177392817728725