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Assessment of lipid profile and kidney function biomarkers in *Plasmodium berghei* infected mice treated with artemisinin-based combination therapy

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Abstract
Malaria pathogenesis is associated with changes in lipid profile and kidney function. The present study was aimed to observe the changes in lipid profile and kidney function biomarkers in *Plasmodium berghei* infected mice treated with artemisinin-based combination drugs i.e., Artesunate + Amodiaquine (ASAQ), Artesunate + Sulfadoxine Pyrimethamine (ASSP) and Artesunate + Lumefantrine (AL). The Total cholesterol (TC), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and triglycerides (TG) were increased whereas high-density lipoproteins (HDL) were decreased in *P. berghei* infected mice. Serum creatinine, urea and potassium ion levels were increased whereas sodium ions and chloride ions were decreased in *P. berghei* infected mice. When these infected mice were treated with ASAQ, ASSP and AL; all the above parameters were restored significantly ($P < 0.05$) to normal values. Among these three drug combinations; ASAQ was the most effective than ASSP and AL in treating *P. berghei* infection in experimental mice.

Keywords: Plasmodium berghei, Lipid Profile, Kidney Function Biomarkers, Artemisinin-based Combination Therapy

1. Introduction

Malaria is a condition that depletes hepatocytes and so remains the prevailing infectious diseases in Sub-Saharan Africa (WHO, 2016) [1]. *Plasmodium falciparum*, the deadliest form of the malarial parasite responsible for the enormous majority of the mortality and morbidity associated with malarial infection. Artemisinin combination therapies (ACTs) are presently the major drugs in the management of malaria caused by *P. falciparum.* Now these treatments continue to be affective in many parts of the world.

Transitory changes in the plasma levels of lipids such as cholesterol and triglycerides have been observed many years ago by many authors in different acute infections (Das *et al*., 1996) [2]. Patients with malarial infection show the wide range of metabolic derangements including changes in serum lipid profile. These changes in lipid profile and their possible correlation with malarial infection has been reported in various studies (Mohanty *et al.,* 1992) [3]. The exact mechanisms resulting in these derangements in serum lipid profile in patient infected with malaria parasite is still poorly understood. Under normal conditions liver ensures homeostasis of lipid and lipoprotein metabolism (Faucher *et al.,* 2002) [4].

Several clinical studies showed lipid profile changes in the setting of both uncomplicated and complicated malaria (Chagnon *et al*., 1985; Maurois *et al*., 1979; Sehadri *et al*., 1981; Vernes *et al*., 1980; Cuisinier-Raynal *et al*., 1990; Faucher *et al*., 2002) [5, 6, 7, 8, 4]. Although the magnitude of changes seems to be related to the severity of malaria in several studies (Davis *et al*., 1993; Mohanty *et al*., 1992) [9, 3]. Others found no correlation between the severity of malaria attacks and the extent of lipid profile changes (Kittl *et al*., 1992; Baptista *et al*., 1996) [10, 11]. These transient lipid profile changes in the parasitaemic phase have been suggested by some researchers as a potential adjuvant diagnostic tool for malaria (Kittl *et al*., 1992; Nilsson-Ehel *et al*., 1990; Badiaga *et al.,* 2002) [10, 12, 13].

The malaria infection is also associated with life threatening complications including hypoglycemia, cerebral malaria and acute renal failure (ARF) (Naqvi *et al*., 2003; Das, 2008; Ogetii *et al*., 2010) [14, 15, 16]. The pathogenesis of ARF is multifactorial and involves a complex interaction of immunological, mechanical, humoral factors, and acute phase reactants (Elam, 2003; Barsoum, 2000) [17, 18]. Cytoadherence of infected red blood cells to the vascular endothelial cells of different host organs, including the kidneys, is reported to alter

microcirculation of these organs which ultimately disrupt their physiological functions (Kyes *et al*., 2001) [19]. Disturbances in electrolyte handling have been reported following malaria infection (Uzuegbu, 2010; Van Wolfswinkel *et al*., 2010; Vannaphan *et al*., 2010) [20, 21, 22]. This hyperkalaemia is linked to increased haemolysis during malaria infection. Hyponatraemia has long been recognized as a complication of malaria that is mediated through multiple mechanisms including inappropriate production of arginine vasopressin (AVP).

A resistant phenotype has been detected in five countries of The Greater Mekong Sub-region: Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Vietnam, as relatively slow parasite clearance rates in patients receiving artemisinin or ACT (White, 2011; Cui *et al.,* 2012; Phyo *et al.,* 2012) [23, 24, 25]. And signs of resistance to the ACT-artesunate sulfadoxine pyrimethamine *in Plasmodium falciparum* have been observed in North-eastern states of India (Operational Manuel for Malaria Elimination in India, New Delhi: 2016) [26].

There have been two case reports of artesunate-resistance in India, occurring in Kolkata and Mumbai (Bhattacharyya *et al.,* 2014; Gogtay *et al*., 2000) [27, 28]. There have been four cases of suspected artesunate-resistant malaria from Andhra Pradesh-Orissa border province in India. Out of the four cases, three cases were from our state Andhra Pradesh i.e., from Vizianagaram and Visakhapatnam districts (Shalini *et al*., 2018) [29].

Hence, the present was taken up to study the efficacy of the artemisinin-based combination drugs in view of growing resistance to antimalarials. The study revealed the changes in lipid profile and kidney function tests in *P. berghei* infected mice and treated mice with respect to commonly used laboratory parameters i.e., total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low density lipoproteins (VLDL), triglycerides (TG), TC/HDL ratio, serum creatinine, serum urea and serum electrolytes such as sodium, potassium and chloride ions.

2. Materials and Methods

2.1 Experimental Animals

Thirty male Swiss albino mice each weighing 25-30 g were divided into 5 experimental groups each with 6 animals (n = 6). Animals were allowed to acclimatize for one week before initiation of the experiment. They were housed in plastic cages with rice husk as beddings, provided with access to commercial pellet food and access to clean drinking water *ad libitum*. The animals were handled in accordance with the guidelines in the Guide for the care and use of laboratory animals (2011) [30]. Animal experiments were designated and approved with Ref. No. ANUCPS/IAEC/AH/Protocol/2/2014 by Institutional Animal Ethics Committee (IAEC) of ANU College of Pharmacy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

2.2 Parasite

Chloroquine sensitive *P. berghei* ANKA strain parasites were maintained by intraperitoneal inoculation of 1×10^7 infected erythrocytes to naïve mice. A standard inoculum consisting of 1×10^7 parasitized erythrocytes was prepared from the infected donor mice with >25% parasitaemia, and used to infect experimental mice.

2.3 Inoculation of Experimental Animals

Parasitized red blood cells used for inoculation were obtained by cardiac puncture from a donor mouse. The infected blood was collected in an anticoagulant and diluted to the desired density in 0.9% normal saline. Each mouse was inoculated with 1×10^7 parasitized red blood cells of *P. berghei* suspension. The infection of the recipient mice were initiated by needle passage of the parasite preparation from the donor to healthy test animals via the intraperitoneal route as described previously (David *et al.,* 2004) [31]. The day of inoculation was defined as Day 0 and subsequent days as Day 1, Day 2, and Day 3 up to Day 28.

2.4 Drugs and Dosage Regimens

In the present work, three Artemisinin-based combination drugs were used namely Atesunate + Amodiaquine (AS+AQ), Artesunate + Sulphadoxine Pyrimethamine (AS+SP), Artemether + Lumefantrine (AL). All the drug dosages were given according to the body weight of mouse by following standards of World Health Organization (WHO).

i) Artesunate+Amodiaquine (ASAQ)

The combination drugs of Artesunate (50 mg) tablet and Amodiaquine Hydrochloride (153.1 mg) tablet from IPCA Laboratories Limited, Mumbai. Artesunate (50 mg) tablet was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 1 mg/ml. And 153.1 mg tablet of Amodiaquine dissolved in 150 ml of distilled water to obtain the stock solution concentration of 1.02 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg + Amodiaquine 10 mg/kg once a day for 3 days. So in the present experiment, the same WHO recommended dosage regimen was followed and administered to the infected mice for 3 days by oral gavage according to the body weight.

ii) Artesunate + Sulphadoxine Pyrimethamine (ASSP)

The combination drugs of Artesunate (200 mg) tablet and Pyrimethamine (25 mg) + Suphadoxine (500 mg) tablet (LARINATE-200 kit) from IPCA Laboratories Limited, Mumbai. Artesunate stock solution (1 mg/ml) was prepared as was in 2.4.1. And Pyrimethamine (25 mg) + Sulphadoxine (500 mg) tablet was dissolved in 100 ml of distilled water to obtain the stock solution concentration of 5.25 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg once daily for 3 days and Sulphadoxine + Pyrimethamine as single dose of 25 mg/kg + 1.25 mg/kg on Day 1, which was administered orally. The above WHO dosage regimen was followed in the present experiment.

iii) Artemether+Lumefantrine (AL)

The third combination drug used was Artemether (20 mg) and Lumefantrine (120 mg) tablet (LUMERAX-20 DT) from IPCA Laboratories Limited, Mumbai, India. The tablet Artesunate (20 mg) and Lumefantrine (120 mg) was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 2.8 mg/ml respectively. The WHO dosage regimen is Artemether 1.5 mg/kg and Lumefantrine 9 mg/kg at 0, 8, 24, 36, 48 and 60 hour. The same WHO regimen was followed and 6 doses were given on 3 consecutive days.

2.5 Animal Groups

The mice were divided into following 5 groups with 6 mice

(n = 6) in each group:

- Group 1 (Control Non-infected): The mice were given only distilled water.
- Group 2 (Infected Non-treated): The mice were infected with *P. berghei* antigen.
- Group 3 (Infected + ASAQ): The mice were first infected with *P. berghei* antigen and then treated with Artesunate+Amodiaquine combination.
- Group 4 (Infected +ASSP): The mice were first infected with *P. berghei* antigen and then treated with Artesunate + Sulphadoxine Pyrimethamine combination.
- Group 5 (Infected + AL): The mice initially were parasitized with *P. berghei* and then treated with Artemether + Lumefantrine combination.

2.6 Study of the course of infection to *Plasmodium berghei* in experimental mice

Thin blood films were prepared on clean slides, initially fixed with methanol. A large drop of blood is put at the center of a clean dry slide. The drop is spread with an applicator slide, and then the smear is thoroughly dried in a horizontal position. Blood smears were stained with Giemsa stain for 5-8 min. Subsequently, distilled water was poured on the surface of the smears to remove excess stain and then dried. A field was selected using x10 objective where the Red Blood Corpuscles (RBCs) were in an evenly distributed monolayer followed by the x100 oil immersion objective. A minimum of 1000 RBCs were counted and among those, number of infected RBCs will be recorded. The percent of infected RBCs (parasitaemia) was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs (Oyewole *et al*., 2010) [32] as follows.

$$\text{Percentage (\%) of Parasitaemia} = \frac{\text{No.of infected RBCs}}{\text{No.of RBCs counted}} \times 100$$

2.7 Collection of blood serum

In all the experimental groups (both infected non-treated and infected-treated mice), parasitaemia was estimated throughout the experimental period daily by observation of Giemsa stained blood smears under the microscope. On 7th day of the experimental period, the required number of mice was euthanized with chloroform and the blood samples were collected through cardiac puncture and serum samples were obtained for estimation of biochemical parameters.

2.8 Estimation of lipid profile and kidney function tests

The lipid profile and kidney function tests were performed by Colorimetric method using reagent kits. The absorbances of all the tests were read in a spectrophotometer (ELICO, Hyderabad, India) and all the assay procedures were according to the kit manufacturer's instruction.

For estimation of lipid profile and serum electrolytes (Na^+, K^+ and Cl^- ions), the reagent kits used were from Beacon Diagnostic Pvt. Ltd., Navsari, Gujarat, India. For estimation of serum creatinine and serum urea, the reagent kits used were from Transasia Bio-medicals Ltd., Solan, Himachal Pradesh, India.

i) The Total Cholesterol (TC) was determined by colorimetric method (Richmond, 1973) [33].

$$\text{Total Cholesterol (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{O.D.of Standard}} \times 200$$

ii) The serum High Density Lipoprotein-Cholesterol (HDL-C) was determined by colorimetric method (Richmond, 1973) [1].

$$\text{HDL Cholesterol (mg/dL)} = \frac{\text{Absorbance of HDL Test}}{\text{Absorbance of Standard}} \times 100$$

iii) The Low Density Lipoprotein-Cholesterol and Very Low Density Lipoprotein-Cholesterol (LDL-C & VLDL-C) was determined by using the equation of Friedewald *et al*. (1972) [34] was used to determine LDL - cholesterol and VLDL - cholesterol concentration.

$$\text{LDL} = \text{TC} - (\text{HDL} + \text{VLDL})$$
$$\text{VLDL} = \text{TG} \div 5$$

iv) The serum Triglycerides (TG) were determined by colorimetric method (Schettler and Nussel, 1975) [35].

$$\text{Triglycerides (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Standard Concentration}$$

v) The serum Creatinine was determined by Alkaline-picrate method using Jaffe reaction (Bowers, 1980) [36].

$$\text{Creatinine (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

vi) The serum Urea was determined by Diacetyl monoxime method using Fearon reaction (Kassirer, 1971) [37].

$$\text{Urea (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

vii) Sodium was determined by the modified method of Maruna (1958) [38].

$$\text{Concentration of Sodium (mEq/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 150$$

viii) Potassium was determined by the modified method of Maruna (1958) [38].

$$\text{Concentration of Potassium (mEq/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 5$$

ix) Chloride ion concentration was determined by the method of Zall *et al.* (1956) [39].

$$\text{Concentration of Chloride (mEq/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

2.9 Statistical analysis

Results of individual parameters were expressed as mean±standard deviation. The comparison between the experimental groups was performed by Student t-test using MINITAB 11.12.32 Bit statistical package and graphs were drawn in MS Excel. The results were statistically significant at $P < 0.05$.

3. Results

3.1 Course of infection to *P. berghei* in experimental mice

i) *P. berghei* Infected Non-treated group

During the study of course of infection, *P. berghei* parasite was given to the experimental mice on Day 0. After inoculation the parasitaemia was first appeared on Day 3 (72hours).Then the parasitaemia was gradually increased up to the peak level on Day 7. On Day 3, initial parasitaemia was 19%, on Day 4 with 23%, on Day 5 with 27%, on Day 6 with 32% and on Day 7 with 36% of parasitaemia. High rate of parasitaemia was observed on 7th day post inoculation after which all the mice died due to heavy infection by Day 8 (Figure 1).

ii) *P. berghei* Infected + ASAQ Treated
In this group, initial parasitaemia was 20% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASAQ combination drug was administered orally. Then the parasitaemia was decreased to 8% on Day 4. On Day 5, the parasitaemia was 0% and so the parasite clearance occurred within 48 hours. No recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in ASAQ treated mice was 2 days (48 hours) (Figure 1).

iii) *P. berghei* Infected + ASSP Treated
In this group, the initial parasitaemia was 21% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASSP drug was administered orally. Then the parasitaemia decreased to 10% on Day 4. On Day 5, the parasitaemia was 2% and on Day 6 with 0%. No recrudescence was observed during follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time of (PCT) in ASSP treated mice was 3 days (72 hours) (Figure 1).

iv) *P. berghei* Infected + AL Treated
In this group, the initial parasitaemia was 19% on Day 3. Then the mice were treated with AL combination drug for 3 consecutive days orally on Day 3, Day 4 and Day 5. On Day 4 the parasitaemia was 11%, on Day 5 parasitaemia decreased to 3% and on Day 6 no parasitaemia was observed. Also no recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in AL treated mice was 3 days (72 hours) (Figure 1).

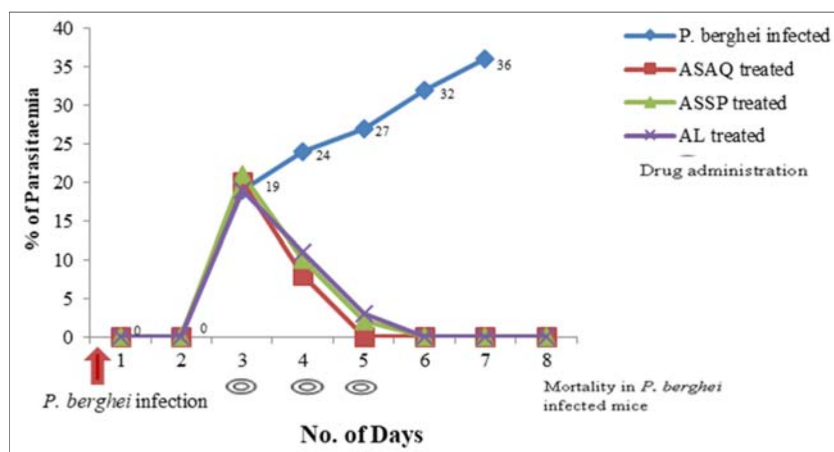

Fig 1: Course of infection to *Plasmodium berghei* in infected non-treated and treated mice during Artemisinin-based combination therapy

In the present study, the changes in lipid profile and kidney function tests were evaluated in control and *P. berghei* infected non-treated and three drug treated groups i.e., ASAQ, ASSP and AL treated mice. The values of all the parameters i.e., lipid profile and kidney function tests were represented in Table 1.

In *P. berghei* infected mice, the above parameters were altered significantly ($P < 0.05$) when compared to the control mice because of peak level of infection on 7th day. In *P. berghei* infected non-treated group; TC, LDL-C, VLDL-C and TG were significantly increased ($P < 0.05$) whereas HDL-C was significantly decreased ($P < 0.05$) when compared to the control group. After treatment with ASAQ, ASSP and AL combination drugs; TC, LDL-C, VLDL-C and TG were significantly decreased ($P < 0.05$) whereas HDL-C was significantly increased ($P < 0.05$) in treated mice when compared to the *P. berghei* infected mice. Thus, the lipid profile parameters were restored to normal levels as in control mice after artemisinin-based combination therapy in experimental mice (Table 1).

In *P. berghei* infected mice, serum creatinine and serum urea levels were significantly ($P < 0.05$) increased when compared to the control group. But after treatment with ASAQ, ASSP and AL combination drugs; serum creatinine and serum urea were significantly decreased ($P < 0.05$) when compared to the *P. berghei* infected mice. Thus, serum creatinine and serum urea levels were restored to normal levels as in control mice after artemisinin-based combination therapy (Table 1).

Among serum electrolytes; sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions were investigated in all the experimental groups. In *P. berghei* infected group, sodium and chloride ion concentrations were significantly decreased ($P < 0.05$) whereas potassium ion concentration was significantly increased ($P < 0.05$) when compared to the control group. After treatment with ASAQ, ASSP and AL combination drugs; sodium and chloride ion concentrations were significantly increased ($P < 0.05$) whereas potassium ion concentration was significantly decreased ($P < 0.05$) when compared to the *P. berghei* infected group. Thus, the serum electrolytes were restored to normal levels as in control mice after artemisinin-based combination therapy in experimental mice (Table 1).

Table 1: Changes in lipid profile and kidney function biomarkers of *Plasmodium berghei* infected non-treated and treated mice with Artemisinin-based combination drugs

S No.	Parameter	Control Non-infected (Normal) (n = 6)	*P. berghei* Infected non-treated (n = 6)	*P. berghei* Infected + ASAQ treated (n = 6)	*P. berghei* Infected +ASSP treated (n = 6)	*P. berghei* Infected + AL treated (n = 6)
Lipid Profile						
1	TC (mg/dL)	94.0±0.589	106.0±0.365 P = 0.0000* t = 34.64	95.0±0.594 P = 0.0000* t = 31.54	96.0±0.730 P = 0.0000* t = 24.49	96.5±0.589 P = 0.0000* t = 27.42
2	HDL-C (mg/dL)	28.0±0.337	20.0±0.337 P = 0.0000* t =28.89	30.0±0.698 P = 0.0000* t = 25.89	28.0±0.523 P = 0.0000* t = 25.73	27.4±0.556 P = 0.0000* t = 23.00
3	LDL-C (mg/dL)	51.6±0.337	69.0±0.258 P = 0.0000* t = 82.02	50.5±0.841 P = 0.0000* t = 42.07	53.2±0.365 P = 0.0000* t = 70.66	54.0±0.416 P = 0.0000* t = 61.24
4	VLDL-C (mg/dL)	14.4±0.250	17.4±0.183 P = 0.0000* t =19.22	14.4±0.350 P = 0.0000* t = 14.82	14.8±0.516 P = 0.0000* t = 9.49	15.0±0.163 P = 0.0000* t = 19.60
5	Triglycerides (mg/dL)	72.0±0.258	85.0±0.171 P = 0.0000* t = 84.15	72.5±0.275 P = 0.0000* t =77.15	74.0±0.258 P = 0.0000* t = 71.23	75.0 ±0.141 P = 0.0000* t = 90.42
6	TC/HDL Ratio	3.3±0.183	5.3±0.171 P = 0.0000* t =16.20	3.1±0.183 P = 0.0000* t = 17.80	3.4±0.183 P = 0.0000* t = 15.40	3.5±0.365 P = 0.0001* t = 9.05
Kidney Function Biomarkers						
7	Creatinine (mg/dL)	0.30±0.008	0.42±0.008 P = 0.0000* t = 20.78	0.35±0.008 P = 0.0000* t = 12.12	0.36±0.008 P = 0.0000* t = 10.39	0.38±0.008 P = 0.0000* t = 6.93
8	Urea (mg/dL)	22.0±0.440	35.0 ±0.337 P = 0.0000* t = 46.95	23.0±0.350 P = 0.0000 t = 49.32	24.0±0.330 P = 0.0000 t = 46.53	24.5±0.330 P = 0.0000 t = 44.41
9	Sodium (mEq/L)	133.0±0.216	115.0±0.698 P = 0.0000* t = 49.30	130.0±0.337 P = 0.0000* t = 38.73	129.0±0.377 P = 0.0000* t =35.36	127.0±0.310 P = 0.0000* t = 31.51
10	Potassium (mEq/L)	3.8±0.258	5.4±0.337 P = 0.0003* t =7.54	3.9±0.337 P = 0.0007* t = 6.30	4.0±0.258 P = 0.0006* t = 6.60	4.2±0.183 P = 0.0008* t = 6.27
11	Chloride (mEq/L)	91.0±0.141	79.0±0.216 P = 0.0000* t = 92.95	90.4±0.330 P = 0.0000* t = 58.14	88.0±0.216 P = 0.0000* t = 58.92	87.0±0.337 P = 0.0000* t = 40.00

The values are expressed as mean of 6 values along with standard deviation and are statistically significant at $P < 0.05$ (*significant) and $t > 2.306$

But out of the three Artemisinin-based combination therapies, ASAQ combination therapy proved to be more efficacious than ASSP and AL combination therapies in restoring the lipid profile parameters and kidney function associated parameters to normal levels.

4. Discussion

In the present study, the course of infection revealed a gradual increase in the level of parasitaemia as the days progressed from 3 to 7 in the *P. berghei* infected mice. This is in agreement with the view that parasitaemia increases progressively after inoculation of the infection until the point of death in the absence of suitable treatment (Trampuz *et al.,* 2003; Breman *et al.,* 2001) [40, 41].

Lipids have been observed to play important role in pathological changes observed in diseased conditions and are implicated in the production of immunity against diseases (Beckwith *et al*., 1975) [42]. Serum lipids primarily bound to lipoproteins can be elevated by an increase in biosynthesis and /or by a decrease in their removal. Both of these processes appear to contribute to the hyperlipidemia that is often produced by some pathological changes. Although the source of increase in erythrocyte lipid component in malaria infection is not from lipid of the parasite there is no indication that the increase in the serum lipid is due to the lipid content of the parasite (Beach *et al.,* 1977) [43].

The malaria parasites have a high requirement for cholesterol and phospholipids for its survival in the human host (Njoku *et al*., 1995) [44]. Circulating HDL-cholesterol particles and erythrocytic membrane are the potential sources of cholesterol and phospholipids for these parasites (Njoku *et al*., 1995) [44]. Erythrocyte phospholipids content has been demonstrated to increase 500 folds following malarial infection (Sherman, 1979) [45]. During the late stage of the parasite development, infected erythrocytes contain 3-5 times more phospholipids than uninfected cells (Labaied *et al*., 2011) [46]. Vial *et al.* (1982) [47] also reported that the infected erythrocytes contain phospholipid synthesizing enzymes. Thus, potent inhibitors of plasmodial phospholipid synthesis were previously characterized as potential target for antimalarial chemotherapy due to its crucial role to the parasite survival (Ben Mamoun *et al*., 2010) [48]. So, it became important to evaluate the total cholesterol and phospholipid contents of the liver because the exoerythrocytic stage of the malaria parasite life cycle occurs in the liver.

In the present study, the total cholesterol TC, LDL VLDL and Triglycerides were significantly increased in the *P. berghei* infected mice when compared to the control mice. This finding is in consistent with the reports by several authors (Oluba *et al*., 2012; Uraku *et al.,* 2015; Igweny *et al*., 2017)

[49, 50, 51]. Similarly, Sirak *et al*. (2016) [52] showed increased serum lipoproteins in *P. falciparum* malaria patients when compared to the healthy controls. In addition another study reported that hyperlipidemia is one of the indicators of malaria infection; the hypothesis for the changes is basically due to increased VLDL production and increased mobilization of free fatty acids from adipose tissue in response to stress (Akanbi, 2013) [53]. However the evidence of higher concentrations of serum lipids in infected group might be due to adipose tissue lipolysis, impairment in lipoprotein lipase system, increase in de novo hepatic fatty acid synthesis, and suppression of fatty acids oxidation in severe infection (Bansal *et al*., 2005) [54].

The increase in the LDL–C level is possibly because of the increased concentration of total cholesterol levels. The increase in total cholesterol, LDL-C, VLDL-C and triglycerides levels during malaria infection have been reported to contribute to the pathogenesis of malaria and this could be dangerous to human health as it is capable of causing atherosclerosis, if necessary treatment is not given. It has been reported that lipoprotein represents a major component of serum needed for the growth of the malaria parasite (Olusegun, 2013) [55]. And LDL-C can be deposited on the walls of the artery leading to arthrosis and other cardiovascular diseases (Mayers *et al*., 2004) [56]. The concentration of HDL-C was significantly lower in *P. berghei* infected mice when compared with control mice. The HDL is synthesized by liver cells (Parks, 2002) [57], hence their lower concentration signifies liver dysfunction resulting in the inability of liver to properly package the lipids (Sunmonu and Oloyede, 2007) [58].

Results from the present study showed that total cholesterol, LDL-C, VLDL-C were significantly lower and HDL-C was significantly higher in infected mice treated with ASAQ, ASSP and AL combination drugs when compared with infected but untreated mice. Oluba *et al*. (2012) [49] had earlier observed a positive correlation between serum and liver lipoprotein cholesterol concentration and parasitaemia level in *P. berghei* infected mice treated with *Ganoderma lucidum*. Oluba (2019) [59] reported that cholesterol, LDL-C, triglycerides and phospholipid were significantly lower in infected mice administered Ganoderma terpenoid extract in combination with chloroquine compared with infected but untreated mice which is in correlation with present finding.

In the present investigation, serum creatinine and serum urea levels were significantly increased when compared to the control mice. Our findings correlate with the previous reports of Uraku and Onuoha (2015) [50] and Pattarapo *et al*. (2017) [60] where increased levels observed during *P. berghei* infection in experimental mice. Similarly Anigboro (2018) [61] reported increased levels of serum urea and serum creatinine in *P. berghei* infected mice when compared to the control mice.

Serum creatinine, serum urea and serum electrolytes are the most responsive biochemical markers used in the assessment of renal tissue damage, because urea and creatinine are excreted through the kidneys. And the electrolytes are reabsorbed in the tubules. This increased serum creatinine and serum urea levels in *P. berghei* infected group suggest possible renal impairment (Koay and Walmsley, 1999) [62] because under normal function the kidney excretes these substances and maintain their levels in the body. During malaria infection renal impairment has been an important life threatening complication of malaria infection (Amet *et al*., 2013) [63]. Propagation of malaria *in vivo* was observed by increasing parasitemia. Moreover, during malaria infection *in vivo*, oxidative stress was occurred, subsequently destruction of erythrocytes was induced as shown by low levels of hematocrit (Becker *et al*., 2004) [64].

After treatment with ASAQ, ASSP and AL combination drugs, serum urea and creatinine values reached to normal level as compared with the control mice. This confirms the findings of Anigboro (2018) [61] where the serum creatinine and serum urea levels were restored upon treatment with *Bambusa vulgaris* leaf extract. And Somsak *et al*. (2013) [65] revealed the restoration of serum creatinine upon treatment with green tea extract in *P. berghei* infected mice; Sibiya *et al*. (2017) [66] reported reduced plasma creatinine concentrations were observed upon treatment with *Syzygium-aromaticum* derived Oleanolic acid in *P. berghei* infected experimental rats as compared with the control rats.

In the present study, serum electrolytes i.e., serum sodium (Na^+) and chloride (Cl^-) ions were significantly decreased and serum potassium (K^+) ions were significantly increased in *P. berghei* infected group. Our findings correlate with the previous reports of Etim *et al*. (2011) [67] where decrease in the levels of Na^+, Cl^- and HCO_3^- observed in untreated patients compared to those of normal controls from Aba, Abia State, Nigeria. Onyesom *et al*. (2015) [68] reported decreased levels of serum sodium and serum chloride ions whereas serum potassium ion concentration elevated due to *P. berghei* infection in mice. Our results are also in agreement with the earlier findings of Sibiya *et al*. (2017) [66] and Anigboro (2018) [61] in experimental mice infected with *P. berghei* infection.

Electrolytes are essential for the standard physiology of life. These are ionized salts (minerals) present in the body fluids and blood circulation. The total body acts like a bioelectric organism and also electrolytes are both the switch and the energy resource for our body (Spence, 1999) [69]. Electrolyte disturbance is known to be the common complication in severe malaria (Jasani *et al*., 2012) [70]. The changes in electrolytes (Na^+ K^+ and Cl^-) concentrations were also reported in Table 1. Correction of fluid amount and electrolyte deficits has been the standard of care for any critically ill patients including those with severe malaria infection. This is because acidaemia, hypokalaemia, hypocalcaemia and hyponatraemia exacerbate myocardial dysfunction and increase the risk of arrhythmias (Khilnami, 1992 and Kumar *et al*., 2001) [71, 72].

It was observed that the electrolytes level (Na^+, Cl^-) decreased significantly in *P. berghei* infected mice with no treatment when compared with the normal control mice, this also indicates that malarial infection affects renal secretion or reabsorption of electrolytes. The decrease in concentration of sodium may be due to losses in urine, because losses in urine are compensated by increase in urea concentrations. This observation has been found to be common in falciparum malaria infection, and occurs in order to maintain constant body osmolality (Mayne, 1994) [73]. Impaired glomerular filtration amidst hyponatraemia reduces the amount of sodium ions available in the renal tubule for potassium exchange and therefore contributes to the elevation of serum potassium concentration. Erythrocytes infected with falciparum malaria are associated with decreased cytosolic concentration of potassium (hypokalemia) (Matthys *et al*., 2008) [74]. A report by Akpan *et al*. (2011) [75] shows low level of serum chloride in malaria infection which is in accordance with the present study. Serious cases of kidney problem associated with

malaria, take the form of nephritic syndrome which gradually progresses to renal failure characterized by metabolic acidosis arising from low level of serum bicarbonate (Marshal and Bangert, 2008) [76].

But after treatment with Artemisin-based combnation therapy; in *P. berghei* infected mice, the serum electrolytes restored to normal level and this restoration is an indication of normal renal activities. This corroborates with the findings of Onyesom *et al*. (2015) [68], Sibiya *et al*. (2017) [66] and Anigboro (2018) [61] in *P. berghei* infected mice after respective treatments.

And a combination therapy would be helpful in simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite (WHO, 2001) [77]. Thus the present study revealed hepatoprotection and kidney function restoration upon administration with artemisinin-based combination drugs. But among the three combinations, ASAQ has shown better restoration of lipid profile and kidney function biomarkers.

5. Conclusions

According to the present study, lipid profile and kidney function biomarkers were altered significantly in *P. berghei* infection. After treatment with ASAQ, ASSP and AL drugs, all the above parameters were restored to normal levels. But among these three artemnisinin-based combination drugs, Artesunate+Amodiaquine (ASAQ) is the most effective drug than Artesunate+Sulfadoxine Pyrimethamine (ASSP) and Artesunate+Lumefantrine (AL) in the present scenario of growing resistance to the antimalarial drugs.

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