Toxicological and biochemical effects of therapeutic doses of Chloroquine, Artecxin, Lonart and P-Alaxin in *P. berghei*-infected mice: A comparative study.

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ABSTRACT

Background: Alterations in hematological indices and biochemical parameters are implicated in malaria and many pharmacologic agents used in the treatment of malaria in the tropics.

Objective: This study was designed to determine and compare *in vivo* antimalarial activity and safety of therapeutic doses of Chloroquine (CQ), Artecxin (ART), P – Alaxin (P-ALA) and Lonart (LON) in *Plasmodium berghei*-infected mice.

Method: Six groups each of five mice (healthy control (non-parasitized non-treated group), parasitized-non-treated (PnT), Parasitized- CQ treated (positive control), parasitized – ART, LON and P-ALA treated groups were used for the study. Antimalarial activity/parasite density in the blood of infected and treated mice was evaluated. Toxicity of drugs was also evaluated with haematological, biochemical parameters and histology.

Results: ART and other antimalarial drugs significantly ($p<0.05$) cleared the parasite density following three days administration of the drugs in the order ART>P-ALA>LON. The drugs ameliorated, malarial infection-induced abnormalities in hematological and biochemical indices. All the antimalarial drugs caused significant elevation ($p<0.05$) in plasma alkaline phosphatase (ALP) and aspartate amino transferase (AST) activities while ART showed no significant difference ($p>0.05$) in alanine amino transferase (ALT) activity when compared with control. Histopathological evaluation revealed severe necro-inflammatory effect of ART on the liver cells while other drugs had mild toxic effects.

Conclusion:
ART has a higher efficacy and antimalarial activity in *Plasmodium berghei*-infected mice compared to LON and P-ALA. However, its toxic effects on liver and kidney cells' architecture suggest that it be recommended in severe cases of malaria infection to preserve life.

Key words: *Plasmodium berghei,* Artecxin, Lonart, P-Alaxin, Toxicity, Chloroquine

INTRODUCTION

Malaria remains a devastating global problem representing a medical emergency as it leads to complications and death if not promptly and appropriately treated. It causes about 250 million cases of fever and approximately 1 million deaths annually, and the vast majority of cases occur in children below 5 years old and pregnant women. Death rate could double in the next 20years if prevalence stays on its present upward course [1-2]. Several interventions have been instituted for prevention and possibly for eradication of malaria. Some of the control measures are provision of mosquito insecticide impregnated nets and insect repellents, spraying of insecticide inside houses and draining of standing or stagnant water where mosquitoes lay their eggs. Successful treatment outcomes are highly predicated on choice of antimalarial drugs employed.

Over the past decade, a new group of antimalarial – the artemisinin compounds, especially artesunate, artemether and dihydroartemisin – have been deployed on an increasingly large scale for treatment of malaria. These compounds produced a very rapid therapeutic response in terms of reduction of the parasite biomass and resolution of symptoms. They are active against multidrug resistant *P.falciparum*, well tolerated by the patients and reduce gametocyte carriage. Hence, they have the potential to reduce transmission of malaria. Several reports have shown that artemisinins cure falciparum malaria in 7 days if used alone [3-5].

Emergence of multidrug resistant *Plasmodium falciparum* (mdrpf) led to the recommendation by WHO that Arteminsinin based Combination therapies (ACTs) are adopted as gold standard medicine for treatment of malaria[6]. Due to relentless increase in resistance of *Plasmodium falciparum* to drugs such as chloroquine, sulfadoxine-pyrimethamine and mefloquine, new drugs with low toxicity, fast action and broad activity against various forms in the life cycle of malaria parasite are continuously being developed. ACTs consist of fixed dose of chemical derivatives of artemisinin, and a longer acting partner drug which ultimately traces itself back to the natural product quinine. The recommended ACTs for treatment of uncomplicated *P. falciparum* malaria by WHO in its new guidelines[2] are Arthemether/Lumefanthrine, Artesunate/Amodiaquine, Artesunate/Mefloquine, Artesunate/Sulfadoxine Pyrimethamine, Dihydroartemisinin/Piperaquine. The need for new drug molecules arises because of the constant threat of emergence of resistance inspite of the efficiency of the

existing medicines. For instance, the signs of resistance to artemisinins are emerging in some parts of Cambodia where patients are taking longer duration to clear their fever[7]. Thus, this showed that there is an early need and urgent priority for development of new chemical entities against new resistant strains of malaria parasite which is currently on-going with series of clinical trials and pre-registration screening [2].

ART is a new ACT drug introduced into Nigeria in 2008 which contains dihydroartemisinin (DHA, 32 mg), piperaquine phosphate (320 mg) and trimethoprim (90 mg).These strengths deviate from the strengths prescribed by WHO for DHA- Piperaquine combination as well as inclusion of trimethoprim (an antifolate) in the regimen. Artecxin combination differed from combination of other existing antimalarial drugs particularly P-Alaxin (40mg Dihydroartemisinin and 320mg piperaquine phosphate). Trimethoprim is an antibiotic used mainly for bladder infections as well as for middle ear infection and traveler's diarrhea [8]. It blocks folate metabolism by binding to the enzyme dihydrofolate reductase[9]. DHA is the active metabolite form of Artemisin and acts by binding tightly to parasite infected erythrocyte membrane to inhibit the sarcoplasmic/endoplasmic reticulum calcium ATPase encoded by *P. falciparum* [10-12]. Piperaquine is related to chloroquine and acts by inhibiting the detoxification by the plasmodium of heme molecules that accumulate in the parasite. The mechanism of action of these drug combinations and their ability to accumulate in the red blood cells being their primary site of action, raised questions regarding the safety of ART. The constituent combination of this drug as an antimalarial drug spurred the current investigation which was aimed at evaluating the antimalarial activity and safety of therapeutic doses of Artecxin compared to P-Alaxin, Lonart and chloroquine (commonly used in Nigeria) in Swiss strain albino mice infected with *Plasmodium berghei*.

METHODS

Drugs and Chemicals

Artecxin (32mg Dihydroartemisinin, 320mg Piperaquine phosphate and 90mg Trimethoprim) was manufactured by Chongqing Tonghe Pharmaceutical Co. LTD, Chongqing, China.P-Alaxin (40mg Dihydroartemisinin and 320mg Piperaquine phosphate) and Lonart (80mg Artemether and 480mg Lumefantrine) were manufactured by Bliss GvsPharma Ltd, India. All other chemicals were of commercially available analytical grade.

Animal Maintenance

Adult male Swiss albino mice (20 – 23g) were obtained from the Animal House, College of Medicine Campus, Idi-Araba,University of Lagos, Nigeria .The mice were kept in well-ventilated standard plastic cages under controlled conditions of 12hr light/12hr dark cycle, acclimatized for 2 weeks while being maintained on standard mouse pellets (Pfizer Livestock Feeds, Lagos, Nigeria) and water *ad libitum*. All experiments were conducted without anaesthesia and the protocol was in conformity to the guidelines of the National Institute of Health (NIH publication 85–23,1985)[13] for laboratory animal care and use. The Guidelines of the Animal Ethic Committee of the College of Medicine, University of Lagos, Nigeria, was also followed in conducting this research study.

Parasite Inoculation

The CQ-sensitive NK 65 strain of *Plasmodium berghei* used in this study was obtained from Dr. O. O. Aina of Malaria Research Laboratory, Department of Biochemistry, National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The mice were infected intra-peritoneally on the first day of the experiment with standard inoculum [1 x 10^7 *Plasmodium berghei* (NK-65 strain**)** infected parasitized red blood cells**]**. The animals were left for three days for incubation of the parasite which was confirmed with thick blood film stained with Giemsa stain and viewed under the microscope at x100 magnification. Parasite density was calculated with the formula: Parasites/microliter blood = Number of parasites counted x 8000 white blood cells)/Number of white blood cells counted.

Experimental design and treatment

Five healthy mice served as control (Group 1) and received 10 mL/kg body weight of normal saline by oral intubation. Twenty five parasitized (PnT) mice were randomly divided into 5 groups of five animals each as follows:
Group 2: PnT untreated mice administered 10 mL/kg body weight of normal saline.
Group 3: PnT mice administered CQ (10.33mg/kg body weightin normal saline).
Group 4: PnT mice administered ART (14.73mg/kg body weight in normal saline).
Group 5: PnT mice administered LON (9.33mg/kg body weight in normal saline).
Group 6: PnT mice administered P-ALA (18mg/kgbody weight in normal saline).

All treatments were administered orally, based on recommended therapeutic dosages, for 3 days and mice were fasted overnight and weighed just before sacrifice by cervical dislocation.

Collection of blood and preparation of plasma

Whole blood samples were collected by retro-orbital puncture using capillary tubes just before sacrifice into labeled lithium heparinized bottles for hematological profile analysis and ethylenediamine tetra-acetic acid (EDTA) anti-coagulated bottles for blood chemistry assays. Plasma was prepared by spinning whole blood samples for 10 minutes at 4,000 rpm in a Cencom Bench centrifuge. Supernatant (plasma) obtained was separated and kept frozen at -80^{0}C. Liver was quickly excised, rinsed in ice-cold 1.15% KCl, blotted and weighed. A portion of the liver was cut and employed into 10% buffered-formaldehyde (formalin) solution to preserve the tissues for histopathological evaluation.

Biochemical Assay

Protein concentrations were determined as described by the method of Lowry *et al*., [14]; alkaline phosphatase, alanine and aspartate aminotransferases activities, urea, creatinine and direct bilirubin levels were analyzed in the obtained plasma using the Roche 912 Chemistry Auto-Analyzer- (Germany).

Haematological analysis

Haematological parameters including red blood cell count, white blood cell count, packed cell volume, haemoglobin concentration, lymphocytes and neutrophils counts were measured using the BC-3200 Auto Hematology analyzer (Mindray Medical International Ltd, Germany).

Histopathological examination
A portion of liver and kidney tissues from each group was collected and preserved in 10% neutral-buffered formalin for histopathological studies. The tissues were processed and embedded in paraffin wax and thin sections of 5-6 µm thickness of liver tissue were cut before staining with hematoxylin and eosin. These thin sections of liver and kidney were made into permanent slides and examined photomicroscopically.

Statistical analysis
Data were statistically computed using Graph Pad Prism 6 Software and expressed as mean ± SEM. Differences between mean values were further analyzed by Tukey Honest Significant Difference (Tukey's HSD) test and values of $P < 0.05$ were considered significant.

RESULTS
Figure 1 depicts the rate of parasite clearance after treatment of *P. berghei*-infected mice with antimalarial drugs. There was total clearance of parasite density in CQ and ART-treated groups by day 2 and no significant ($p<0.05$) difference in clearance rate of parasitized red blood cells of LON and P-ALA-treated groups were obtained compared to CQ and ART-treated groups on day 3. Statistically significant ($p<0.05$) decrease in parasite density of PnT group occurred on day 3 compared to day 2.
The activities of alanine (ALT) and aspartate amino transferases (AST); alkaline phosphatase (ALP) and total protein (TP) concentrations in plasma of antimalarial-treated *P. berghei* infected mice were as presented in Table 1.Significant increases ($p<0.05$) in activities of AST (114.7%), ALP (29%) and levels of total protein (33.8%) were obtained in the plasma of PnT-untreated compared to control mice. Plasma AST activities of all antimalarials-treated mice except P-ALA were not significantly different from one another and PnT group but significantly ($p<0.05$) higher than in control (CQ = 103%, Artecxin = 130%, Lonart = 123%). Furthermore, AST activity was significantly lowered ($p<0.05$) by P-ALA treatment (-28.4%) than in PnT-untreated but higher than in control mice by 53.7%, respectively. The antimalarials- CQ, LON, and P-ALA also caused significant increases ($p<0.05$) of (41%), (140%), and (47%) respectively, in activities of plasma ALT as compared to PnT-untreated mice. However, there was no significant ($p<0.05$) difference in the activity of plasma ALT of parasitized non-treated, ART -treated and healthy control mice. The levels of plasma total protein in ART- and LON-treated mice were not significantly ($p<0.05$) different from healthy control mice but were significantly ($p<0.05$) increased and decreased by treatment with P-ALA (12%) and CQ (11%), respectively.When compared with PnT-untreated mice, total plasma protein levels decreased significantly ($p<0.05$) by 33, 22, 23 and 16 % respectively, after treatment of *P. berghei*-infected mice with CQ, ART, LON and P-ALA.The activity of alkaline phosphatase in the plasma was increased by 29 % in the *P. berghei* -infected untreated mice compared to control. All the antimalarial treatments reduced the activity of this enzyme non-significantly ($p>0.05$) compared to *P. berghei*-infected untreated mice except in CQ-treated mice where a slight increase in this enzyme activity was observed. Similarly, plasma levels of urea and direct bilirubin were non-significantly ($p>0.05$) increased while creatinine was decreased non-significantly in the PnT-untreated mice compared to control. All antimalarial treatments reversed the infection- induced increases in direct bilirubin levels except LON which decreased the levels to below normal baseline. Furthermore, significant ($p<0.05$) decreases in creatinine and urea levels were observed in the ART, LON and CQ-treated PnT-infected mice compared to PnT untreated and control while P-ALA treatment increased creatinine and urea levels higher than in control and PnT-untreated mice,

Hematological profile of PnT untreated and antimalarial –treated mice were as shown in Table 2. *P. berghei* infection significantly reduced ($P<0.05$) RBC, PCV, MCV, MCH and haemoglobin concentration by 52, 51, 21, 21 and 50 %, respectively, compared to healthy control. All the antimalarial treatments significantly reversed ($P<0.05$) the levels of these parameters by over 68 % compared to healthy control mice. CQ indicated an outright reversal and treatment proved most effective and rapid in the CQ and ART treated groups as PCV, RBC counts and MCH tended towards normal. There was a marked reduction of 18 % in WBC as reflected in the neutrophils count (39% reduction) of the PnT-untreated group, but it was restored to basal by all antimalarial treatments (Table 2).
Plate 1 shows the photomicrograph of the kidney sections of PnT untreated and antimalarial –treated mice. Healthy control (Plate 1A) kidney micrographs showed normal renal glomeruli and tubules while the PnT- untreated (PnT) kidney (Plate 1B) have numerous parasitized red cells. In PnT- CQ and PnT-LON treatments (Plate 1C and 1E) there were preserved renal architecture, interstitial nephritis and severe cortico-medullary congestion extending to involve the pelvi-calyceal system. However, there was cortical haemorrhage, congested glomeruli and moderate congestion of the medullary vessels. The ART- treated PnT (Plate 1D) kidney section has preserved renal tissue with focal cortical inflammatory cell infiltration. P-ALA-treated PnT kidney section (Plate 1F) has preserved renal architecture, diffused cortical and medullary vascular congestion/inflammation extending to involve the glomeruli and pelvi-calyceal system, as well as interstitial nephritis .
Plate 2 shows the photomicrographs of livers from mice infected with *P. berghei* parasites compared with four different antimalarial treatments. The healthy control liver (plate 2A) shows normal hepatic architecture while the PnT, CQ-, LON-, and P-ALA treated PnT groups in Plates 2B, 2C, 2E and 2F, respectively show focal and piecemeal necrosis of heptocytes with moderate, diffuse parenchymal, portal congestion, fatty change (steatosis) and pockets of neutrophilic and lymphocytic aggregates within the parenchyma and around the portal tracts. However, malaria pigments and haemorrhage were also seen within parasitized hepatocytes in PnT (Plate 2B). The ART-treated PnT (Plate2D) liver section has severe necroinflammation of hepatic lobules and portal tracts. The hepatic arteries are dilated and filled with blood.

DISCUSSION
The development of ACT regimens is to achieve rapid schizontocidal activity by means of the selected artemisinin compound together with a longer antimalarial effect associated with the different mechanism of action and longer half-life of the selected partner agent [15]. The search for effective anti-malarial chemotherapeutic agents targets identifying drugs of low toxicity, fast action and broad activity against various forms in the life cycle of malaria parasite [16-17].
In the present study, the therapeutic parasite clearance by CQ (positive control) and Artemisinin based combination therapies administered (tested groups) were in consonance with expected outcomes since artemisinin-

based compounds are established antimalarial drugs, and their efficacy had been confirmed[4,18]. However, there is no information on their comparative strength and toxicity on the host cells. The pattern of efficacy of these drugs on parasite density which showed total parasite clearance by Day 2 (Figure 1) and their rapid onset of actions rank the antimalarial activity of ART next to CQ which was used as positive control in this experiment. The antimalarial activities elicited by ART could be attributed to the combined action of dihydroartemisinin, piperaquine and the antibiotic effects of trimethoprim contained in it. A comparison of the parasite clearance rate of P-ALA (dihydroartemisinin and piperaquine) with that of ART (dihydroartemisinin, piperaquine and trimethoprim) clearly suggests that the antibiotic activity of trimethoprim contributed immensely to the high degree of total parasitemia clearance observed by day 2 unlike in P-ALA-treated parasitized mice. The water-soluble dihydroartemisinin is known to be rapidly absorbed from the gastrointestinal tract and it is the active metabolite of all artemisinin compounds [19]. Thus, the time lag required for the oxidative demethylation of Arthemether to dihydroartemisinin[20] as well as the antibiotic activity of trimethoprim might be accountable for difference of 24 hours between the total parasitemia clearance by ART and LON. A slight drop in parasite density of parasitized-non-treated group observed on day 6 post inoculation could be an indication that parasitemia may induce increased lymphocytes circulation and their phagocytic activities may cause reductions in parasite density[21]. This is also in line with reports by Ganiyu *et. al.*[22]which showed progressional increase in parasitaemia from day 3 to 8 with a sharp fall and subsequent peak parasitaemia obtained on 11th day.

The measurement of the activities of various enzymes in tissues and body fluids play a significant role in the investigation of diseases and tissues cellular damage[23]. It is also a means of assessing drug or herbal safety as well as toxicity risk. Alkaline Phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum [24] which is often employed to assess the integrity of the plasma membrane [25]. The administration of the ACTs to *P. berghei*-infected mice resulted in increased ALP activity in the plasma in the order CQ>LON> P-ALA>ART. These increases indicate a form of injury to hepatocytes membranes by the antimalarial drugs and may hinder permeability and transportation of ions and molecules across the hepatocyte membranes [26].

Alanine (ALT) and Aspartate aminotransferases (AST) are useful marker enzymes in assessing damage to the liver [27]. Their presence in the plasma gives information on tissue injury and organ dysfunction [27, 28]. The observed increase in AST activity is in the order ART>LON>CQ> P-ALA(Table 1). The elevation in plasma AST following drug administration may be due to leakage of AST from the hepatocytes into the blood or increase in functional capacity of the liver. Increased plasma ALT activity in drug treated mice especially LON, P-ALA and CQ could be a pointer to toxicity of these drugs to hepatic cells while increasing their membrane permeability. This is in correlation with findings of [29]that elevated ALT and AST activities in liver parenchyma are associated with drug-induced CYP450 in rat, dog and human. However, ALT activity decreased significantly in ART treated group compared to other treated groups possibly because of inclusion of trimethoprim (antifolate) in ART which might be interfering with ALT synthesis. The significant increase in plasma total protein of parasitized non-treated group compared to other groups might be connected with the proteins contributed by the malarial parasites present in the mice (Table 1). In this regard, infections have been previously reported to result in hyperproteinaemia and hyperglobulinaemia in mice and rabbits respectively [30-31].

Creatinine and urea have been implicated in kidney diseases such as acute glomerulo-nephritis, nephrosclerosis and tubular necrosis[32]. Marked increases in the levels of plasma total protein and urea of parasitized mice were obtained (Table 1). Thus, implying that there is probably an increase in protein synthesis and degradation to serve as alternative source of fuel or the combined effects of the contributed proteins by the parasite hence causing increased urea formation as was noticed in the parasitized group. The total protein tended towards normal in all the treatment groups and the level of urea, creatinine and direct bilirubin were reversed to almost normal levels after treatment as compared to parasitized mice except in ART-treated group in which creatinine and urea levels were reduced. These are contrary to previous reports that artesunate, P-ALA and DHA caused significant increases in the serum creatinine, urea and total protein levels when administered to healthy rats. Findings from this study suggest that the reversal of these markers of kidney function to normalcy is probably due to the total parasite clearance in the mice by these drugs. However, ART might be interfering with urea synthesis and causing partial loss in functional tubular excretion[33]. The photomicrographs of kidney sections obtained from parasitized and antimalarial treated mice also showed focal cortical inflammatory cells and interstitial nephritis while the renal architecture was retained in all the antimalarial-treated mice (Plate 1).

The erythrocytes play a vital role in the merozoites invasion during malarial infection and the parasite's life cycle. This erythrocytic stage of the parasite destroys the membrane integrity of the host RBC and leaves it for phagocytic action by the sensitized macrophages. The parasite digests the host haemoglobin as its source of amino acid building blocks while in the host RBC. Several reports on the haematological profile in healthy rat models administered DHA or/and Artesunate or CQ for different periods have indicated either increase or decrease in some haematological indices including RBC count, haemoglobin concentration and PCV [33- 34]. Contrary to these previous reports, findings from the present study indicated marked reductions in PCV, HB, RBC and WBC counts in *P. berghei* infected mice whereas significant ($p<0.05$) increases in these haematological indices occurred up to near basal to different extents after 3 days of treatment with the different antimalarials (Table 2). Similarly, the photomicrographs of the liver sections confirmed the presence of a lot of neutrophilic and lymphocytic aggregates within the parenchyma and around the portal tracts of the non-treated and antimalarial-treated parasitized mice (Plate 2). In particular, ART increased the levels of WBC by 15 % over the control which is indicative of leukocytosis[35]. Conversely, P- ALA and LON caused non-significant reductions in lymphocyte count as compared to ART. During malaria, WBC counts are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis. Leukocytosis is typically reported in a fraction of cases and may be associated with concurrent infections and/or poor prognosis. Studies have shown that the rise or fall of WBC count is dependent on the

organism, if there has been prior exposure or not, and on the type of species of malaria parasite used for infection [36].

CONCLUSION

Artecxin has similar efficacy and antimalarial activity as chloroquine in *P. berghei* infected mice. However, it is very toxic to liver and kidney cells' architecture. Hence, Artecxin should be recommended for treatment in severe cases of malaria infection when life saving option far outweighs the adverse effects of this drug on patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

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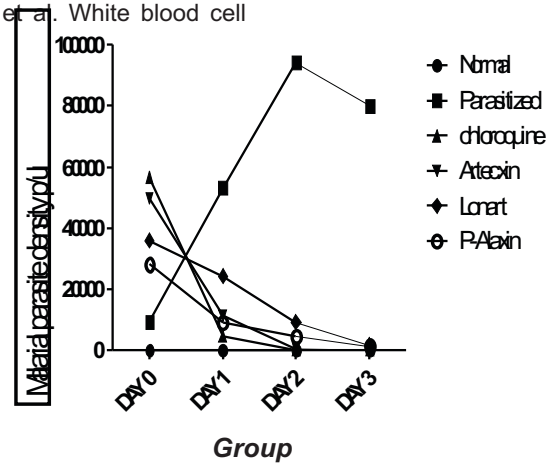

Figure 1: Rate of Parasite Clearance After Treatment of *P. Berghei*-infected Mice With Antimalarial Drugs

Table 1.Biochemical parameters in the plasma of *P. berghei*-infected mice treated with therapeutic doses of CQ, ART, LON and P-ALA.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)	D.B (µmol/L)	CREAT (µmol/L)	UREA (mmol/L)
Normal	41.9±5.1^{a}	21.4±1.3^{a}	75.9± 4.1^{a}	57.4± 0.6^{a}	0.2± 0.01^{a}	0.9±0.01^{a}	24.5±0.30^{a}
PnT	90 ±0.2^{b}	20.1±0.7^{a}	98± 4.1^{b}	76.8± 0.1^{b}	0.3± 0.03^{a}	0.87±0.05^{a}	29±1.05^{a}
PnT+CQ	85 ± 4.8^{b}	28.4± 1^{b}	100± 2.4^{c}	51.2± 1.2^{c}	0.2± 0.02^{a,}	0.70±0.06^{b}	19.9±1^{b}
PnT+ART	96.2 ±1.8^{b}	23.3± 0.4^{a}	83.7± 2.4^{a}	59.7± 0.8^{a}	0.2± 0.01^{a}	0.62±0.02^{c}	20.3±2.1^{b}
PnT+LON	93.4 ±0.6^{b}	48.4± 0.4^{c}	96.6± 1.6^{b}	58.9± 0.3^{a}	0.1± 0.01^{c}	0.68±0.04^{b}	19.6±2.2^{b}
PnT+ALA	64.3 ±4.2^{c}	29.6± 2.2^{b}	95.2± 4.1^{b}	64.4± 0.1^{d}	0.2± 0.01^{a}	0.95±0.05^{a}	31.6±2.6^{c}

Data are presented as Mean ± SEM. Values with different superscript a, b, c , d ----- in each row are significantly different ($p<0.05$). AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, ALP – Alkaline phosphatase, TP – Total protein, D.B- Direct bilirubin, Creat- Creatinine. PnT- Parasitized, CQ- Chloroquine (10.33mg/kg body weight), ART- Artecxin (14.73mg/kg body weight), LON- Lonart (9.33mg/kg body weight), and P-ALA - P-Alaxin (18mg /kg body weight).

Table 2: Haematological parameters in the blood of *P. berghei* –infected mice treated with therapeutic doses of certain antimalarials

Parameter	Control	PnT	PnT + CQ	PnT + ARTX	PnT + LON	PnT + P-ALA
Hb$^{\alpha}$	13.3 ± 0.20^{a}	6.7 ± 0.30^{b}	11.7 ± 0.51^{a}	9.6 ± 0.22^{c}	8.9 ± 0.47^{c}	9.1 ± 0.46^{c}
PCV$^{\pi}$	40.3 ± 0.63^{a}	19.8 ± 0.97^{b}	35.7 ± 1.23^{a}	29.0 ± 0.68^{c}	26.2 ± 1.32^{c}	27.5 ± 1.32^{c}
WBC$^{\mu}$	9737.5± 375^{a}	8010 ±131.2^{a}	8058.3 ± 428^{a}	11200 ± 400^{b}	7240 ± 48^{a}	7762.5 ± 677^{a}
RBC$^{\Omega}$	5.5 ± 0.12^{a}	2.7 ± 0.15^{b}	5.0 ± 0.08^{a}	4.0 ± 0.26^{c}	4.1 ± 0.23^{c}	4.2 ± 0.16^{c}
MCV$^{\Delta}$	73.1 ± 1.06^{a}	57.4 ± 1.59^{b}	71 ± 1.76^{a}	73.5 ± 3.87^{a}	64.1± 2.65^{a,b}	65.5 ± 2.08^{a,b}
MCH$^{\delta}$	24.2 ± 0.26^{a}	19.1 ± 0.57^{b}	23.3 ± 0.79^{a}	24.2 ± 1.27^{a}	21.8 ± 1.14^{a,b}	21.7 ± 0.65^{a,b}
MCHC$^{\alpha}$	33.1 ± 0.13^{a}	33 ± 0.19^{a}	32.7 ± 0.34^{a}	32.6 ± 0.32^{a}	34 ± 0.65^{a}	33.1 ± 0.13^{a}
NEUT$^{\pi}$	71.5 ± 2.10^{a}	43.6 ± 4.15^{b}	78.2 ± 1.19^{a}	72.5 ± 1.15^{a}	79.2 ± 1.28^{a}	79.8 ± 0.63^{a}
LYMPH$^{\pi}$	26.8 ± 2.17^{a}	55.8 ± 3.9^{b}	21.2 ± 1.30^{a}	26.3 ± 1.23^{a}	19.6 ± 1.08^{a}	19.5 ± 0.29^{a}

Values are mean (n=5) ± SEM. Values with different superscripts a,b,c.... in each row are significantly different at $p < 0.05$. α= g/dl, π = %, $\mu = 1\times10^{6}$/L, $\Omega = 1\times10^{12}$cells/L, Δ = fL/cell, $^{\delta}$ = pg/cell.
PnT- Parasitized, CQ- Chloroquine (10.33mg/kg body weight), ART- Artecxin (14.73mg/kg body weight), LON- Lonart (9.33mg/kg body weight), P-ALA - P-Alaxin (18mg/kg body weight).HB- haemoglobin, PCV- packed cell volume, WBC- white blood cell count, RBC- red blood cell count, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin concentrations, NEUT- neutrophils counts, LYMPH- Lymphocyte counts.

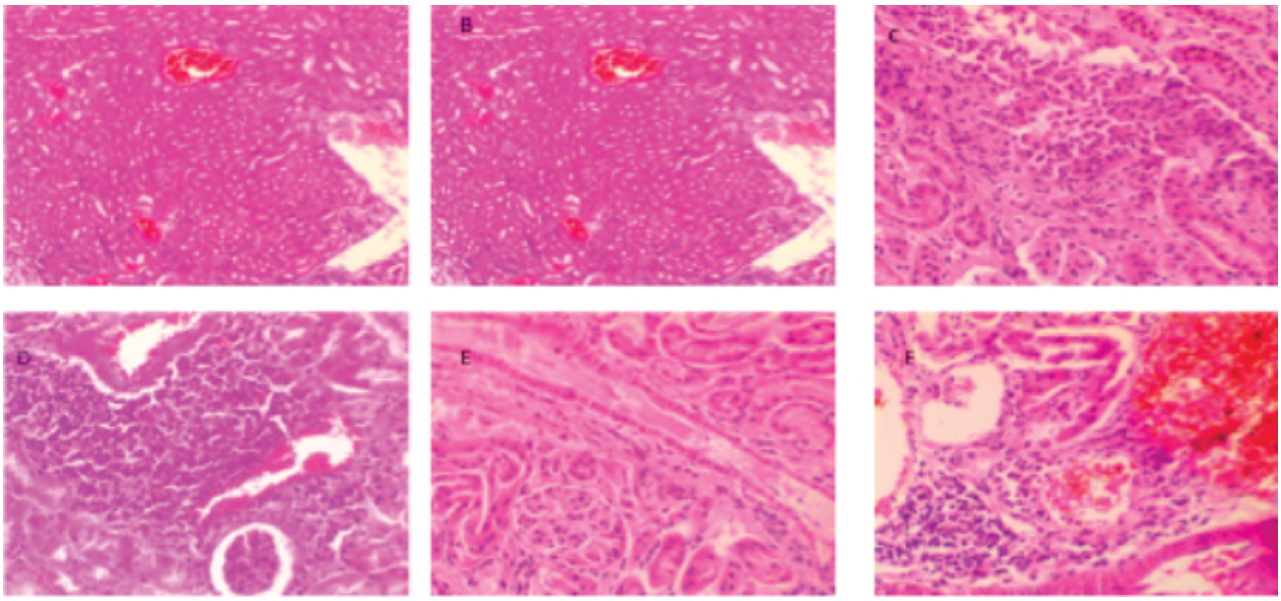

Plate 1: Photomicrographs of representative kidney sections of A – Control (Normal Saline); B - *P. berghei* infected mice (untreated); C - *P. berghei* infected mice + CQ; D - *P. berghei* infected mice + ART; E - *P. berghei* infected mice + LON; F - *P. berghei* infected mice + P-ALA. Haematoxylin and Eosin stained. Magnification × 40

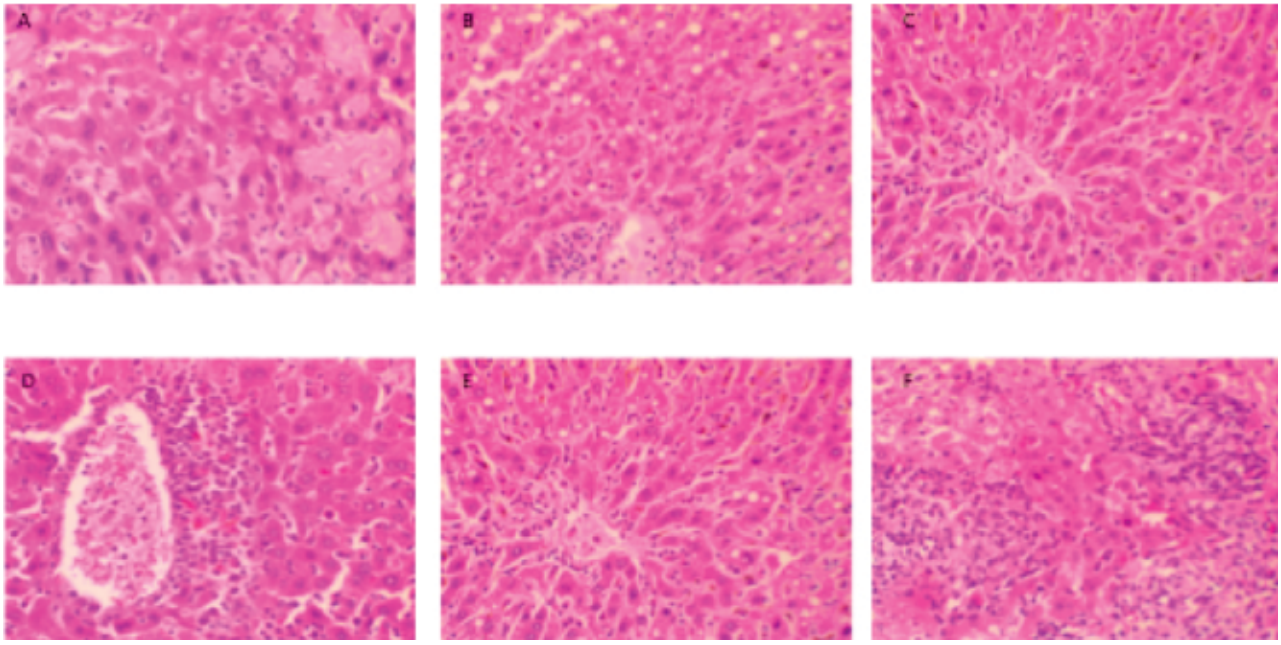

Plate 2:Photomicrographs of representative liver sections of A – Control (Normal Saline); B - *P. berghei*- infected mice (untreated); C - *P. berghei* infected mice + CQ; D - *P. berghei*-infected mice + ART; E - *P. berghei*-infected mice + LON; F - *P. berghei*-infected mice + P-ALA. Haematoxylin and Eosin stained. Magnification x40.