Original Article

Investigating the Comparative Effects of Six Artemisinin-based Combination Therapies on *Plasmodium*-induced Hepatorenal Toxicity

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Abstract

Background: Too many artemisinin-based combination therapies (ACTs) are available, thus creating a dilemma on the most preferred for the treatment of malaria. **Aim:** We compared the effect of six ACTs in mitigating *Plasmodium*-induced hepatorenal toxicity in experimental malaria. **Materials and Methods:** Forty adult male Swiss mice allotted into eight groups: Group 1 (normal control [NC] uninfected and untreated), Group 2 (parasitized nontreated – [PNT]), and Groups 3–8 received *Plasmodium berghei* inoculum. After 72 h, the initial parasitemia was established. Groups 3–8 were administered oral therapeutic doses of artesunate-amodiaquine (AA), artesunate-mefloquine (AM), artesunate-sulfadoxine-pyrimethamine (ASP), artemisinin-piperaquine (AP), dihydroartemisinin-piperaquine (DP), and artemether-lumefantrine (AL) per kg bodyweight, respectively, as standard regimen, and final parasitemia determined. Animals were euthanized via chloroform inhalation and blood collected for hepatorenal analysis. Liver and kidney were dissected out for histology. **Results:** Parasitemia was significantly ($P < 0.05$) decreased in tests compared to PNT, except in ASP group. Liver enzymes were significantly ($P < 0.05$) increased in PNT compared to tests and NC. Hyperplastic cells and portal tract inflammation were prominent in ASP group, but mild to moderate in other treated groups. Urea-creatinine were significantly ($P < 0.05$) increased in PNT compared to treated groups. The Na^+ and Cl^- were significantly ($P < 0.05$) reduced in PNT, with significantly ($P < 0.05$) increased K^+ compared to NC and treated groups. Glomerulonephritis and glomerulus splitting was observed in PNT, while moderate distortions were observed in treated groups. The AA and AM groups had good kidney histoarchitecture. **Conclusion:** Parasitemia decreased in all the treatment groups except in PNT and ASP groups which had severe hepatorenal distortions. Hepatorenal histoarchitecture were mildly distorted in the AA, AM and AL-administered groups with lower hepatorenal indices comparable to NC. The least elevated liver enzymes were in AA and AM. In decreasing order ASP > DP > AL > AP > AM > AA.

Keywords: Artemisinin-based combination therapies, kidney, liver, malaria, mice

Introduction

Malaria remains a world health problem with millions of cases each year, especially in African countries, and malaria-associated deaths reached 445,000 with nearly half the world's population at risk of the infection.[1] In Nigeria, malaria is responsible for 25% infant and 30% childhood mortality, and it is the country with the highest number of malaria cases,[2] with 60% of outpatients and 30% hospitalization attributed to malaria.[3] Infants under 5 years, pregnant women, patients with HIV/AIDS, nonimmune migrants, and travelers have high level of susceptibility to malaria.[4]

Malaria is classified as complicated or uncomplicated,[5] the uncomplicated malaria is the mild form of the disease and presents as a febrile illness with headache, tiredness, muscle pains, abdominal pains, rigors, nausea, and vomiting. If left untreated, malaria can rapidly develop into severe form (complicated) with anemia, hypoglycemia, renal failure, pulmonary edema, convulsions, coma, and liver dysfunction, and eventually death.[6] The main objective of malaria treatment

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is to prevent the patient from dying, prevent disabilities, and recrudescence.

In rodents, *Plasmodium berghei* and *Plasmodium vinckei* cause malaria infection and can be used as comparable genetic models to humans.[7] Rodent *Plasmodium* parasites vary in virulence depending on the parasite species, the strain of mice or species of rodent,[8] species causing virulence in rodents; *P. berghei, Plasmodium yoeli, P. vinckei,* and *Plasmodium chabuadi*.[9] The vector of these parasites in the wild is the *Anopheles stephensi* mosquito.[10] *P. berghei* is used as a model organism for the investigation of malaria because of its similarity to the species which cause human malaria. In addition, *P. berghei* has a similar life cycle as the species that infect humans and cause a disease in mice which has signs and symptoms similar to those of human malaria.[11]

The liver is an important organ in the hepatic stage of the *Plasmodium* life cycle, and malaria hepatopathy may manifest as jaundice, hepatomegaly, elevated liver enzymes, portal tract inflammation, hyperplastic Kupffer cells, cholestasis, cell necrosis, and deposition of hemozoin pigment.[12,13]

Acute renal failure (ARF) commonly occurs as a complication of *Plasmodium falciparum* infection and occasionally in *Plasmodium vivax* infection[14] and is associated with the risk of chronic kidney disease and high mortality.[15] Histological features of malaria-associated acute kidney injury include acute tubular necrosis with interstitial nephritis and glomerulonephritis.[16] In patients suffering from repeated episodes of malaria infection, chronic kidney disease has been associated with the infection.[17]

There are currently five artemisinin-based combination therapies (ACTs) recommended by the World Health Organization (WHO), namely, artesunate-amodiaquine (AA), artesunate-mefloquine (AM), artesunate-sulfadoxine-pyrimethamine (ASP), arteme ther-lumefantrine(AL),and dihydroartemisinin-piperaquine (DP).[1,18] However, artemisinin-piperaquine (AP) is currently not approved but commercially available in Nigeria. The toxicity of ACTs has been evaluated in humans[19] and in animal experiments.[20] Artemisinin-related hepatotoxicity and liver malfunction have previously been documented.[21] ACT-associated renal toxicity includes; reduced glomerular filtration rate and the accumulation of products of metabolism, with glomerular distortion.[22] There is a need to investigate the available ACT brands to determine how they fare in mitigating the negative effects of *P. berghei* in an experimental murine malarial model.

Materials and Methods

Experimental animals

Forty male Swiss albino mice weighing between 20 and 30 g were obtained from the Animal House of the Faculty of Pharmacy, University of Uyo, Uyo. The animals were acclimatized for 2 weeks prior to the start of the experiment in the Animal House of the Faculty of Basic Medical Sciences, College of Health Sciences, Annex Campus, University of Uyo, Uyo. There were well-ventilated and clean cages and maintained under optimum environmental conditions of temperature 25°C ± 5°C and 12 h' light/dark cycle. All animals were allowed access to food (rat mash; Vital Feeds® from Grand Cereals Limited, Jos, Plateau State, Nigeria) and drinking water *ad libitum*. This experiment was carried out in accordance to the guidelines for the care and use of laboratory animals.[23] Animals were placed in eight groups with each group having five animals, based on their bodyweights. The groupings were as follows: group 1 normal control (NC) was uninfected and untreated but received distilled water 5 ml/kg as placebo, Group 2 (parasitized nontreated – [PNT] with 1×10^6 *P. berghei* and distilled water 5 ml/kg as placebo), while test groups 3–8 received inoculum 1×10^6 *P. berghei* intraperitoneally, then 3 days later orally received therapeutic doses of AA (Camosunate® 5.71 mg), AM (Artequin® 6.43 mg), ASP (Simbcure® 25.36 mg then 2.86 mg, day 1 and 2, respectively), AP (Artequick® 12.5 mg), DP (P-alaxin® 5.14 mg) and AL (Coartem® 8 mg) per kg bodyweight, respectively. The administrations were for 3 days except in Group 5 which was for 2 days.

Experimental design

Acquisition of donor mice

The donor mice were acquired from the Animal House of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo. Donor mice were infected with the *P. berghei ANKA* chloroquine-resistant strain, which obtained from the National Institute of Medical Research, Yaba, Lagos State, Nigeria.

Drug acquisition and preparation

Six brands of ACTs were obtained from manufacturers namely; AA (Camosunate®, Front Pharmaceutical Plc., Anhui, China), AM (Artequin™, Acino Pharma AG, Liesberg, Switzerland), ASP (Simbcure®, Ruinian Qianjin Pharmaceutical Co. Ltd., Jiangsu Province, China), AP (Artequick® Artepharm Co. Ltd., China), DP (P-alaxin™, Bliss GVS Pharma Ltd., Maharashtra, India), and AL (Coartem®, Novartis Saglik, Basle, Switzerland). Therapeutic doses of ACTs were administered orally to the animals using a calibrated cannula. Each tablet was dissolved in distilled water and administered based on the animals' weight, using the formula = weight of rat/1000 × dose/stock.

Parasite inoculation and estimation

Forty Swiss male mice (5 - 6 weeks) were used for the study. The normal control had 5 mice, which were uninfected and untreated receiving placebo. The test groups (2-7) consisted of 35 mice, 5 per group which were infected with *P. berghei*-ANKA 10^6 in 200 µl of sterile phosphate-buffered saline i.p.[24] This was prepared by determining both the percentage parasitemia and the erythrocyte count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations. Stained thin blood smears were obtained from the tail vein of the mice onto clean frosted slides, Giemsa-stained and viewed under oil immersion at × 100, and parasitemia determined by the direct enumeration method.[25]

Determination of liver and kidney functions

Serum was obtained by centrifuging the whole blood at 1200 g for 15 min at room temperature. The kinetic

determination of serum aspartate transaminase (AST), alanine transaminase (ALT), ASP, urea, creatinine, potassium, sodium, and chloride concentrations was performed in accordance with the method earlier described in literature.[26]

Histopathological assessment

After inducing the unconscious state by chloroform inhalation, the liver and kidney were dissected out, weighed, and fixed in 10% buffered formalin. The paraffin wax blocked tissues were sectioned at 5 μ using rotary microtome (Microtome Thermo Scientific – Microm HM 325, England) and were stained with hematoxylin and eosin,[27] images were obtained with Amscope digital camera (MU 1000, China) attached to a light microscope (Olympus-CX31, Japan).

Statistical analysis

Data were analyzed using one-way analysis of variance and a *post hoc* test with Student's Newman. Values were considered statistically significant at $P < 0.05$.

Results

Effect of artemisinin-based combination therapies on organosomatic indices in murine experimental malaria

Organ weight was reduced in the PC group compared with other experimental groups. Change between the initial and final bodyweights showed a decrease in PC and treated groups, compared to NC as presented in Table 1.

Effect of artemisinin-based combination therapies on parasite density in murine experimental malaria

After a 3-day postinfection assessment of parasitemia, all treated groups except ASP demonstrated high parasite clearance compared to NC group, as presented in Table 2.

Effect of artemisinin-based combination therapies on liver function in murine experimental malaria

Liver enzymes ALT and AST increased significantly in the PC group compared to NC and decreased in ACT-treated groups compared to PC; however, alkaline phosphatase (ALP) was significantly increased in ACT-treated groups compared to PC, as presented in Table 3.

Effect of six artemisinin-based combination therapies on renal function in murine experimental malaria

An increase in urea, creatinine, and potassium concentrations was observed in the PC group compared with other experimental groups, while there was a marked decrease in sodium and chloride concentrations in the PC compared to NC and treated groups [Table 4].

Discussion

The bodyweight decrease of parasitized groups, compared to NC, may be attributed to the adverse effect of the parasite on the overall bodyweight of the animals.[28,29] Kidney weights indicated that PNT had the lowest weight of 0.17 g, while NC had highest organ weight (0.21 g). An organ weight loss has been associated with tissue shrinkage, together with cellular degeneration and atrophy,[30] and these tissue distortions (mild to severe) were demonstrated in this study. Decrease in body and organ weights are likely due to loss of appetite, inadequate glycogen synthesis, glycogen storage, metabolic malfunction,[31] as well as parasite-mediated hemolysis, toxins that alter metabolism causing liver damage and anoxia leading to muscle wasting.[32]

Parasite clearance was in the following sequence; AM > DP > AP > AA > AL. The ASP had no positive effect. The effectiveness of any antimalaria medication is measured by rate of parasite clearance, estimated by the level of parasitemia.[33] Hyperparasitemia was established in this study before treatment, and PNT had the highest percentage parasitemia increase, likely due to the continuous parasite multiplication,[28] while AA, AM, AP, DP, and AL had a percentage decrease, except ASP, thus confirming that ACTs are efficient in parasite clearance.[34,35] This result corroborates earlier human studies that ASP is not efficient in parasite clearance,[36] possibly due to the half-life of artesunate which ranges from 0.15 to 0.21 h (equivalent of

Table 1: Effect of artemisinin-based combination therapies on the body and organ weights (g) in mice experimental malaria

Group	Initial bodyweight	Final bodyweight	Percentage change in bodyweight	Kidney weight	Kidney organosomatic index	Liver weight	Liver organosomatic index
NC	26.8	27.4	+2.19	0.21±0.01	0.76	1.90±0.27	6.93
PNT	21.6	20.4	−5.88	0.17±0.01	0.83	1.49±0.36	7.30
PbAA	24.0	22.8	−5.00	0.19±0.01	0.83	1.72±0.16	7.54
PbAM	25.0	22.2	−12.73	0.19±0.01	0.85	1.39±0.02	6.26
PbASP	27.6	26.4	−4.55	0.20±0.01	0.75	1.40±0.06	5.30
PbAP	32.4	30.2	−7.28	0.23±0.01	0.76	2.02±0.07	6.69
PbDP	28.0	26.2	−6.87	0.21±0.01	0.80	1.78±0.17	6.79
PbAL	26.0	24.0	−8.33	0.21±0.02	0.87	1.66±0.41	6.92
P value	-	-	-	0.051	-	0.059	-

Data from the study were expressed as mean±SEM. NC – Normal control; PNT – Parasitized nontreated; PbAA – Parasitized treated with artesunate-amodiaquine; PbAM – Parasitized treated with artesunate-mefloquine; PbASP – Parasitized treated with artesunate-sulfadoxine-pyrimethamine; PbAP – Parasitized treated with artemisinin-piperaquine; PbDP – Parasitized treated with dihydroartemisinin-piperaquine; PbAL – Parasitized treated with artemether-lumefantrine; SEM – Standard error of mean

Table 2: Effect of artemisinin-based combination therapies on the parasitemia in murine experimental malaria

Group	Initial parasitemia	Final parasitemia	Percentage change in parasitemia
NC	0.00±0.00	0.00±0.00	0.00
PNT	72.50±3.19	78.44±4.94	+7.57
PbAA	60.02±5.59	9.92±1.06***	−83.47
PbAM	74.22±2.02	6.48±0.50***	−91.27
PbASP	67.78±5.33	70.30±0.01	+3.58
PbAP	73.86±3.21	11.80±0.73***	−84.03
PbDP	64.64±2.70	7.16±0.60***	−88.92
PbAL	76.04±4.00	15.88±1.20***	−79.12
P value	-	0.000	-

Data from the study were expressed as mean±SEM. ***SS compared to PNT and PbASP groups. NC – Normal control; PNT – Parasitized nontreated; PbAA – Parasitized treated with artesunate-amodiaquine; PbAM – Parasitized treated with artesunate-mefloquine; PbASP – Parasitized treated with artesunate-sulfadoxine-pyrimethamine; PbAP – Parasitized treated with artemisinin-piperaquine; PbDP – Parasitized treated with dihydroartemisinin-piperaquine; PbAL – Parasitized treated with artemether-lumefantrine; SEM – Standard error of mean; SS – Statistically significant

Table 3: Effect of artemisinin-based combination therapies on liver enzymes (U/L) activities of mice experimental malaria

Group	AST	ALT	ALP
NC	130.19±0.05	60.89±0.06	60.58±0.22
PNT	141.83±0.26***	72.90±0.03***,d	63.08±0.04m
PbAA	133.12±0.24c	62.71±0.14i	65.08±0.67n
PbAM	133.43±0.32c	65.77±0.15e	64.60±0.09
PbASP	135.65±0.23a	65.36±0.17g	66.41±0.27k
PbAP	133.75±0.09c	66.30±0.20d	66.60±0.47k
PbDP	136.25±0.09a	63.41±0.24h	67.88±0.13j
PbAL	134.79±0.24b	65.02±0.22f	65.33±0.13l
P value	0.000	0.000	0.000

Data from the study were expressed as mean±SEM. ***SS compared to all test groups and NC; aSS compared to NC, PbAA, PbAM, PbAP, and PbAL; bSS compared to NC, PbAA, PbAM, and PbAP; cSS compared to NC; dSS compared to all other test groups and NC; eSS compared to all other test groups and NC except PNT and PbAP; fSS compared to NC, PbAA, PbASP, and PbDP; gSS compared to NC, PbAA, and PbDP; hSS compared to NC and PbAA; iSS compared to NC; jCompared to all other test groups and NC; kSS compared to NC, PNT, PbAA, PbAM, PbAL; lSS compared to NC and PNT; mSS compared to NC and PbAM; nSS compared to NC and PNT. NC – Normal control; PNT – Parasitized nontreated; PbAA – Parasitized treated with artesunate-amodiaquine; PbAM – Parasitized treated with artesunate-mefloquine; PbASP – Parasitized treated with artesunate-sulfadoxine-pyrimethamine; PbAP – Parasitized treated with artemisinin-piperaquine; PbDP – Parasitized treated with dihydroartemisinin-piperaquine; PbAL – Parasitized treated with artemether-lumefantrine; SEM – Standard error of mean; SS – Statistically significant

9–13 min),[37] and this must have contributed to its effect on the parasitemia; however, the effect was not sustained by the partner drug. The effectiveness of ASP to induce parasite clearance depends on the level of parasite resistance to sulfadoxine and pyrimethamine, thus limiting its usage.[38]

Hepatotoxicity may arise from parasite and ACTs independently and/or synergistically.[18] Hepatic damage/inflammation may be reactive oxygen species (ROS) mediated, by erythrocytes via immunological response to invasion by the schizonts, as shortly after digestion of red blood cells (RBCs) by the parasites, the ROS is regurgitated back to the liver cytosol where they induce cytosolic and membranous damages.[39,40] In extreme cases, the high parasite density overwhelms the liver regenerative potential.[41]

The drug-induced hepatic damage may arise during ACTs biotransformation which produces ROS, such as peroxides and hydroxyl during the endoperoxide bridge cleavage, thus inducing oxidative stress on the membrane of hepatocytes, causing cytolysis and leakage of liver enzymes.[42] The PNT had significantly ($P < 0.05$) increased serum AST and ALT compared to the treated groups and is indicative of greater damage induced by the parasite than the ACTs.[43] ACTs can ameliorate hepatic damage by mitigating parasitemia via endoperoxide bridge cleavage by heme iron species that results in the generation of ROS against the parasites.[44]

The enzymes AST and ALP are predominantly found in the liver, cardiac muscles, skeletal muscles, kidney, and testes,[45] and their high levels could be indicative of extrahepatic damage and to a lesser degree a sign of hepatotoxicity.[46] Using ALT as the principal biomarker of hepatic damage, the order of the ACTs mitigating the effect on hepatocellular damage was AA > DP > AL > ASP > AM > AP. The least efficacy profile was demonstrated by AP compared to other ACTs due to the cumulative elevated liver enzyme levels, and it should be noted that AP is not one of the WHO-approved ACTs possibly due to the artemisinin content which has poor water and lipid solubility, hence low bioavailability, whereas piperaquine has high lipophilicity and is implicated in membrane damage.[47]

Although AA had the least effect on ALP levels, and it contains two water-soluble component drugs; AA,[47] it rarely influences fatty acid metabolism. The increase in ALP levels in PNT was not significant compared to NC but was significantly less than levels in treated groups. Liver ALP levels are normally supposed to increase simultaneously with AST and ALT, due to its role in their catalytic activity. Hence, any condition that induces an increase in liver AST and ALT should definitely induce a corresponding increase in liver ALP and vice versa.[48] Increased serum ALP is an indication of cholestasis which arises from hepatobiliary damage and it is characterized by the regurgitation of bile into the bile canalicular. The link of increased ALP levels in the treated groups with cholestasis compared with Al-Salahy *et al*. and Wulkan and Leijnse.[13,49] The marginal rise in the PNT ALP level indicates that the ACTs have greater effect on cholestasis damage than that induced by the *Plasmodium* parasite. This finding, while agreeing with views that increase in liver enzymes, is directly proportional to increase in malaria parasite density, indicates that ALP does not fit into this general assumption.[50,51] ALP in mice liver is localized mainly in the canalicular membrane of

Table 4: Effect of artemisinin-based combination therapies on the renal function in murine experimental malaria

Group	Urea (mg/dl)	Creatinine (mg/dl)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
NC	11.60±0.08	0.62±0.01	138.52±0.22	4.13±0.12	99.90±0.38
PNT	18.33±0.06***	0.92±0.01***	116.32±0.79[g,h]	5.29±0.03***	90.70±0.14[l,m]
PbAA	12.50±0.06	0.65±0.01[e]	129.36±0.63	4.79±0.01[i]	95.50±0.26[l,m]
PbAM	13.68±0.10[b]	0.74±0.10[d]	125.37±0.71[g]	4.53±0.02[k]	96.91±0.32[l]
PbASP	13.98±0.03[a]	0.66±0.01[e]	130.59±0.57	4.66±0.01[k]	97.22±0.12[l,n]
PbAP	12.52±0.04	0.69±0.01[d]	131.43±0.94	4.59±0.01[k]	97.94±0.67[l,n]
PbDP	12.36±0.04	0.64±0.01	125.23±0.88[g]	4.71±0.01[j]	95.34±0.17[l,m]
PbAL	13.55±0.04[c]	0.66±0.01[e]	132.94±0.69[f]	4.64±0.02[k]	96.64±0.34l
P value	0.000	0.000	0.000	0.000	0.000

Data from the study were expressed as mean±SEM. ***SS compared to all test groups and NC; [a]SS compared to NC, PbAA, PbAM, PbAP, PbDP, and PbAL; [b]SS compared to NC, PbAA, PbAP, PbDP, and PbAL; [c]SS compared to NC, PbAA, PbAP, and PbDP; [d]SS compared to all groups except PNT; [e]SS compared to PbAA, PbASP, and PbAL; [f]SS compared to all groups except NC and PbAP; [g]SS compared to PbAA, PbASP, and PbAP; [h]SS compared to PbAM and PbDP; [i]SS compared to NC, PbAM, and PbAP; [j]SS compared to NC and PbAM; [k]SS compared to NC; [l]SS compared to NC; [m]SS compared to PbAM, PbASP, PbAP, and PbAL; [n]SS compared to PbAA and PbDP. NC – Normal control; PNT – Parasitized nontreated; PbAA – Parasitized treated with artesunate-amodiaquine; PbAM – Parasitized treated with artesunate-mefloquine; PbASP – Parasitized treated with artesunate-sulfadoxine-pyrimethamine; PbAP – Parasitized treated with artemisinin-piperaquine; PbDP – Parasitized treated with dihydroartemisinin-piperaquine; PbAL – Parasitized treated with artemether-lumefantrine; SEM – Standard error of mean; SS – Statistically significant

the hepatocytes, whereas in the human liver, it is found in the endothelial cells around the portal and central veins as well as in the sinusoids around the central veins and sometimes around the bile canaliculi.[49] Transaminases are mainly cytosolic enzymes with AST predominantly found in mitochondria and partially in the cytoplasm, whereas ALT is predominant in the cytoplasm.[52] Thus, ALP is a good marker for canaliculi and membrane damages.

The groups that demonstrated improved serum transaminase levels also demonstrated mild inflammation (AL, DP, AM, and AA), whereas the groups that demonstrated elevated serum transaminases also demonstrated massive inflammation (ASP and PNT). The liver histology in Figure 1, demonstrated that PC showed severe alterations (that is severely affected) with prominent inflammation as a result of the parasite lodging in the liver, necrosis caused by direct toxic damage to hepatocytes,[53] and distorted sinusoids. In the AA-, AM-, AP-, and DP-treated groups, prominent pathological features, such as the presence of congested sinusoids which may be as a result of accumulation of byproduct of the metabolism of hemoglobin by the parasite,[54] were observed, and necrosis was observed which can be due to the mechanism of action of artesunate, wherein lipid peroxides produced accumulate and become toxic to membrane structure leading to change in permeability, and organelle disintegration leading to necrosis,[55] and the presence of hemozoin may be as a result of lysis of blood cell.[28] The ASP treated showed massive lymphoid aggregate which can be related to the sulfonamide content that causes allergy.[56] In Table 2, the high percentage increase in parasitemia even after ASP treatment indicates inflammation due to drug resistance.[53]

The PNT and ASP groups demonstrated the absence of Kupffer cells (Kc) due to the hepatoimmune evasion mechanism expressed by the parasites via the interaction between their circumsporozoite protein and the Kc surface proteins which consequently downregulated the expression/function of Kc major histocompatibility complex-1.[57] On the other hand, other treated groups aside ASP demonstrated increasing the presence of Kc indicating that the parasite clearance mediated by these ACTs alleviated the inhibitory effect of the *Plasmodium* parasites on the Kc and thus boosted Kc-induced immunological response aiding in the destruction of the remaining parasites.

The rise in serum urea and creatinine indicates kidney malfunction which could either be due to underlying distortions, injury to the glomerulus or damage to functioning nephrons, thereby reducing glomerular filtration.[22] There was a significantly higher level of urea (18.33 mg/dl) and creatinine (0.92 mmol/dl) in PNT. In the treated groups, the lowest urea level (12.36 mg/dl) was in DP, while the highest level was (13.08 mg/dl) in the ASP-treated group. Collectively, this study shows a decreasing trend following ACT administration on the serum urea levels compared to NC which correlates with previous studies, but an increase in urea level following AM administration in a nonmalarial study has been reported.[58] There was no significant change in creatinine level in the treated groups compared to NC (0.62 mg/g), but AM had the widest variation in concentration (0.74 mg/g). These results deduce that ACTs do not appear to increase serum creatinine levels,[34] although there is a report of significant increase of creatinine level (67%) following DP administration in noninfected rats.[45]

Surge in urea levels in PNT and ASP groups arises from increased eryptosis (apoptosis of injured RBCs) which increases amino acid efflux in the pool leading to excess amino acids deamination to form ammonia which is converted to urea via the urea cycle with the final reaction involving the hydrolysis of arginine by arginase to produce urea,[59] and hence the decrease parasitemia correlates with the decrease urea in the other treated groups.

A dysfunction of renal tubules or poor glomerular filtration will ultimately lead to significantly lower electrolyte levels. Na^+ and

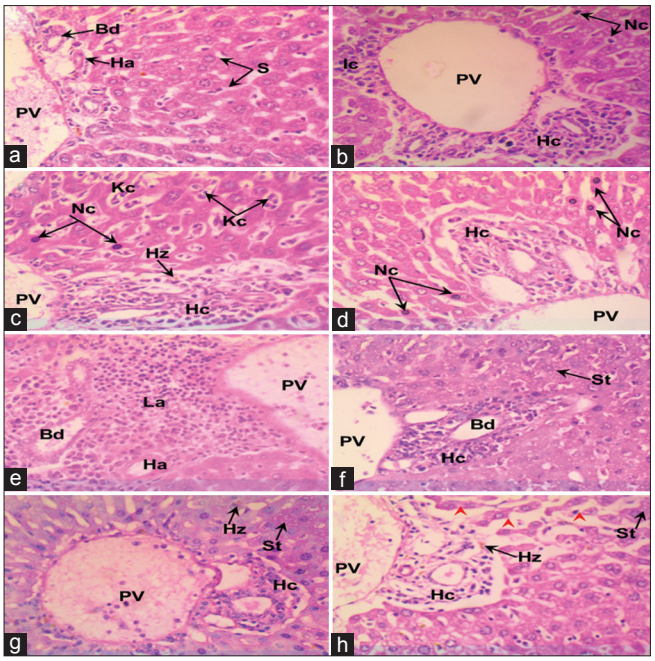

Figure 1: Photomicrograph of the liver section of normal control (a), parasitized nontreated (b) and artemisinin-based combination therapies-treated groups ([c-h] for parasitized treated with artesunate-amodiaquine, parasitized treated with artesunate-mefloquine, parasitized treated with artesunate-sulfadoxine-pyrimethamine, parasitized treated with artemisinin-piperaquine, parasitized treated with dihydroartemisinin-piperaquine, and parasitized treated with artemether-lumefantrine, respectively) showing PV – portal vein; Bd – Bile duct; Ha – Hepatic artery; Hz – Hemozoin; St – Steatosis; Ic – Inflammatory cells; S – Sinusoids; La – Lymphoid aggregate; Nc – Necrotic cell; Kc – Kupffer cell; Red arrowhead – expanded sinusoids

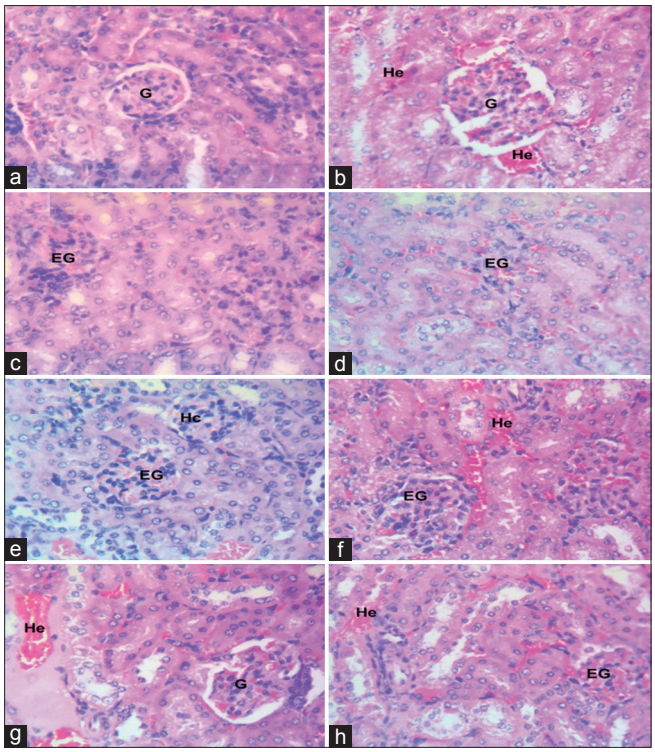

Figure 2: Photomicrograph of the kidney section of normal control (a), parasitized nontreated (b), and artemisinin-based combination therapies-treated groups ([c-h] for parasitized treated with artesunate-amodiaquine, parasitized treated with artesunate-mefloquine, parasitized treated with artesunate-sulfadoxine-pyrimethamine, parasitized treated with artemisinin-piperaquine, parasitized treated with dihydroartemisinin-piperaquine and parasitized treated with artemether-lumefantrine, respectively) showing G – Glomerulus; Hc – Hyperplastic cells; He – Hemorrhage; EG – Expanded glomerulus

Cl^- were significantly lower in PNT than in all other groups. This correlates with the findings from studies performed on mice.[60] However, serum electrolyte post-ACT treatment was within range when compared with NC group, but lower results were observed in Na^+ concentration of AP (125.37 mg/dl)- and DP (125.23 mg/dl)-treated groups. These results contrast studies performed on uninfected rats, which reported elevated serum electrolytes.[61]

Kidney histology shown in Figure 2 demonstrated increased urinary spaces, coupled with glomerular distortion, acute tubular necrosis, presence of hemorrhages, and glomerular basement membrane thickening in the PNT and treated groups (ASP, AP, DP, and AL), all of which had common features of the malaria parasite infection of the kidney,[28] leading to increased urea and creatinine concentrations and lowered levels of electrolytes which are indications of renal challenge and possible ARF.[22] These changes are due to increased parasite infection of erythrocytes which ultimately cause microcirculation blockade.[16] During repair, the damaged tissues are replaced mainly through the regeneration of cells.[53] Hypercellularity is due to intracapillary leukocytes and proliferation of intrinsic glomerular cells.[53]

Conclusion

This study is the first to demonstrate the activity of six commercially available ACTs on biochemical and histological alterations in hepatorenal profile of experimental malaria model, and it has established that AA, AM and AL induced the least elevation of liver enzymes and urea-creatinine levels (best safety profile); the most ameliorative liver histology was observed in AA, AM, and AL with the least in ASP- and AP-treated groups. For the kidney histology, AA and AM had the most ameliorative effect; likewise, ASP had the least positive effect compared to other groups which indicated milder distortions. Thus, comparatively AA, AM and AL, respectively, had the most mitigating effect on parasite-induced hepatorenal toxicity based on biochemical and histological findings.

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Conflicts of interest

There are no conflicts of interest.

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