Research Article

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ASSESSMENT OF THE SUB-ACUTE AND DELAYED TOXICITY OF ARTEMETHER-LUMEFANTRINE COMBINATION IN RATS

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ABSTRACT
Considering the current surge in the use of artemether-lumefantrine combination (AL), as a treatment regimen for malaria infection, this research elucidated its sub-acute and delayed toxicity profile. Adult albino wistar rats were randomly placed in 7 groups (n=8). Groups 1-3 received oral AL 14, 28 and 56 mg/kg and were used for the sub-acute toxicity study. Groups 4-6 equally received oral AL 14, 28 and 56 mg/kg and were used for the delayed toxicity study. Animals in group 7 served as control. Treatment was given for 7 days; animals for the sub-acute tests were sacrificed on day 8, while animals for the delayed toxicity test were sacrificed on day 15. Parameters evaluated include random blood sugar levels, alanine transaminase, aspartate transaminase, alkaline phosphatase, bilirubin, cholesterol, serum electrolytes and hematological indices. The liver, kidney and heart were also subjected to histopathological evaluation. The random blood sugar level was only significantly (P<0.05) elevated in the sub-acute phase but not in the delayed phase. The AL treated rats had a marginal but non-significant increments in Na^+, serum cholesterol, urea and liver enzymes in both the sub-acute and delayed phases. The AL had no effect on total and conjugated bilirubin, but reduced K^+, Cl^- and HCO_3^-. There was mild increase in hemoglobin, packed cell volume, reticulocyte, total white blood cell and lymphocyte, and a decrease in neutrophil counts. Histology sections showed dose-related increase in severity of hepatic congestion and inflammation. Renal sections showed no significant changes. However, about 25% of animals that received 14, 28 and 56 mg/kg of AL respectively had granular and eosinophilic hyaline casts in renal tubules. There were no remarkable histopathologic changes in the heart in both sub-acute and delayed phases. However, one animal that received 56 mg/kg in the sub-acute phase had organizing fibrinous pericarditis, with intense lymphocytic infiltration and tubular coagulative necrosis. Though oral administration of normal to quadruple strength of AL affected vital organs and clinical parameters, no significant deleterious toxic effect was observed.
Keywords: Artemether, lumefantrine, sub-acute toxicity, delayed toxicity,

INTRODUCTION

Artemether–lumefantrine (AL) was the first fixed dose artemisinin based combination therapy (ACT) to meet the WHO's pre-qualification criteria for efficacy, safety and quality.[1,2] Based on WHO's current recommendation for the use of ACT,[3,4] 20 countries, 7 of which are in Africa have included ACT as 1st and 2nd line chemotherapy for malaria. The AL is now used as first-line treatment of uncomplicated *Plasmodium falciparum* in many regions worldwide.[5]

The AL is a fixed dose preparation of artemether (20 mg) and lumefantrine (120 mg) in the ratio 1:6. Artemether has strong blood schizontocidal and moderate gametocytocidal activities and its activity like other artemisinin derivatives, depends on the intact peroxide bridge of the molecule.[6] However, monotherapy with artemisinin or it derivatives is associated with significant incidences of recrudescence, unless enhanced dose regimens are used (the safety of which are yet to be established). Lumefantrine (benflumetol) is an aryl amino alcohol which belongs to the same chemical class as quinine, mefloquine and halofantrine.[7-10] Lumefantrine is a class II blood schizontocide, which inhibits heme polymerization.[10,11] The combination of artemether, that rapidly reduces parasite biomass, with longer-acting lumefantrine, that eliminates residual parasites, has proven to be highly effective in achieving parasitologic cure, symptom relief, and reduction of gametocyte carriage.[12] Furthermore, comparison of concentration profiles after intake of artemether or lumefantrine alone or in combination demonstrated no sign of pharmacokinetic interaction between the two drugs.[13] The AL is approved for the treatment of uncomplicated malaria in adults, children and infants, and non-immune travelers returning from malaria endemic regions. It is reported as one of the most successful malaria treatment regimen, highly effective, and well tolerated, providing cure rates of up to 97%, even in areas of multi-drug resistance.[14,15]

Most common adverse effects of AL include gastrointestinal (abdominal pain, anorexia, nausea, vomiting and diarrhea) and central nervous system (headache, dizziness) effects. Others include vertigo, asthenia, myalgia and arthralgia, pruritus and rash. More than 90% of these reported adverse effects, many of which are typical of the clinical symptomatology of acute malaria, were rated mild to moderate in severity.[9,12,15-19] The 6-dose regimen of AL was better tolerated than, and as effective as, artesunate-mefloquine against multi-drug–resistant falciparum malaria.[8,20] Furthermore, AL was better tolerated than the comparator drugs used in some trials. Higher incidences of vomiting and pruritus were reported with chloroquine. Dizziness, nausea and vomiting were more common with mefloquine while dizziness, abdominal pain, nausea and vomiting were more frequent with quinine.[8]

Considering the acclaimed efficacy and widespread use of AL, the knowledge of its sub-acute and delayed toxicity patterns will be essential in comparative risk assessment

and management of malaria patients. Hence this study set out to evaluate the toxicity profile of normal and high doses of AL in rats.

MATERIALS AND METHODS

Adult albino wistar rats (100-145 g) of either sex bred in the Laboratory Animal Facility of the College of Medicine, University of Nigeria, Nsukka were used for the study. The animals were housed in metal cages and allowed to acclimatize for one week under standard conditions of temperature (25 ± 3°C) with a 12:12 h natural light:dark cycle before commencement of the experiment. The animals were maintained freely on standard pellets (Guinea Feed Nigeria, PLC) and water. All animal experiments were in compliance with International Guidelines for handling experimental animals.[21] Animal studies were conducted with prior permission obtained from the National Health Research Ethics Committee (NHREC) of the University of Nigeria Teaching Hospital, Enugu, with protocol ethical clearance number NHREC/05/01/2008B.

Drug

Artemether-lumefantrine (Coartem® Novartis Pharma AG, Basel, Switzerland).

Sub-acute toxicity test

Varying doses of AL calculated using the standard six dose regimen and the single dose per administration of 14 mg/kg (N) were administered. Adult rats of both sexes were randomly grouped (n=8) to orally receive 14 (N), 28 (2N) or 56 mg/kg (4N) of AL respectively, control group received distilled water (5 ml/kg). Treatment was given for 7 days. Animals were sacrificed on the 8th day after blood collection by cardiac puncture; also appropriate tissues were collected for histopathological studies.

Delayed toxicity test

Adult rats of both sexes were randomly grouped (n=8) to orally receive 14, 28 or 56 mg/kg of AL, control group received distilled water (5ml/kg). Treatment was given for 7 days and animals were sacrificed on the 15th day and assessed for delayed toxic effects.

Clinical Assessment

All the animals were weighed and examined for clinical signs at the start and end of the experiment.

Biochemical studies

Blood samples collected from treated and control animals into the appropriate containers were used to perform the following biochemical tests:

Random blood sugar

Random blood sugar (RBS) level was estimated using glucose oxidase technique.[22]

Liver function tests

Serum samples from both treated and control animals were analyzed for bilirubin (total and conjugated) using the diazo method,[23] alanine (ALT) and aspartate (AST) transaminases,[24] and alkaline phosphatase (ALP)[25] using standard procedures.

Kidney function tests

Serum electrolytes (Na^+, K^+, HCO_3^- and Cl^-) and urea were evaluated using standard techniques.[26] The Na^+, K^+, were estimated with flame photometer, while HCO_3^- and Cl^- were determined by titration method. Urea was estimated using the diacetyl monoxime method.[27]

Serum cholesterol

Serum cholesterol was determined by the endpoint enzymatic colorimetric method using a kit, Cromatest® (Linear Chemicals, Spain).

Hematological Studies

Blood samples collected from treated and control animals into EDTA bottles were analyzed for hemoglobin, hematocrit (packed cell volume), reticulocyte and white blood cell (total and differential) counts using standard procedures. Hemoglobin count was done using the cyanmethemoglobin method (colorimetric method),[28] while hematocrit was estimated using a microhematocrit. Reticulocytes were counted on a slide smear after staining with brilliant cresyl blue/ supravital stain. White blood cells were counted using improved Neubauer chamber (Tiefe Marienfeld, Germany).

Histopathological Studies

Liver, heart and kidney of treated and control animals were collected for histopathological examination. The tissues were placed in 10% formol saline on dissection. Sections of the tissues were processed using automatic tissue processor; and used to prepare paraffin wax embedded and microtone sections of 5-6 µm. These were stained and studied using routine Haematoxylin and Eosin stains.

Statistical Analysis

The results obtained were analyzed with SPSS version 16.0 using One Way ANOVA and expressed as Mean ± Standard Error of Mean. Differences between means of treated and control groups were evaluated further using LSD Post hoc test, and considered significant at $P<0.05$.

Table 1: Effects of AL on random blood sugar (RBS) level

Test Phase	Dose (mg/kg)	RBS (mmol/L)
Sub-acute	14	10.11±0.46*
	28	10.36±0.44*
	56	10.20±0.38*
Delayed	14	5.04±0.26
	28	5.33±0.27
	56	5.56±0.21
Control	-	5.41±0.17

n=8; *$P<0.05$ (ANOVA, LSD post hoc); RBS = random blood sugar

Table 2: Effects of AL on hepatic function and serum cholesterol

Test Phase	Dose (mg/kg)	Liver Enzyme			Bilirubin		Cholesterol (mm/L)
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB	CB	
Sub-acute	14	15.75±0.31	17.75±0.65	26.25±0.59	8.6±0.00	4.3±0.00	3.25±0.07
	28	15.75±0.75*	17.88±0.79	27.63±0.50	8.6±0.00	4.3±0.00	3.49±0.11
	56	15.88±0.64*	18.75±0.80	29.25±0.65	8.6±0.00	4.3±0.00	3.43±0.19
Delayed	14	17.00±0.57	20.00±0.38	31.38±0.65	8.6±0.00	4.3±0.00	3.70±0.19
	28	18.86±0.55	21.38±0.65	38.25±0.86	8.6±0.00	4.3±0.00	3.75±0.25
	56	19.50±0.60	22.50±0.53	41.25±0.70	8.6±0.00	4.3±0.00	3.99±0.17
Control	-	14.25±0.31	17.13±0.35	24.13±0.44	8.6±0.00	4.3±0.00	2.39±0.04

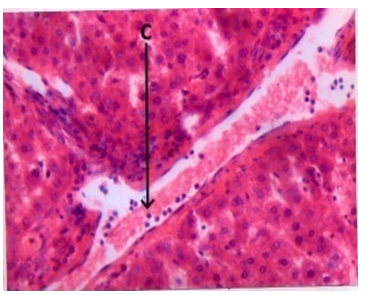
n=8; **P*<0.05 (ANOVA, LSD post hoc).

Table 3: Effects of AL on serum electrolytes and urea (renal function tests)

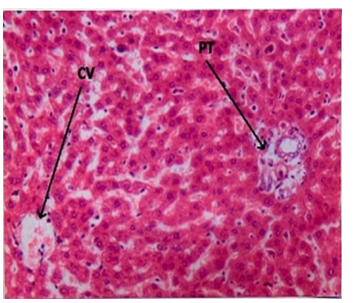
Test Phase	Dose (mg/kg)	Serum electrolyte and urea (mmol/L)				
		Na^+	K^+	Cl^-	HCO_3^-	Urea
Sub-acute	14	147.63±0.10	4.58±0.09	96.63±0.50	22.88±0.44	4.86±0.11
	28	152.13±1.96	4.35±0.08	97.13±0.61	22.88±0.48	5.71±0.17
	56	166.25±1.68	4.66±0.09	97.00±0.33	23.75±0.37	6.54±0.24
Delayed	14	143.63±0.26	4.43±0.14	97.75±0.25	26.75±0.31	5.53±0.16
	28	144.63±0.56	4.44±0.08	99.00±0.73	23.88±0.30	6.83±0.18
	56	143.38±1.0	4.38±0.10	98.75±0.56	24.25±0.41	7.26±0.10
Control	-	141.25±0.56	4.64±0.08	102.63±0.46	26±0.19	4.76±0.11

Table 4: Effects of AL on haematological parameters

Test Phase	Dose (mg/kg)	Hb	PCV	Reticulocyte Count (%)	White blood cell count		
					TWBC x10^3 cells /L	Neutrophil (%)	Lymphocyte (%)
Sub-acute	14	13.95±0.77	42.00±2.33	3.13±0.23	9.09±0.25	21.25±4.06	78.75±4.06
	28	11.75±0.62	36.13±1.88	2.25±0.49	9.66±0.33	30.63±5.18	69.38±5.18
	56	11.98±0.80	36.13±2.41	2.13±0.40	10.10±0.99	20.63±1.84	79.38±1.84
Delayed	14	13.76±0.63	41.75±1.93	2.00±0.27	9.66±0.39	36.13±2.74	63.63±2.81
	28	13.75±1.10	41.88±3.30	2.00±0.27	10.21±0.40	38.50±3.42	61.25±3.41
	56	13.89±0.95	42.25±2.79	1.63±0.32	10.74±0.83	41.13±2.02	58.88±2.02
Control	-	12.06±0.21	36.88±0.93	0.56±0.08	7.49±0.29	64.50±2.24	35.50±2.24

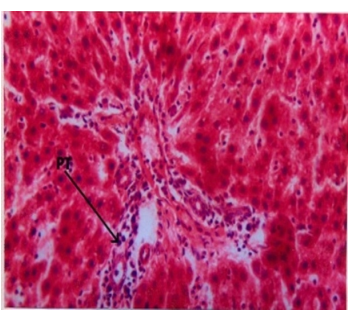


Liver section of AL (14 mg/kg) treated rat showing mild central vein congestion with mild vascular lymphocytosis and lymphocytic pavementing (C).

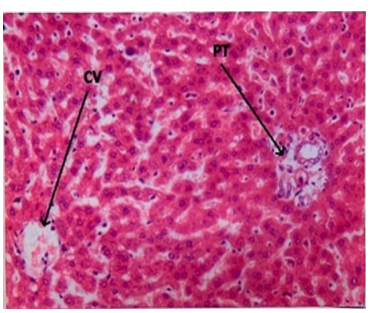


Liver of Control animal showing normal liver parenchyma with central vein (CV) and portal triad (PT).

Figure 1: Sub-acute effects of AL (14 mg/kg) on rat liver

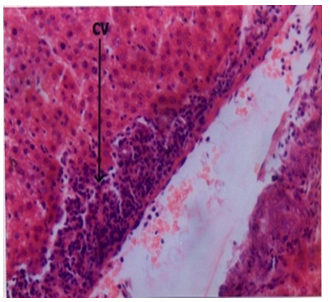


Liver section of AL (28 mg/kg) treated rat showing mild to moderate lymphocytic infiltration of the portal triad (PT).

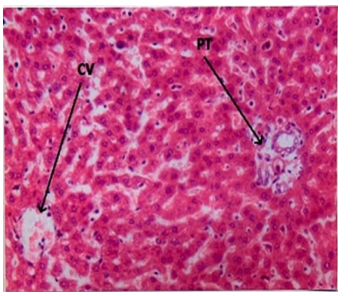


Liver of Control animal showing normal liver parenchyma with central vein (CV) and portal triad (PT).

Figure 2: Sub-acute effects of AL (28 mg/kg) on rat liver

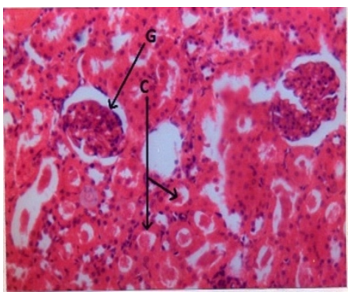


Liver section of AL (56 mg/kg) treated rat showing severe lymphocytic infiltration around a central vein (CV).

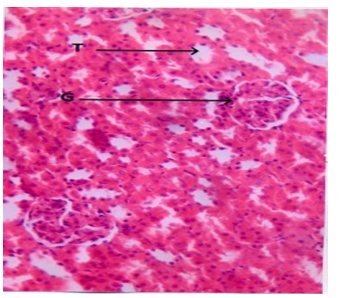


Liver of Control animal showing normal liver parenchyma with central vein (CV) and portal triad (PT).

Figure 3: Sub-acute effects of AL (56 mg/kg) on rat liver

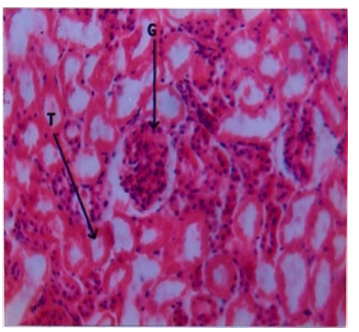


Kidney section of AL (28 mg/kg) treated rat showing granular and eosinophilic hyaline casts (C) within renal tubules. The glomeruli (G) appear unremarkable.

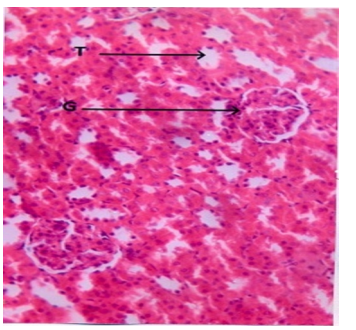


Kidney of control animal showing normal renal parenchyma with glomeruli (G) and tubules (T).

Figure 4: Sub-acute effects of AL (28 mg/kg) on rat kidney

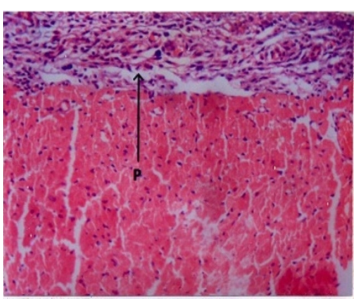


Kidney section of AL (56 mg/kg) treated rat showing diffuse coagulative necrosis of tubular epithelium with granular and eosinophilic hyaline casts (C) in some tubules. The glomeruli (G) are spared.

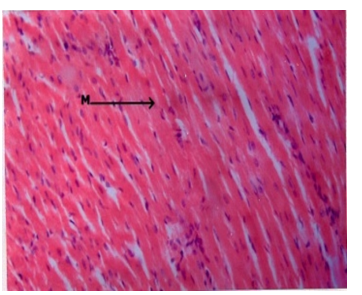


Kidney of control animal showing normal renal parenchyma with glomeruli (G) and tubules (T).

Figure 5: Sub-acute effects of AL (56 mg/kg) on kidney

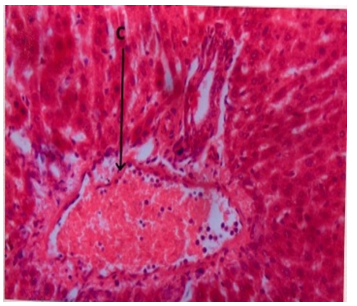


Heart section of AL (56 mg/kg) treated rat showing organizing fibrinous pericarditis (P), with intense lymphocytic infiltration

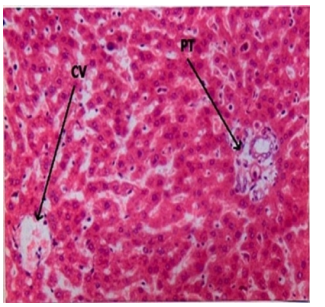


Heart of control animal showing normal myocardial fibers (M) in a longitudinal section.

Figure 6: Sub-acute effects of AL (56 mg/kg) on rat heart

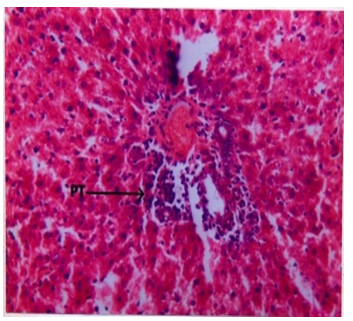


Liver section of AL (28 mg/kg) treated rat showing mild lymphocytic infiltration of the portal triad (C).

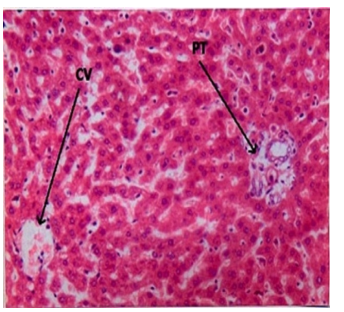


Liver of Control animal showing normal liver parenchyma with central vein (CV) and portal triad (PT).

Figure 7: Delayed effects of AL (28 mg/kg) on rat liver

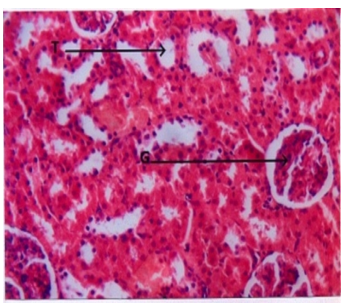


Liver section of AL (56 mg/kg) treated rat showing moderate lymphocytic infiltration around the portal triad (PT).

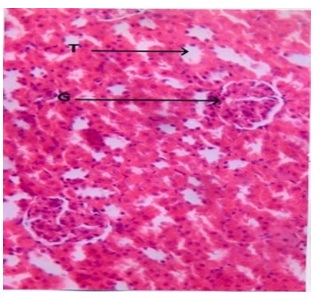


Liver of Control animal showing normal liver parenchyma with central vein (CV) and portal triad (PT).

Figure 8: Delayed effects of AL (56 mg/kg) on rat liver

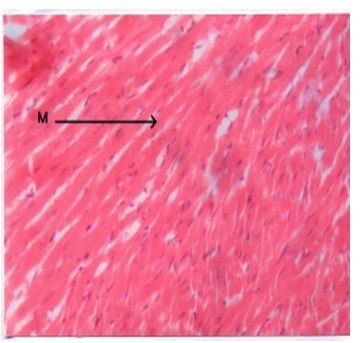


Kidney section of AL (56 mg/kg) treated rat showing no remarkable histopathological changes in the glomeruli (G) and tubules (T).

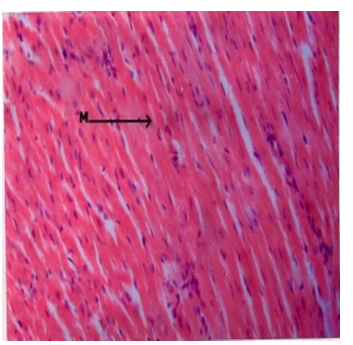


Kidney of control animal showing normal renal parenchyma with glomeruli (G) and tubules (T).

Figure 9: Delayed effects of AL (56 mg/kg) on rat kidney



Heart section of AL (56 mg/kg) treated rat showing no remarkable pathological changes in the myocardium (M).



Heart of control animal showing normal myocardial fibers (M) in a longitudinal section.

Figure 10: Delayed effects of AL (56 mg/kg) on rat heart

RESULTS

Clinical signs

No animal was found dead or in a moribund state during the experiment.

Effects of AL on random blood sugar

The AL elicited a significant ($P<0.05$) increase in the random blood sugar level in the sub-acute phase compared to control (Table 1). However, in the delayed phase, there was slight and non-significant reduction in RBS of 14 and 28 mg/kg treated rats; while rats that received 56 mg/kg had a slight increase in RBS relative to control (Table 1).

Effects of AL on hepatic function and serum cholesterol

There was dose-related and non-significant increases in levels of cholesterol, ALT, AST and ALP in AL treated rats compared to control rats in both sub-acute and delayed phases. The increase was higher in delayed than in sub-acute phase (Table 2). However, the levels of total and conjugated bilirubin were the same in both treated and control animals (Table 2).

Effect of AL on renal function

The AL elicited a marginal, dose-related and non-significant elevation of Na^+, but reduced K^+, Cl^- and HCO_3^- compared to control animals. There was mild but non-significant increase in blood urea, which was greater in delayed than sub-acute phase (Table 3).

Effect of AL on haematological parameters

In the sub-acute phase, AL 14 mg/kg caused a mild increase in Hb and PCV, while animals that received higher doses of 28 and 56 mg/kg had slightly lower Hb and PCV. However, in the delayed phase, AL elicited dose-related increase in Hb and PCV compared to control. The AL also increased the reticulocyte, total white blood cells and lymphocyte, but reduced the neutrophil counts (Table 4).

Histopathological effects of AL

Subacute toxicity

Histological sections of the liver of AL treated rats revealed dose-related increase in severity of hepatic congestion and inflammation. There was mild central vein congestion with mild vascular lymphocytosis and lymphocytic pavementing in animals exposed to normal dose of AL (14 mg/kg) (Figure 1), while those administered 28 mg/kg had mild central and portal vein congestion with vascular lymphocytosis (lymphotic pavementing) and mild to moderate portal tract inflammation (lymphocytic infiltration) (Figure 2). Animals exposed to quadruple strength of AL (56 mg/kg) had central and portal vein congestion, severe vascular lymphocytosis and lymphotic pavementing, with severe periportal and perivenular inflammation (lymphocytic infiltration) (Figure 3).

The renal sections of animals exposed to 14 or 28 mg/kg of AL showed no significant changes compared to control, however, two animals in each group (25%) had granular and eosinophilic hyaline casts in renal tubules respectively (Figure 4). Three of the eight animals exposed to quadruple strength of AL showed no remarkable histopathological changes, two of the animals had only granular and eosinophilic hyaline casts in renal tubules, while one animal showed diffuse coagulative necrosis (loss of cell nuclei and preservation of cell outline) of tubular epithelium (acute tubular necrosis) with granular and eosinophilic hyaline casts in the renal tubules; the glomeruli were spared (Figure 5).

The cardiac sections of animals exposed to 14 and 28 mg/kg of AL showed no remarkable histopathological changes compared to control. However, the animal that received 56 mg/kg AL and presented with diffuse coagulative necrosis of the tubular epithelium also showed organizing fibrinous pericarditis with intense lymphocytic infiltration (Figure 5 & 6), while the hearts of the other seven animals showed no remarkable histopathological changes.

Delayed toxicity

Histological sections of the livers of animals exposed to normal strength of AL (14 mg/kg) showed varying features. One of the animals showed focal areas of chronic inflammation (lymphocytic) around central veins, two showed central vein congestion, one showed portal vein congestion and four showed no remarkable histopathological changes. Histological sections of the livers of animals exposed to double strength of AL (28 mg/kg) showed varying features. Three of the animals showed mild central vein congestion, one showed mild portal tract inflammation (Figure 7), and four showed no remarkable histopathological changes. Histological sections of the livers of animals exposed to quadruple strength of AL (56 mg/kg) also showed varying features. Two of the animals showed severe portal tract inflammation (lymphocytic infiltration), three showed moderate portal tract inflammation and three showed mild portal tract inflammation (Figure 8).

The renal section of one animal exposed to AL (14 mg/kg) showed granular and eosinophilic hyaline casts in renal tubules, while seven animals had no remarkable histopathological changes. However, rats given 28 and 56 mg/kg respectively exhibited no remarkable renal histopathological changes (Figure 9).

The cardiac sections of all the animals given 14, 28 or 56 mg/kg had no remarkable histopthological changes (Figure 10).

DISCUSSION

Consequent on the rise in the use of AL as a treatment regimen for malaria infection, this study sought to elucidate the sub-acute and delayed toxicity profile of AL for comparative risk assessment in recipients. Artemisinins like other therapeutic agents are not devoid of unwanted, adverse and toxic effects. Studies in animals have reported toxic effects such as neurotoxicity[29,30] and contragestational effects in animals.[13,31]

The significant increase in random blood sugar (RBS) in the sub-acute phase suggests a hyperglycemic effect. This implies that in the treatment of malaria in hypoglycemic conditions, AL may have an advantage over other antimalarials like chloroquine, amodiaquine, etc, which exert hypoglycemic effect. On the other hand, there is need for caution, monitoring and supervision during its use in diabetics. Nevertheless, the reduction in RBS in the delayed phase may indicate a recovery phase.

The AL elicited dose-related and insignificant mild elevations of liver enzymes which were greater in the

delayed phase, but the values fell within the normal range of enzyme activity. The result is consistent with earlier reports that artemisinin derivatives cause elevations in serum levels of hepatic enzymes.[32-34] The AL had no effect on total and conjugated bilirubin, but elicited a moderate and non-significant increase in blood cholesterol level in rats. The increase in cholesterol level was dose-dependent and higher in the delayed phase. These suggest that AL may be devoid of any effect on bilirubin, and may increase cholesterol level. Drugs produce a wide variety of clinical and pathological hepatic injury; increase (changes) in biochemical markers such as ALT, ALP and bilirubin are indicators of hepatotoxicity. Generally, hepatotoxicity is defined as rise in either ALT level more than three times of upper limit of normal (ULN), ALP level more than twice ULN, or total bilirubin level more than twice ULN when associated with increased ALT or ALP.[35,36] Liver damage is further characterized into hepatocellular (predominantly initial alanine transferase elevation) and cholestatic (initial alkaline phosphatase rise) types. However, they are not mutually exclusive and mixed type of injuries is often encountered. Elevation of serum bilirubin level of more than 2 times ULN with associated transaminase rise indicates severe hepatotoxicity.[35,36] Since the levels of these biochemical markers of hepatotoxicity are within normal range, AL may be devoid of hepatotoxicity.

The stimulatory effect of normal dose of AL (14 mg/kg) on Hb concentration and PCV may be beneficial in antimalarial treatment as malaria causes anaemia. However, the inhibitory effect of higher doses on hematopoiesis needs further study. Artemisinin derivatives such as artesunate and dihydroartemisinin (DHA) have been reported not to cause significant effects on RBC counts, hemoglobin and hematocrit.[37] The AL increased reticulocyte counts in both acute and delayed phases, with greater increase in the acute phase; this also points to and reinforces the ability of AL to increase RBC as reticulocytes are immature red blood cells. This may suggest enhancement of erythropoiesis. Furthermore, the increase in Hb and reticulocyte count in AL treated rats infer that treatment with AL may not trigger hemolysis; this is consistent with previous finding.[12] However, artemether has been reported to cause a decrease in reticulocyte count.[38]

The AL also caused a significant dose dependent increase in TWBC count in both the sub-acute and delayed phases. However, the neutrophils were decreased, while there was substantial increase in lymphocyte count. The finding of leucocytosis, lymphocytosis and neutropenia in AL treated rats is consistent with reports from studies with artesunate and DHA.[37,39] The presence of leucocytosis and lymphocytosis suggest that AL may stimulate inflammatory response. The neutropenia is consistent with earlier reported clinical observations.[19] Many drugs are known to cause neutropenia (as an isolated event) probably due to depressed granulopoiesis or accelerated granulocytic removal or destruction secondary to immunologically mediated injury to the neutrophils.[40]

The AL may be devoid of effects on renal function as it elicited minute increases in levels of Na^+ and urea, but decreased K^+, Cl^- and HCO^{3-}. However, artesunate and DHA have been reported to significantly increase serum creatinine,[37,41] which is a measure of renal toxicity.[42,43]

In the sub-acute phase, the animals presented dose dependent histopathologic effects in the liver which include central and portal vein congestion, lymphocytosis as demonstrated by vascular lymphocytosis and lymphocytic pavementing (diapedesis), and lymphocytic infiltration of the portal tract and central vein. These increased in severity with higher doses. In the delayed phase, there was also dose related increase in severity of hepatic congestion and inflammation as the dose of AL was increased from N to 4N. However a global look reveals an overall improvement in the pathological features of the liver sections in the delayed relative to the sub-acute phase. The various degrees of vascular congestion and lymphocytosis elicited by AL in the liver suggest ability to elicit periportal and perivenular inflammatory response as lymphocytes migrate to areas of (chronic) inflammation. This finding is also consistent with the observation of leucocytosis and lymphocytosis in the haematology tests. About 8% of reported adverse reactions involve the liver.[44] This reflects its central role in the metabolism and excretion of many drugs. Virtually any type of liver disease may be caused by drugs. Histopathological patterns were devoid of features of any particular hepatic disease, but only showed periportal and perivenular inflammation which increased with higher doses. However, there was improvement after 7 days (delayed phase) as shown by changes in the degree of inflammation; mild-moderate to mild (28 mg/kg) and severe to moderate (56 mg/kg). It is known that on the removal or suppression of an injurious agent, there is reduction in release of chemical mediators and stimuli for inflammation, and ultimately resolution of inflammation. Results suggest resolution of inflammation, and hence a recovery in the delayed phase.

Drug-induced renal diseases are common, and about 20 – 30% of all cases of acute renal failure are drug-induced.[44] The kidney is particularly prone to damage from drugs and chemicals due to its notably very high blood flow in relation to its size, and its excretory role for these agents and their metabolites.[44,45] Hence the renal toxicity profile of any drug is highly valuable. In the sub-acute phase, there were granular and eosinophilic hyaline casts in renal tubules of 25 % of animals administered N, 2N and 4N of AL respectively. However, one animal administered 56 mg/kg showed features of acute tubular coagulative necrosis in addition to granular and eosinophilic hyaline casts; this isolated finding needs further study. All the animals were devoid of significant delayed renal effects, except one (14 mg/kg) that had granular and eosiniphilic hyaline casts in the renal tubules. This improved picture as demonstrated by AL being devoid of any delayed renal effect suggests a recovery phase. Coagulative necrosis is a common type of cell death in the presence of exogenous stimuli such as stress and chemicals. Hyaline is a non-specific marker of cell injury, and is widely used as a descriptive histologic term. Furthermore, a multitude of mechanisms produce hyalinization leading to various implications, hence its presence does not connote a specific renal toxic effect/disease. Nevertheless, the presence of granular and eosinophilic hyaline casts within

renal tubules may result in the thickening and narrowing of tubular lumen.

There were no remarkable histopathological changes in the heart, but the same animal administered 56 mg/kg, with acute tubular coagulative necrosis, also showed features of organizing fibrinous pericarditis with intense lymphocytic infiltration; this isolated finding needs further study. Pericarditis is the epicardial manifestation of inflammation. Also no significant histopathological changes were observed in the heart in the delayed phase. The observed dose related and non-significant increase in AST with greater increase in the delayed phase than the sub-acute phase is also worthy of mention here, as AST is one of the plasma cardiac markers that increase sequentially after acute myocardial infarction.[46] Previous studies of serial electrocardiograms from over 700 patients (children and adults) administered the 6 dose regimen of AL showed that the frequency of QTc interval prolongation was similar to or lower than that observed with chloroquine, mefloquine, or artesunate plus mefloquine; and significantly lower than that of halofantrine. These changes were considerably less than asymptomatic and no adverse clinical cardiac events were reported.[16,47-50]

In conclusion, the once daily oral administration of increasing doses of AL to rats for one week, elicited varying sub-acute and delayed hematological, biochemical and histopathological effects. The AL elicited hyperglycemic effect, as well as stimulatory effects on haemopoeisis and immune response, but no significant sub-acute and delayed toxic effects on the liver, kidneys and heart.

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