Biokemistri
Vol. 33, No. 4, December 31, 2021
Printed in Nigeria

0795-8080/2021 $10.00 + 0.00

http://journals.niseb.org
Also available online at http://www.bioline.org.br/bk

BKR 33402

Assessment of hepatotoxicity effects of long term administration of Synriam™ in rats

David C. Nwikwe* and Elizabeth A. Balogun

Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

E-mail: ea_balogun@yahoo.com; Contact: +2348033589068
E-mail: davidnwikwe@gmail.com; Contact: +2348066941371
*Author for Correspondence

ABSTRACT: Synriam™ antimalarial drug (SAD), a combination of arterolane malate (150 mg) (a short-acting drug that is effective against all parasite blood stages) and piperaquine phosphate (750 mg) (a slow, long-acting drug that kills residual parasites), has been proven to be safe and effective for malaria treatment in human. However, dearth data is available on its safety in animals. This study investigated the toxicity of SAD on selected liver indices in rats. Thirty-five adult Wistar rats were randomized into five groups (n=7). Group A-Control, Group B-E SAD-treated with 4.0, 8.0, 16.0 and 32.0 mg/kg body weight (bwt.) SAD, respectively, for 28 days. The rats were sacrificed 24 h after the last administration. Liver and blood (for serum extraction) were collected using standard methods. SAD-treated groups compared favorably (p>0.05) with control for organ-body weight ratio, liver and serum activities of aminotransferases, gamma-glutamate transferase, glutamate dehydrogenase, lactate dehydrogenase, sodium-potassium-adenosine triphosphatase, calcium-magnesium-adenosine triphosphatase, and serum globulin levels of subjects. However, SAD treatment showed significant effects (p<0.05) on liver and serum activities of alkaline phosphatase, serum protein, albumin and bilirubin levels. SAD treatment also showed mild effects on the hepatocytes but with no fatty degeneration. This study therefore provides evidence that long term therapeutic dose administration of SAD does not predispose to liver dysfunction in rats.

Keywords: Synriam™, antimalarial, arterolane malate, piperaquine phosphate.

Introduction

Malaria, being an ancient disease, is an endemic protozoal blood infection caused by a mosquito-borne Apicomplexan parasite transmitted to humans during the bite of an infected female *Anopheles* mosquito (34). It remains a major public health issue in sub-Saharan Africa and considerably contributes to child morbidity and mortality. Children under five accounted for 67% out of the 94% cases of malaria deaths recorded in sub-Saharan Africa in 2018 (59). This disease respectively contributed 5% and 18% of under-five deaths worldwide and in sub-Saharan Africa. Although this figure was approximately halved from 2010 to 2018 for children under-five in the world as result of the intensified control measures including vector control and drug-based interventions (35, 41). This progress marked in most sub-Saharan Africa countries is nonetheless hindered by the challenge of achieving an early diagnostic and appropriate treatment of malaria cases to prevent the occurrence of complications and death (35, 55).

For the parasite to complete its life cycle, it requires both female Anopheles mosquito (the definitive host or vector) and human (an intermediate host) involving ten morphological changes in five different tissues of hosts (28). Some of the clinical symptoms of malaria ranges from acute febrile illness with fever associated with chills, headache, and vomiting to deadly complications like severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria which can eventually lead to death (1).

However, *falciparum* malaria therapy is dependent on the severity of infection, status of the host and drug sensitivity pattern in the locality. Moreover, selection of the appropriate treatment agent depends on the local antimalarial drug resistance patterns, government treatment guidelines, tolerability, availability and gametocidal activity (3). Poor drug quality, incorrect dosing, non-compliance with duration of dosing regimen, drug interactions, unpredictable or poor absorption and misdiagnosis are some of the leading conditions to malaria treatment failure which contribute to the development of resistance by parasites. Furthermore, factors responsible for decrease in the effectiveness of immune system in clearing parasite residuum after treatment likewise increase survivorship and intensification of resistance (3). The rate at which resistance develops in a given area is dependent on some factors such as the intensity of transmission, initial prevalence of mutations, intensity of drug pressure, population movement between areas or its strains, etc. Use of an alternative drug reduces drug pressure, which is thought to be the single most important factor in the development of resistance (3).

World Health Organization (WHO), however, recommended artemisinin-based combination therapies (ACTs) as the first-line therapy for uncomplicated *P. falciparum* in malaria-endemic regions as result of its quick reduction of parasitaemia (50, 57). The success of ACT has been revealed in reduced acute parasite index over the recent years. The major threat today is the possibility for resistance to arise in *falciparum* against artesunate or its partner drug. Some of the measures intended at preventing drug resistance mostly focus on reducing overall drug pressure through more selective and restrictive use of drug, improved prescribing and follow up practices, improved patient compliance and use of drug combinations (3). Artemisinin derivatives in ACT are used in combination with a long acting, more slowly eliminated partner drug that prevents infection recrudescence (49). Artemisinins, however, are subject to demand and supply problems since it is derived from plant source (33, 50, 57). These facts therefore point towards a need to identify a new alternative, effective and affordable antimalarial remedy (3).

Arterolane maleate (AM) is a rapid, short-acting antimalarial drug that is effective against all blood stages of the parasites. Conversely, piperaquine phosphate (PQP) is a proven effective and well-tolerated slow, long-acting antimalarial drug that kills residual parasites (49, 51). The efficacy, pharmacokinetic profile, tolerability and low cost of PQP make it a promising partner drug for use in ACT (49). AM has been combined with PQP in a fixed-dose combination commercially referred to as Synriam™ drug. The combination (150 mg AM and 750 mg PQP) has been proven to be effective and safe for the treatment of acute uncomplicated *falciparum* malaria. This combination provides antimalarial activity at different time windows that will prevent the emergence of resistance to either drug. Its mechanism of action is different from those of artemisinins (51). Both the mechanisms of action and of resistance of PQP have not been well characterized, but are likely to be similar to those of the 4-aminoquinolines. It has however been shown to be active against highly chloroquine-resistant *P. falciparum*, and also highly lipid-soluble with its oral bioavailability lower when given without any food (14). Valecha *et al.* (51) and Toure *et al.* (49) have reported the efficacy and safety of the clinical trial of the drug in patients with acute uncomplicated *P. falciparum* malaria.

This study thereofore aimed at investigating the safety of prolonged administration of Synriam™ drug in the liver of rats.

Materials and Methods

Chemicals and reagents

Assay kits for total protein, albumin, bilirubin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-gamma-glutamate transferase (GGT), sodium-potassium-adenosine triphosphatase (Na^+-K^+-ATPase), and calcium-magnesium-adenosine triphosphatase (Ca^{2+}-Mg^{2+}-ATPase) were obtained from Sigma-Aldrich, USA, and Randox Laboratories Ltd., Co-Antrim, UK. All other reagents used were of analytical grade.

Animals and animal grouping

Thirty-five adult Wistar rats with an average weight of 150 ± 5 g were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. They were housed in plastic cages and acclimatized for 2 weeks before the commencement of the experiment (temperature 25-30ºC, relative humidity 40-45%, and 12 h) with free access to pellets (Top Feeds, Ilorin, Nigeria) and tap water *ad libitum*. The research adhered strictly to the Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985). The rats were randomly divided into five groups (A-E), consisting of seven rats each. Group A-Control received 0.5 mL distilled water (the vehicle). Groups B to E received the same volume of drug corresponding to 4.0, 8.0, 16.0, and 32.0 mg/kg body weight, respectively. Both the distilled water and drug were administered orally once daily using oropharyngeal cannula for a period of 28 days.

Drug and drug preparation

Synriam™ drug was obtained from Mosaaj Pharmacy, Ilorin, Nigeria, manufactured by Ranbaxy Laboratories Limited, Plot No. B-2, Madkai Industrial Estate, Ponda, Goa, India. The drug was pulverized. The tablets were ground into fine powder using mortar and pestle, and then transferred into an air-tight bottle for storage.

Sample collection and preparation

All the rats in the various experimental groups were sacrificed 24 h after the completion of their daily doses (28 days). Under ether anesthesia, the neck of rats was quickly cleared of fur and skin to expose the jugular vein. These animals were made to bleed through their cut jugular vein. Blood was collected into clean, dry centrifuge (plain) tubes and was mixed thoroughly before been centrifuged at 3000 *g* for 15 min using a Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The serum was thereafter aspirated into clean, dry, sample bottles using Pasteur pipettes and kept frozen before it was used for assay. The rats were thereafter quickly dissected, and the liver removed and cleaned of blood. The tissue was homogenized in 0.25 M sucrose solution (1:5 w/v) as described by Ngaha *et al*. (32). The homogenate was transferred into specimen bottles and kept frozen for 24 h before analyses.

Determination of liver biochemical indices

The biochemical parameters were determined using standard methods described for serum protein (22), serum albumin (17), serum globulin (47), serum bilirubin (63), ALP (60), GGT (45), aminotransferases (38), LDH (61), GDH (43), Na^+-K^+-ATPase (7, 39), and Ca^{2+}-Mg^{2+}-ATPase (19, 39). The liver was prepared for histopathological analysis according to the procedure described by Krause (27) and stained with hematoxylin and eosin (H & E). The photomicrographs were captured at ×400 with software.

Statistical analysis

Data were presented as mean of seven determinations ± SEM. Statistical analysis were carried out using one-way analysis of variance (ANOVA) followed by Duncan's *Post-hoc* multiple comparisons, using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA). Differences were considered statistically significant at $p<0.05$. Graphs were created using GraphPad Prism 6 software for Windows (GraphPad Software, California, USA).

Results

The mathematically expressed organ to body weight ratio of the rats orally administered 4.0, 8.0, 16.0 and 32.0 mg/kg body weight drug showed no significant difference ($p>0.05$) compared to control (Figure 1).

The serum protein and albumin levels in the treated rats increased significantly ($p<0.05$) compared to control. Meanwhile, the serum globulin levels in the treated rats showed no significant difference ($p>0.05$) compared to control. Whilst a significant decrease ($p<0.05$) was observed in the total bilirubin levels of the treated rats compared to control, a significant decrease ($p<0.05$) was also observed at 4.0, 8.0 and 16.0 mg/kg body weight of the direct bilirubin levels. However, at 32.0 mg/kg in the direct bilirubin levels, there was no significant difference ($p>0.05$) compared to control (Table 1).

None of the doses of the drug administered showed any significant ($p>0.05$) alteration in the liver activities of ALT, AST, GGT, GDH, LDH, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase compared to control, except the AST activity at 32.0 mg/kg which increased significantly ($p<0.05$) compared to control. However, the liver ALP activity showed a significant decrease ($p<0.05$) compared to control. However, while the serum ALP activity increased significantly ($p<0.05$), the AST activity significantly decrease ($p<0.05$) compared to control. The serum ALT activity, at 4.0 and 8.0 mg/kg, showed no significant difference ($p>0.05$) compared to control, while a significant decrease ($p<0.05$) was observed at 16.0 and 32.0 mg/kg. Also, none of the doses of the serum GGT, GDH and LDH activities showed any significant ($p>0.05$) alteration compared to control (Figures 2-9).

Finally, oral administration of the drug for 28 days produced no histopathological changes at 4.0 mg/kg body weight dose, but showed mild deterioration without fatty degeneration at 8.0 and 16.0 and 32.0 mg/kg body weight (Plate 1a-e).

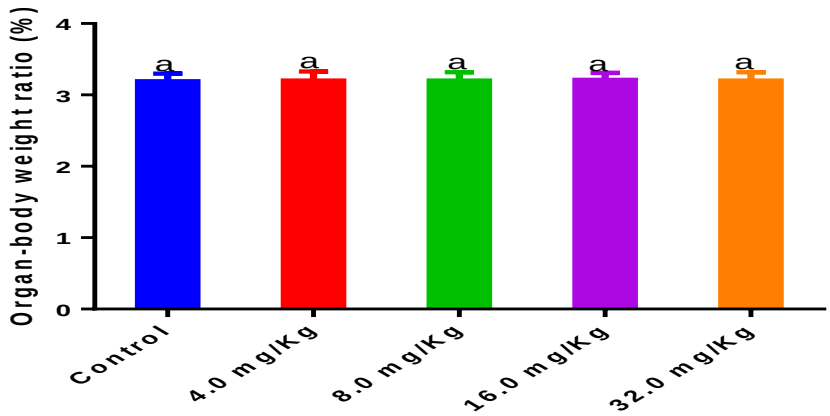

Figure 1: The organ-body weight ratio (%) of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters similar to the control are not significantly different ($p>0.05$).

Table 1: Effects of prolonged oral administration of drug on selected liver function indices of rats

Parameters	Control	Drug (mg/kg body weight)			
		4.0	8.0	16.0	32.0
Total protein (g/L)	15.56 ± 1.32[a]	22.04 ± 1.21[b]	22.48 ± 2.02[b]	23.57 ± 1.86[b]	21.14 ± 1.92[b]
Albumin (g/L)	8.83 ± 0.81[a]	15.98 ± 0.72[b]	16.55 ± 0.58[b]	18.08 ± 0.66[b]	16.03 ± 0.80[b]
Globulin (g/L)	6.73 ± 0.25[a]	6.06 ± 0.17[a]	5.93 ± 0.20[a]	5.49 ± 0.18[a]	5.11 ± 0.14[a]
Total bilirubin (µmol/L)	11.03 ± 0.24[a]	3.97 ± 0.30[b]	4.72 ± 0.28[b]	5.13 ± 0.19[b]	4.86 ± 0.21[b]
Direct bilirubin (µmol/L)	6.35 ± 0.85[a]	1.16 ± 0.13[b]	2.02 ± 0.18[b]	1.95 ± 0.15[b]	5.88 ± 0.16[a]

Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

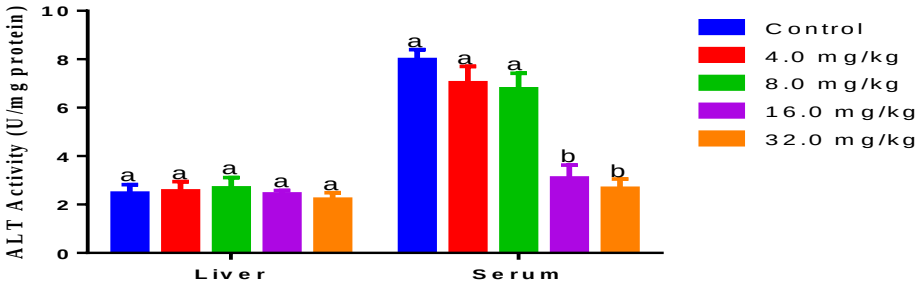

Figure 2: Alanine transaminase (ALT) activities in liver and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

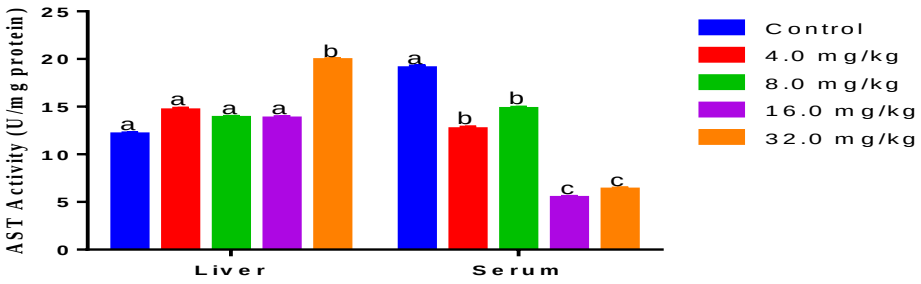

Figure 3: Aspartate transaminase (AST) activities in liver and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lower case letters different from that of the control are significantly different (p<0.05).

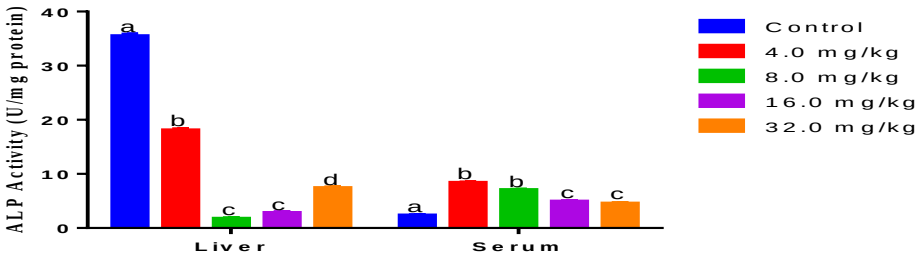

Figure 4: Alkaline phosphatase (ALP) activities in liver and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

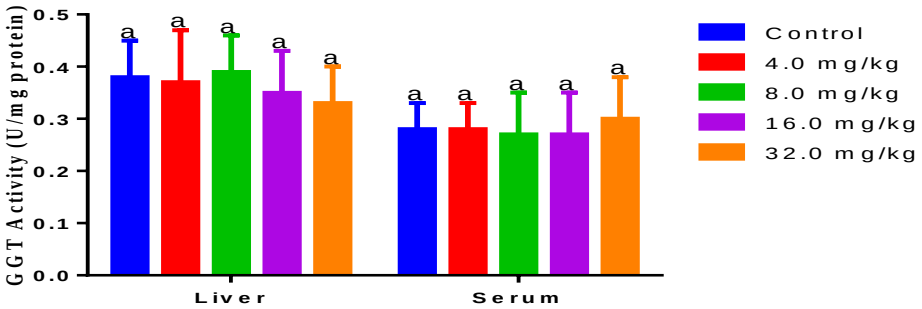

Figure 5: Gamma-glutamate transferase (GGT) activities in liver and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

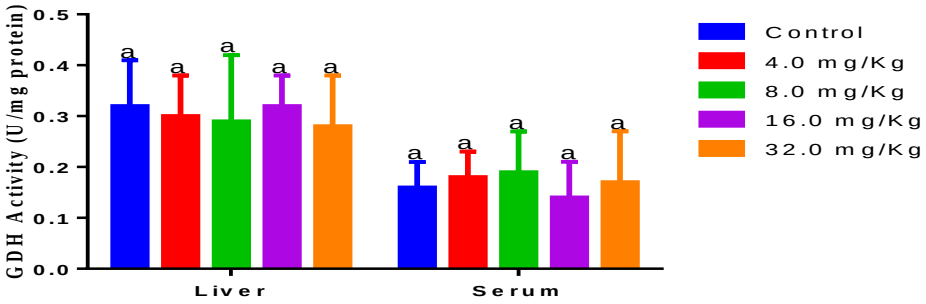

Figure 6: Glutamate dehydrogenase (GDH) activities in liver and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

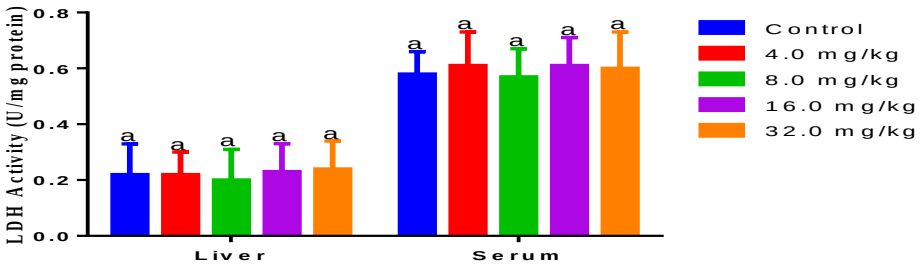

Figure 7: Lactate dehydrogenase (LDH) activities in kidneys and serum of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

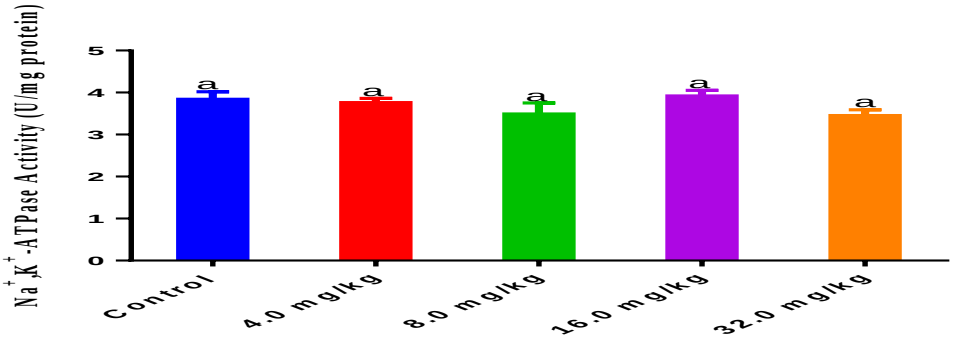

Figure 8: Na^+-K^+-ATPase activities in liver of rats following prolonged oral administration of drug. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters similar to that of the control are not significantly different (p>0.05).

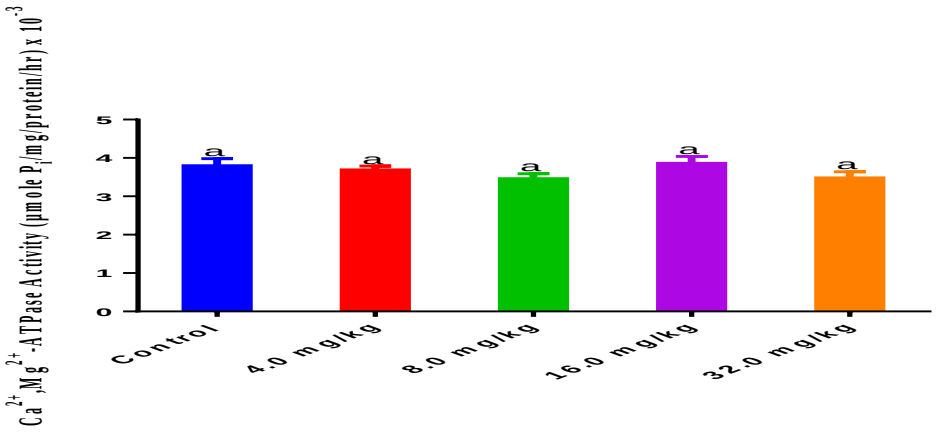

Figure 9: Ca^{2+}-Mg^{2+}-ATPase activities in liver of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters similar to that of the control are not significantly different (p<0.05).

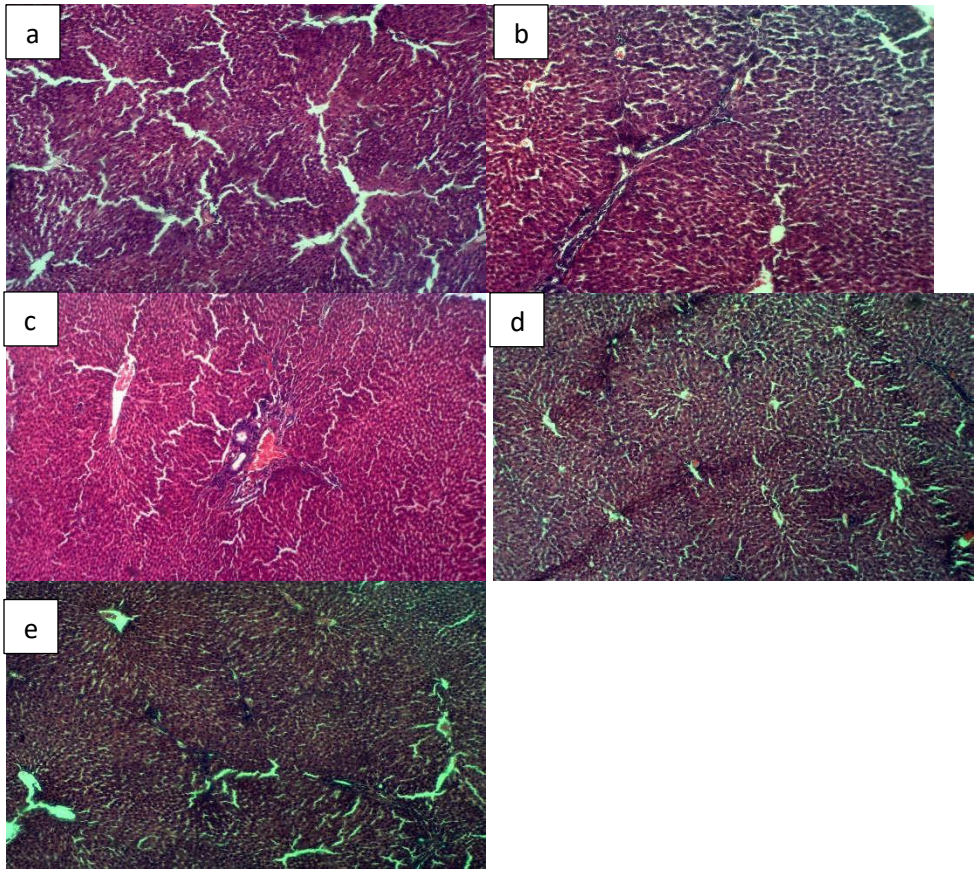

Plate 1: (a) Photomicrograph of the liver of rat administered distilled water for 28 days. The liver is normal with no pathological changes. (b) Photomicrograph of the liver of rat administered 4.0 mg/kg body weight of drug for 28 days. The hepatocyte is normal with no pathological damage. (c) Photomicrograph of the liver of rat administered 8.0 mg/kg body weight of drug for 28 days. The hepatocyte showed mild deterioration with no fatty degeneration. (d) Photomicrograph of the liver of rat administered 16.0 mg/kg body weight of drug for 28 days. The hepatocyte showed pathological changes with no fatty degeneration similar to rat administered 8.0 mg/kg. (e) Photomicrograph of the liver of rat administered 32.0 mg/kg body weight of drug combination for 28 days. The hepatocyte showed pathological changes with no fatty degeneration similar to rats administered 8.0 and 16.0 mg/kg.

Discussion

WHO (58) reported that Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths reported annually (approximately 30% of the total malaria burden in Africa), while 97% of the total population (approximately 173 million) is at risk of infection. WHO recommends artemisinin-based combination therapies (ACTs) as first-line action for the treatment of uncomplicated malaria caused by *falciparum* parasite or by chloroquine (CQ)-resistant *vivax, ovale, malariae*, and *knowlesi* (64). Synriam^{TM} drug containing fixed dose combination of arterolane maleate (150 mg) and piperaquine phosphate (750 mg) exhibits a rapid onset of action, potent activity against all erythrocytic stages of *P. falciparumn.* It was synthesized as an alternative to artemisinin, and has advantages of once-daily dosing and low pill burden as well as a long duration of post-treatment prophylaxis (50). Valecha and co-workers (52) reported its efficacy in phase III trial in patients with acute, uncomplicated *vivax* compared to CQ. Vennerstrom *et al.* (53) and Dong *et al.* (16) reported the *in vitro* and *in vivo* antimalarial potency of the drug in *falciparum*. Therefore, this study aimed at

establishing the hepatocellular toxicity of the prolonged administration of this drug commonly sold in Nigeria as an alternative antimalarial on various liver indices in rats.

Change in organ-body weight ratio is a sensitive indication of chemically or pathologically induced alterations in organs and determination of this ratio enables the assessment of alteration in size of an organ relative to the body weight of the biological subject (65, 66). Increase in organ body weight ratios of the liver may suggest hepatocellular hypertrophy (67, 68, 69). Synriam^{TM} caused no significant alteration in organ-body weight ratios of the liver probably indicating that the drug neither caused inflammation nor constriction of the hepatocyte.

Total protein content of serum basically consists of albumin and globulin. An alteration in the level of total protein of a tissue gives insight into functional changes that possibly occurred in such tissue. Reduction in the level of total protein in organs with corresponding elevation in serum could imply cellular damage or toxicity to those organs (26). Total protein, albumin and bilirubin concentrations in the serum can indicate the state of the liver (20). Serum total protein constitutes 60% albumin and 40% globulin. Serum albumin binds and transport many sparingly soluble metabolic products such as fatty acids, unconjugated bilirubin and foreign bodies in blood and helps in maintaining the osmotic pressure of cells (30, 44). Reduction in serum level of albumin is an indication of impaired synthesis of albumin arising from weakened liver function (62), and such reduction causes accumulation of fluids in interstitial spaces, resulting in oedema (5). Globulins act as transport molecules and perform immune functions. The serum level of globulin (especially the immunoglobulin fraction) is raised after a biological system is exposed to antigen, thereby acting as an indicator of immune response (48).

The significant increase in serum protein and albumin levels of the treated group compared to untreated group might have resulted from the *in situ* production of enzymes or a leakage of some of the enzymes from the liver to the serum (2). Therefore, this may imply that the synthetic capability of the liver was altered, thereby altering the availability of these biomolecules to perform their cellular roles of immunity, transport and osmotic pressure normal control (70).

The non-significant difference observed in the globulin level of SAD-treated rats compared to untreated group could imply that the drug did not hinder the hepatocyte from performing its normal functions such as transport of molecules and response to immune functions. Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cell (RBC). It is a lipid-soluble pigment that binds non-covalently to albumin to increase its solubility in the plasma and serves as a biomarker of hepatic function (6).

Bilirubin remains in cells until rendered water-soluble through conjugation by a specific transferase enzyme which is primarily located in the endoplasmic reticulum and the conjugated bilirubin is readily excreted in the bile (54). Elevated serum levels of bilirubin could be due to overproduction resulting from excessive degradation of haem (pre-hepatic hyperbilirubinemia) or decreased conjugation, culminating in failure of its excretion due to liver damage caused by diseases or xenobiotics (hepatic hyperbilirubinemia). Likewise, obstruction of the excretory ducts of the liver (extra-hepatic cholestasis) can also result in hyperbilirubinemia (post-hepatic hyperbilirubinemia) (18).

The significant decrease in both total and conjugated bilirubin concentrations for the treated group compared to untreated could be as a result of the cumulative effect of the drug on the liver leading to impairment in the conjugation of bilirubin. In the circulation of blood, bilirubin is bound to serum albumin, which prevents its potential toxicity thought to be caused by free bilirubin (10). It is rapidly and selectively taken up by the liver, biotransformed upon conjugation with glucuronate (15), and secreted into bile. Thus, bilirubin is converted into bilirubin glucuronic acid in the liver and excreted along with bile. In liver disease, however, or in instances of obstruction in the biliary duct system, bilirubin excretion usually becomes impaired, causing a rise in the serum bilirubin concentration. The non-significant difference observed in the conjugated bilirubin at 32.0 mg/kg could infer inability of the drug to cause haem degradation at this particular dose and that the conjugation functions of the hepatocytes were not altered.

Tissue damage results in the release of some enzymes into the blood. A mild and reversible cellular alteration such as inflammation results in increased permeability of cell membrane, hence, cytoplasmic

enzymes could be released into extracellular fluid whereas a much higher alteration or cellular death could result in release of organelle-based enzymes such as mitochondrial enzymes into extracellular fluid (9). Alkaline phosphatase (ALP) is a membrane-bound enzyme found in hepatobiliary tissues and cytoplasmic membranes of cells. It generates inorganic phosphate ions from organic phosphate esters and can serve as biomarker in assessing the integrity of membrane (25, 36). Gamma-glutamyltransferase (GGT) catalyzes the transfer of gamma-glutamyl functional group from glutathione to an acceptor, such as amino acid. They are concentrated in hepatobiliary ducts cells and endoplasmic reticulum of hepatocytes and their activities are elevated in the blood in various hepatobiliary diseases (31). The decrease in liver ALP and the restored normalcy in GGT activities may imply that the drug did not alter the integrity of the hepatocyte membranes.

Aminotransferases are intracellular enzymes with alanine aminotransferase (ALT) being more hepatocyte-specific than aspartate aminotransferase (AST). They are pyridoxal phosphate-requiring enzymes that function in amino acid biosynthesis, catalyzing the transfer of amino group from an amino acid to α-keto acid, thereby forming another amino acid (30). They are normally localized within the cells of the liver, heart, kidney, muscles and spleen. They are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage to the liver (4). Alterations in activities of these enzymes in liver with concomitant rise in blood suggest damage of hepatic cells (24, 40). In this study, the liver ALT and AST (except at 32.0 mg/kg) activities produced no alterations in the hepatic cells throughout the period of the experiment, while serum AST and ALT (at 16.0 and 32.0 mg/kg) activities decreased. This may imply that the drug may not have caused leakage from the hepatocytes to the circulation, hence may not be responsible for the fluctuation in the concentrations of these enzymes in the blood.

The amino group of glutamate is removed as NH_4^+ during oxidative deamination reaction catalyzed by glutamate dehydrogenase (GDH), a mitochondrial enzyme. The enzyme also catalyses the reductive amination of α-ketoglutarate to form glutamate (40) which is favoured by high cellular energy level while low cellular energy level favours the oxidative deamination reaction to form α-ketoglutarate (12). The activity of the enzyme could serve to supply the tricarboxylic acid (TCA) cycle with α-keto acids, and to remove ammonia from circulation for synthesis of urea. Mitochondrial enzyme such as GDH has elevated activity in serum in cases of mitochondrial damage and dysfunction (29, 56). The activity of GDH in serum is also elevated during necrosis (36). High levels of this enzyme could be found in mammalian liver, kidney, brain, and pancreas. Throughout the period of the experiment, the drug caused no observable alterations in the activities of this enzyme in the liver and blood indicating that it caused no mitochondrial damage in the hepatic cells.

Lactate dehydrogenase (LDH), on the other hand, plays essential role in anaerobic glycolysis, during which pyruvate generated from glucose is reduced to lactate by LDH, utilizing NADH as cofactor and generating NAD^+. It is predominantly found in heart, kidney, liver, skeletal muscle, and erythrocyte but in smaller amount in brain (13). The serum activity of LDH is elevated in cardiac, hepatic, renal and skeletal muscle injuries or diseases, but highest level of its elevation is seen in pernicious anaemia, haemolytic disorders (25) and myocardial infarction (13). Elevated serum activity could also imply that the cell membrane has been ruptured leaking some of the enzyme into the blood. This could indicate acute or chronic cell damage, although low level are not always harmful but are rarely found (42). The LDH activities not altered throughout the period of this study may imply that the drug did not cause any harm to the tissue and probably did not cause excessive production of lactate in the body of the rats.

Na^+-K^+-ATPase, also known as sodium pump, is primarily responsible for maintaining high extracellular sodium concentration relative to intracellular concentration, and high intracellular potassium ion concentration relative to extracellular concentration. The action of this enzyme ensures generation and maintenance of transmembrane gradients of sodium and potassium which is vital for the normal resting membrane potential (11). The electrochemical gradient created by this enzyme provides energy for transport of metabolites, nutrients, and ions across membrane (46). Compounds such as β-blockers (e.g. propranolol) that prevent phosphorylation of the pump can inhibit its activity. Inhibition of sodium pump by compounds such as ouabain, cause Mg^{2+}-ATPase to swap their action by utilizing magnesium ion to

sustain energy metabolism and maintain membrane potential (37). Similarly, Ca^{2+}-Mg^{2+}ATPase is a membrane-bound enzyme responsible for maintaining intracellular calcium ion homeostasis through active transport (23), and inhibition of the enzyme activities could result in tissue abnormalities and cell death (21, 37). Since no significance difference was observed in the treated groups orally administered the drug compared to the untreated group, this could indicate that the membrane of the tissue in production of energy for action potential through active transport was not altered thereby permitting transport of nutrients, metabolites, and ions across membrane.

The photomicrograph of the liver obtained revealed that the integrity of the hepatocyte membranes was not compromised, except at 16.0 and 32.0 mg/kg where mild histopathological changes with fatty degeneration were observed. Since the drug has been proven to be safe, the mild effects observed in some of the parameters may probably not wholly have resulted from the drug but from other factors such as the animals' health status, diet, environment, etc.

Therefore, from the liver data reported in this study, Synriam™ drug, having significantly different mechanism of action from artemisinins, has been proven to be safe in rats. The safety of this drug at the administered doses in this study corroborates the study of Toure *et al.* (49) who reported its safety and efficacy in paediatric patients (children 6 months to 12 years) with uncomplicated malaria in a fixed dose combination for 28 days.

Conclusion

Synriam™ drug, when administered once daily at doses of 4.0, 8.0, 16.0 and 32.0 mg/kg body weight for rats (25-200 mg; doses approved for a 70 kg man) is safe with minimal side effect.

Acknowledgement

We thank the laboratory technologists, Dr. Nnaemeka Tobechukwu Asogwa and University of Ilorin 2016/2017 Biochemistry Final Year students for their expertise and assistance throughout the laboratory aspects of this study.

Author's Contributions

EAB: Conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials and analysis tools and revised and edited the paper.
DCN: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; drafted the manuscript.

Ethics

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Adeoye AO, Bewaji CO, Ademowo GO. *In vivo* antimalarial activity of *Adansonia digitata* stem bark extract and some fractions. *International Journal of Toxicological and Pharmacological Research,* 2017; 9(1); 26-32.
2. Adesokan AA, Akanji MA. Effect of administration of aqueous extract of *Enantia chlorantha* on the activities of some enzymes in the small intestine of rats. *Nigeria Journal of Biochemistry and Molecular Biology,* 2003, 18(2); 103-5.
3. Aditya S, Nandha R, Sekhri K, Tyagi S. Arterolane maleate and piperaquine phosphate: a new option in the treatment of *plasmodium falciparum* malaria. *Journal of Drug Discovery and Therapeutics,* 2013, 1(3); 66-9.
4. Appidi JR, Yakubu MT, Grierson DS, Afolayan AJ. Toxicological evaluation of aqueous extract of *Hermania incana Cav*. Leaves in male wister rats. *African Journal of Biotechnology*; 2008, 8(10); 2016-20.
5. Arneson WL, Brickell JM. Assessment of liver function. In: Arneson, W.L. and Brickell, J.M. (eds.), *Clinical Chemistry: A Laboratory Perspective*, F.A. Davis Company, Philadelphia, 2007, pp. 233.
6. Awad Jr. WM. Iron and Heme Metabolism. In: Devlin, T.M. (Ed.), *Textbook of Biochemistry with Clinical Correlations*, 7th edition, Wiley, Hoboken, 2010.

7. Bewaji CO, Olorunsogo OO, Bababunmi EA. Comparison of the membrane-bound (Ca^{2+}, Mg^{2+})-ATPase in erythrocyte ghosts from some mammalian species. Comparative Biochemistry and Physiology Part B: *Comparative Biochemistry*, 1985, 82(1); 117-22.
9. Brickell JM, Arneson WL, Mass D. Overview of clinical chemistry. In: Arneson, W.L. and Brickell, J.M. (eds.), *Clinical Chemistry: A Laboratory Perspective*, F.A. Davis Company, Philadelphia, 2007, 22.
10. Carducci C, Birarelli M, Leuzzi V, Carducci C, Battini R, Cioni G, Antonozzi I. Guanidinoacetate and creatine plus creatinine assessment in physiologic fluids: an effective diagnostic tool for the biochemical diagnosis of arginine: glycine amidinotransferase and guanidinoacetate methyltransferase deficiencies. *Clinical Chemistry*. 2002, 48(10); 1772-8.
11. Cheng C, Kuo E, Huang C. Extracellular Potassium Homeostasis: Insights from Hypokalemic Periodic Paralysis. *Seminars in Nephrology*, 2013, 33(3); 237-47.
12. Coomes MW. Amino Acid Metabolism. In: Devlin, T.M. (Ed.), *Textbook of Biochemistry with Clinical Correlations*, 7th edition, Wiley, Hoboken, 2010.
13. Crook MA. Clinical Biochemistry and Metabolic Medicine. 8th edition, CRC Press, 2013, 270-81.
14. D'Alessandro U. Progress in the development of piperaquine combinations for the treatment of malaria. *Current Opinion in Infectious Diseases*, 2009, 22; 588-92.
15. Demura S, Yamada T, Yamaji S, Komatsu M, Morishita K. The effect of L-ornithine hydrochloride ingestion on human growth hormone secretion after strength training. *Advances in Bioscience and Biotechnology*, 2010, 1; 7-11.
16. Dong Y, Wittlin S, Sriraghavan K, *et al.* The Structure-Activity Relationship of the Antimalarial Ozonide Arterolane (OZ277). *J. Med. Chem*, 2010, 53; 481-91.
17. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, 1971, 31(1); 87-92.
18. Ferrier DR. Conversion of Amino Acids to Specialized Products. In: Harvey, R.A. (Ed.) *Biochemistry: Lippincott's Illustrated Reviews*, 6th edition, Lippincott Williams & Wilkins, 2014, 277.
19. Fleschner CR, Kraus-Friedmann N. The effect of Mg^{2+} on hepatic microsomal Ca^{2+} and Sr^{2+} transport. *European Journal of Biochemistry*, 1986, 154(2); 313-20.
20. Ganong WF. Review of Medical physiology. 21st edition, Lange medical books: Mc Graw-Hill medical publishing division, London, 2006.
21. Geering K. Na, K-ATPase. *Current Opinion in Nephrology and Hypertension*, 1994, 6, 434.
22. Gornall AG, Bardawill CT, David MM. Determination of serum protein by means of Biuret reaction. *Journal of Biological Chemistry*, 1994, 177; 751.
23. Gutierres JM, Carvalho FB, Schetinger MRC, *et al.* Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rats. *International Journal of Developmental Neuroscience*, 2014, 33; 88-97.
24. Honda A, Komuro H, Hasegawa T, *et al.* Resistance of metallothionein-III null mice to cadmium-induced acute hepatotoxicity. *Journal of Toxicological Sciences*, 2010, 35; 209-15.
25. Johnson-Davis K, McMillin GA. Enzymes. In: Bishop, M.L., Fody, E.P. and Schoeff, L.E. (eds.), *Clinical Chemistry; Techniques, principles, correlations*, 6th edition, Lippincott Williams & Wilkins, Philadelphia, 2010, 281-308.
26. Kaneko JJ, Harvey JW, Bruss ML. Clinical Biochemistry of Domestic Animals. 5th edition, Academic Press, San Diego, 1997.
27. Krause WJ. The art of examining and interprinting histologic preparations. A student handbook. Partheton Publishing Group, UK., 2001, 9-10.
28. Mackinnon MJ, Marsh K. The selection landscape of malaria parasites. *Science*, 2010, 328; 866-71.
29. McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H. Acute liver failure study group. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. *Hepatology*, 2014, 60; 1336-45.
30. Metzler DE. Biochemistry: The chemical reactions of living cells. 2nd edition, Elsevier Academic press, San Diego, 2003.
31. Murray RK. Metabolism of Xenobiotics. In: Murray, R.K *et al.* (eds.), *Harpers Illustrated Biochemistry*, 29th edition, McGraw-Hill, 2012, 676.
32. Ngaha EO, Akanji MA, Madusolumuo MA. Studies of correlations between chloroquine-induced tissue damage and serum enzyme changes in rats. *Experimental*, 1989, 45; 143-6.
33. Noorden RV. Demand for malaria drug soars. *Nature*, 2010, 466; 672-3.

34. Nureye D, Assefa S. Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia. *The Scientific World Journal,* 2020, Volume 2020, Article ID 12953811-17.
35. Ouédraogo M, Kangoye D, Samadoulougou S, Rouamba T, Donnen P, Kirakoya-Samadoulougou, F. Malaria Case Fatality Rate among Children under Five in Burkina Faso: An Assessment of the Spatiotemporal Trends Following the Implementation of NC Programs. *Int. J. Environ. Res. Public Health*, 2020, 17, 1840.
36. Panteghini M, Bais R. Enzymes. In: Burtis, C.A., Ashwood, E.R. and Bruns, D.E. (eds.), *Tietz fundamentals of clinical chemistry*, 6th edition, Saunders Elsevier, St. Louis, 2008, 317-36.
37. Ravindran VS, Kannan L, Venkateshvaran K. *In-vitro* modulation of Na^+ K^+, Mg^{++} ATPases and AchE by sea anemone toxic proteins. *Indian Journal of Geo-Marine Sciences*, 2012, 41(5); 451-6.
38. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *American Journal of Clinical Pathology*, 1957, 28; 56-63.
39. Ronner P, Gazzotti P, Carafoli E. A lipid requirement for the (Ca^{2+}-Mg^{2+})-activated ATPase of erythrocyte membranes. *Archives of Biochemistry and Biophysics*, 1977, 179(2); 578-83.
40. Rosenthal MD, Glew RH. Medical biochemistry: human metabolism in health and disease. *John Wiley & Sons*, 2011.
41. Rowe AK, Rowe SY, Snow RW, *et al.* The burden of malaria mortality among African children in the year 2000. *Int. J. Epidemiol*, 2006, 35; 691-704.
42. Schueren F, Lingner T, George R, Hofhuis J, Gartner J, Thoms S. "Peroxisomal lactate dehydrogenase is generated by translational read-through in mammals". eLife 3: e03640, 2014.
43. Shimizu H, Kuratsu T, Hirata F. Purification and some properties of glutamate dehydrogenase from *Proteus inconstans*. *Journal of Fermentation Technology*, 1979, 57; 428.
44. Snider MD, McGarry JD, Hanson RW. Lipid Metabolism I: Synthesis, Storage, and Utilization of Fatty Acids and Triacylglycerols. In: Devlin, T.M. (Ed.), *Textbook of Biochemistry with Clinical Correlations*, 7th edition, Wiley, Hoboken, 2010.
45. Szasz G. A Kinetic Photometric Method for Serum γ-Glutamyl Transpeptidase. *Clinical Chemistry*, 1969, 15; 124-36.
46. Therien AG, Goldshleger R, Karlish SJ, Blostein R. Tissue-specific distribution and modulatory role of the gamma subunit of the Na,K-ATPase. *Journal of Biological Chemistry*, 1997, 272(51); 32628-34.
47. Tietz NW. Clinical Guide to Laboratory Tests, WB Saunders, Philadelphia, Pa, USA, 3rd edition, 1995.
48. Timbrell JA. Principles of Biochemical Toxicology. 4th edition, Informa Healthcare, New York, 2009, 248.
49. Toure OA, Rulisa S, Anvikar A, *et al.* Efficacy and safety of fixed dose combination of arterolane maleate and piperaquine phosphate dispersible tablets in paediatric patients with acute uncomplicated *Plasmodium falciparum* malaria: a phase II, multicentric, open-label study. *Malar J*, 2015, 14; 469.
50. Toure OA, Valecha N, Tshefu A, *et al.* A Phase 3, Double-Blind, Randomized Study of Arterolane Maleate–Piperaquine Phosphate vs Artemether-Lumefantrine for *Falciparum* Malaria in Adolescent and Adult Patients in Asia and Africa. *Clinical Infectious Disease*, 2016, 1-8.
51. Valecha N, Krudsood S, Tangpukdee N, *et al.* Arterolane maleate plus piperaquine phosphate for treatment of uncomplicated *Plasmodium falciparum* malaria: A comparative, multicenter, randomized clinical trial. *Clinical infectious diseases*, 2012, 55; 663-71.
52. Valecha N, Savargaonkar D, Srivastava B, *et al.* Comparison of the safety and efficacy of fixed-dose combination of arterolane maleate and piperaquine phosphate with chloroquine in acute, uncomplicated *Plasmodium vivax* malaria: a phase III, multicentric, open-label study. *Malar J*, 2016, 15; 42.
53. Vennerstrom JL, Arbe-Barnes S, Brun R, *et al.* Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature*, 2004, 430(7002); 900-4.
54. Vítek L, Ostrow JD. Bilirubin Chemistry and Metabolism; Harmful and Protective Aspects. *Current Pharmaceutical Design*, 2009, 15; 2869-83.
55. Walker K. A review of NC methods for African malaria vectors. *Environ. Health Proj.*, 2002, 2; 618-27.
56. Weemhoff JL, Woolbright BL, Jenkins RE, *et al*. Plasma biomarkers to study mechanisms of liver injury in patients with hypoxic hepatitis. *Liver International*, 2017, 37(3); 377-84.
57. WHO. Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000–2010. Geneva, Switzerland: World Health Organization, 2010.
58. WHO. World malaria report 2014. Geneva: World Health Organization; 2014.
59. WHO. World Health Organization; World Malaria Report 2019. Available online: https://www.who.int/publicationsdetail/world-malaria-report-2019.

60. Wright PJ, Leathwood AA, Plummer DT. Enzymes in rat urine: Alkaline phosphatase. *Enzymologia*. 1972, 42(4); 317-27.
61. Wróblewski F, Ladue JS. Lactic Dehydrogenase Activity in Blood. *Proceedings of the Society for Experimental Biology and Medicine*, 1955, 90(1); 210-3.
62. Zargar R, Raghuwanshi P, Rastogi A, Koul AL, Khajuria P, Ganai AW, Kour S. Protective and ameliorative effect of sea buckthorn leaf extract supplementation on lead induced hemato-biochemical alterations in Wistar rats. *Veterinary World*, 2016, 9(9); 929-34.
63. Jendrassik L, Grof P. Determination of bilirubin in blood. *Biochemische Zeitchrift*, 1938, 297(8).
64. WHO. Malaria Policy Advisory Committee and Secretariat. Malaria Policy Advisory Committee to the WHO: conclusions and recommendations of September 2013 meeting. *Malar J*, 2013; **12:** 456.
65. Sellers, RS, Mortan D, Michael B, *et al.* Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. *Toxicologic Pathology*, 2007, 35(5), 751-5.
66. Michael B, Yano B, Sellers RS, *et al.* Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicologic Pathology*, 2007, 35(5), 742-50.
67. Amacher D, Schomaker S, Boldt S, Mirsky M. Relationship among microsomal enzyme induction, liver weight, histological change in cynomolgus monkey. *Food Chem Toxicol*, 2006, (4); 528-37.
68. Juberg D, Mudra D, Hazelton G, Parkinson A. Effect of fenbuconazole on cell proliferation and enzyme induction in the liver of female CD1 mice. *Toxicol & Applied Pharmacol*, 2006, 214(2), 178-87.
69. Greaves P. Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation. 4th edition, Academic Press, San Diego, 2012.
70. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. *Cellular & Molecular Immunology*, 2016, 13, 267-76.