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Assessment of Prolonged Administration of Synriam™ on Haematological Parameters and Lipid Profiles of Rats

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ABSTRACT: A potential antimalarial prospect should have high efficacy with low toxicity. Synriam™ antimalarial drug has been proven to be safe and effective for the treatment of malaria in human with dearth information on rats. This study investigated its potential toxicity from prolonged administration on lipid profiles and haematological indices in rats. Thirty-five Wistar rats were randomized into five groups (A – E) of seven rats each. Animals in group A (control) received 0.5 mL distilled water, while groups B, C, D, and E respectively received 4.0, 8.0, 16.0 and 32.0 mg/kg bwt of the drug once daily for 28 days. The concentrations of serum TC, LDL and atherogenic index showed significant (p<0.05) decrease, while serum TRIG, HDL, and VLDL concentrations had no effect (p>0.05). The drug also had no effect (p>0.05) on the levels of the red blood cell count, PCV, Hb, MCV, MCH, and MCHC. Similarly, it had no effect (p>0.05) on the levels of the WBC, lymphocytes and neutrocytes, while the platelet counts had slight (p<0.05) decrease. Hence, Synriam™ is relatively safe at doses of 4.0-32.0 mg/kg bwt in rats when taken once daily with minimal effects on the lipid profiles and haematological indices.

Keywords: Antimalarial, Arterolane malate, Piperaquine phosphate, Synriam™, Toxicity

Introduction

Malaria is still the most prevalent and devastating parasitic disease in several tropical countries (Wang *et al.,* 2017). It is an ancient protozoal blood infection caused by a mosquito-borne Apicomplexan parasite, which is transmitted to humans during the bite of an infected female *Anopheles* mosquito species (Nureye and Assefa, 2020). *Plasmodium falciparum* infection caused 99% death in human malaria cases worldwide in 2015. Symptoms and pathologies of *P. falciparum* malaria are entirely due to parasite stages that infect and remodel host red blood cells (RBCs) (WHO, 2015; Bhattacharjee *et al.,* 2018; Hernández-Castañeda *et al.,* 2021).
Plasmodium infection is thought to have a history of longer than 10 thousand years in malaria endemic areas where some populations by way of increasing resistance to malaria, have developed protective genetic mutations that lead to altered protein expression in RBCs, such as haemoglobin S (HbS) (the basis of sickle cell anaemia) and glucose-6-phosphate dehydrogenase deficiency. This genetic resistance is inheritable, implying that RBCs are at the forefront of responding to the selective pressure imposed by malaria (Allison, 1954; Ruwende and Hill, 1998; Bunn, 2013; Wang *et al.,* 2017). Several laboratorial abnormalities however occur in the cause of malaria infection such as anaemia, thrombocytopenia, methemoglobinemia, and blood lipid levels (Baird, 2013; Dias *et al.,* 2016; Gallagher, 2022).
Extracellular vesicles have been suggested to play crucial roles in the transportation of molecules among cells for signal transduction, transcriptional regulation during immune responses, and inflammatory reactions. High levels of circulating microparticles (MPs) have been detected in patients with malaria, and higher levels of MPs seemed to be linked to the progress of immunopathological lesions in cerebral malaria. Reflecting *in vivo*

findings, data from *in vitro* culture have shown that exosome-like vesicles derived from *P. falciparum*-infected RBCs (*Pf*RBCs) act as messengers, setting up communication between parasites during asexual blood stages and helping direct the parasites to develop into sexual stages (Robbins and Morelli, 2014; Wang *et al.,* 2017). Data from mouse models also show that reticulocyte-derived vesicles that contain parasite protein are involved in modulating the immune response during *P. yoelii* infection and provide immunoprotection for the host against the attack of a lethal *P. yoelii* strain (Wang *et al.,* 2017).

Variations in serum lipid profile have been associated with genetics and with infectious and inflammatory diseases as well as in chronic or acute conditions (Dias *et al.,* 2016). Malaria parasite uses cholesterol and phospholipids for survival in its human host, where circulating high-density lipoprotein (HDL) particles and erythrocytic membrane are the potential sources of cholesterol, while the source of phospholipids is erythrocytic membrane (Njoku *et al.,* 1995; Labaied *et al.,* 2011; Sirak *et al.,* 2016). Most plasma apolipoproteins, endogenous lipids, and lipoproteins have their origin in the liver, and this depends on cellular integrity and functionality of the hepatocytes (Jiang *et al.,* 2006). Chylomicrons, very low-density lipoproteins (VLDL), low-density lipoprotein (LDL), HDL, and free fatty acids are known to be the major lipid component lipoproteins in plasma. A population-based study on common lipid parameters showed that cholesterol levels tend to be lower among Africans, where malaria parasite is endemic, than in several other parts of the world. Lower levels of lipids, with hypolipoproteinemia, such as total cholesterol (TC), HDL, and LDL was observed in patients suffering from malaria caused by *falciparum* species (Fielding and Fielding, 2002; Sirak *et al.,* 2016). This implies that patients with this disease present hypocholesterolemia, decreased levels of HDL and LDL, which are accompanied by increased levels of triglycerides, and VLDL. Such lipid aberrations are transient, occurring in the most prevalent species of *Plasmodium* as well as in complicated or non-complicated cases (Dias *et al.,* 2016; Amah *et al*., 2021).

Haematological alterations are some of the most common complications that cause changes in major cell lines such as RBCs, white blood cells (WBCs), and platelets. Blood cells infected with malaria parasites are usually associated with increased haematological parameters values. Despite causing complication and malaria pathology, haematological changes such as anaemia, thrombocytopenia, and leukocytosis or leukopoenia have been reported but the degree of alterations varies according to the level of malaria endemicity, nutritional status, background hemoglobinopathy, socio-demographic factors, and malaria immunity (Sirak *et al.,* 2016). The extent of serum lipid and haematological parameters changes during malarial infection and their underlying biological mechanisms in level of parasitaemia remain unclear. This therefore calls for further investigation about malaria and its association with lipid and haematological parameters, which could be important for management of malaria-infected patients, and better understanding in this relationship is also crucial in the development of potential antimalarial regimen (Sirak *et al.,* 2016; Megabiaw *et al*., 2022).

Artemisinins, despite being combined with other drugs, is faced with the challenge of mismatch in demand and supply since it is derived from plants. These facts therefore call for an urgent need for development of novel synthetic antimalarial regimens with similar antimalarial potency (Ridley, 2002; Shanks, 2006; Valecha *et al.,* 2010; Belete, 2020).

Synriam™ is a commercially available antimalarial drug containing a fixed-dose combination of arterolane maleate (AM) and piperaquine phosphate (PQP). The combination (150 mg AM and 750 mg PQP) has been proven to be effective and safe for the treatment of acute uncomplicated *P. falciparum* malaria (Valecha *et al.,* 2010; Toure *et al.,* 2016). It is a synthetic trioxolane that is affordable, easy to synthesize, and rapid-acting oral antimalarial drug. It exhibits potency and a rapid onset against all erythrocytic stages of *falciparum* and *vivax* (Valecha *et al.,* 2010; Toure *et al.,* 2016). The combination provides antimalarial activity at different time windows that will prevent the emergence of resistance to either drug. It has its mechanism of action different from those of artemisinins (Valecha *et al.,* 2012). It has been reported to show *in vitro* potency higher than that of chloroquine, mefloquine, artemether, and artesunate against K1 (chloroquine-resistant) and NF54 (chloroquine-susceptible) strains of *falciparum*, as well as being highly effective and safe *in vivo* against *Plasmodium berghei* in mice (Valecha *et al.,* 2010).

Arterolane maleate is a novel antimalarial agent with IUPAC name cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methyl-propyl) amino] carbonyl] methyl]-1', 2', 4'-trioxaspiro [4, 5] decane maleate, developed by Ranbaxy Research Laboratories (Haryana, India) (Vennerstrom *et al.,* 2004). It is the first fully synthetic oral antimalarial compound having rapidly acting parasiticidal activity similar to artemisinin derivatives. It is highly active against laboratory adapted *falciparum* strains, rodent parasites *in vivo*, and field isolates from Gabon (Vennerstrom *et al.,* 2004; Kreidenweiss *et al.,* 2006). It is cytotoxic to all parasite stages of *falciparum* at 100 × IC_{50} (0.91 ± 0.12 ng/mL) level, exposed for 6 h or longer. The distribution between RBCs and plasma is 1.5 for uninfected RBCs and up to 270 for infected RBCs. Safety pharmacology studies conducted indicate that AM is safe and does not produce any clinically significant effect on behavioural parameters or the cardiovascular system (Maerki *et al.,* 2006; Gautam *et al.,* 2011). PQP is a slow absorption, long mean terminal elimination half-life and large mean volume distribution antimalarial drug that prevent recrudescent infections

(D'Alessandro, 2009). It has been proven to be an effective and well-tolerated antimalarial drug and its efficacy, tolerability, pharmacokinetic profile and low cost makes it a promising partner drug for use with short and rapidly acting antimalarial agents (Toure *et al.*, 2015; Valecha *et al.*, 2016). Some of the advantages of the combined drug over ACTs are its once-daily dose and low pill burden as well as a long duration of post-treatment prophylaxis (Toure *et al.*, 2016). Gautam *et al.* (2011) and D'Alessandro (2009) respectively established the clinical efficacy and safety of AM and PQP in patients with uncomplicated *falciparum* malaria. Meanwhile, Valecha *et al.* (2012) and Toure *et al.* (2015) have reported the efficacy and safety of the clinical trial of the combination in patients with acute uncomplicated *falciparum* malaria.

This study thereofore aimed at elucidating the toxicity of prolonged administration of Synriam™ in the lipid profiles and haematological parameters of adult Wistar rats.

Materials and methods

Chemicals and reagents: Assay kits for total cholesterol, triglycerides, high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) 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 (*Rattus norvegicus*) with an average weight of 150 ± 5.0 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 - 30 ^{o}C, 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. The animals in group A (control) were given 0.5 mL distilled water (the vehicle), while those in groups B-E were administered with the same volume of drug corresponding to 4.0, 8.0, 16.0, and 32.0 mg/kg body weight. Both the distilled water and drug were administered orally once daily using oropharyngeal cannula for a period of 28 days.

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 tablets were ground into fine powder using mortar and pestle, and then transferred into an air-tight bottle for storage.

Determination of biochemical indices: The biochemical parameters were determined using standard methods described for total cholesterol (Tietz, 1995), triglycerides (Tietz, 1995), high density lipoprotein (Friedwald *et al.*, 1972), low density lipoprotein (Friedwald *et al.*, 1972), very low density lipoprotein (Friedwald *et al.*, 1972), and atherogenic index (Lamarche *et al.*, 1996). The haematological parameters such as red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), neutrophils (NEUT), lymphocytes (LYM) and platelets count (PLT) were determined using automated haematology analyser (SYSMEX XS-1000i model, SYSMEX Corporation, Japan) after whole blood from each rat was collected in EDTA sample bottle.

Sample collection and preparation: All the animals 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 being 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.

Statistical analysis: Data obtained were presented as mean ± S.E.M. (n=7) replicates. Statistical analysis were carried out using one-way analysis of variance (ANOVA) and compared 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 effect of prolonged oral administration of Synriam™ on selected red blood cell parameters: The drug combination at the doses of 4.0, 8.0, 16.0 and 32.0 mg/kg body weight did not significantly (p>0.05) alter the concentrations of RBC, PCV, Hb, MCV, MCH and MCHC compared to control (Table 1).

Table 1: Effects of prolonged oral administration of drug combination on selected red blood cells indices of rats

Parameters	Control	Dose (mg/kg body weight)			
		4.0	8.0	16.0	32.0
RBC (10^{12}/L)	8.41 ± 0.10[a]	8.76 ± 0.10[a]	8.63 ± 0.08[a]	8.22 ± 0.06[a]	8.26 ± 0.11[a]
PCV (%)	45.37 ± 0.30[a]	47.30 ± 0.40[a]	45.40 ± 0.32[a]	44.13 ± 0.20[a]	47.43 ± 0.20[a]
Hb (%)	11.40 ± 0.20[a]	11.37 ± 0.10[a]	12.17 ± 0.10[a]	11.96 ± 0.13[a]	12.20 ± 0.17[a]
MCV (10^3/µL)	54.50 ± 0.31[a]	53.67 ± 0.29[a]	56.00 ± 0.28[a]	53.97 ± 0.18[a]	58.40 ± 0.29[a]
MCH (pg)	14.47 ± 0.16[a]	13.97 ± 0.12[a]	15.53 ± 0.13[a]	15.53 ± 0.20[a]	15.20 ± 0.11[a]
MCHC (g/dL)	25.53 ± 0.12[a]	24.37 ± 0.10[a]	26.10 ± 0.17[a]	24.67 ± 0.11[a]	25.40 ± 0.15[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).

The effect of prolonged oral administration of Synriam™ on selected white blood cell and platelet parameters: Similarly, in Table 2, administration of graded doses of the drug did not significantly (p>0.05) alter the levels of WBC, LYM and NEU in a dose-dependent manner, when compared to control. The PLT level at all doses revealed a significant (p<0.05) decrease in a dose-dependent manner compared to control (Table 2).

Table 2: Effects of prolonged oral administration of drug combination on selected white blood cells indices and platelet count of rats

Parameters	Control	Dose (mg/kg body weight)			
		4.0	8.0	16.0	32.0
WBC (10^9/L)	18.20 ± 0.17[a]	21.37 ± 0.14[a]	16.73 ± 0.20[a]	15.50 ± 0.13[a]	20.53 ± 0.15[a]
LYM (%)	92.37 ± 0.37[a]	93.17 ± 0.23[a]	95.43 ± 0.18[a]	92.17 ± 0.23[a]	94.97 ± 0.45[a]
NEU (%)	8.41 ± 0.10[a]	8.76 ± 0.14[a]	7.63 ± 0.08[a]	8.22 ± 0.06[a]	8.26 ± 0.11[a]
PLT (10^3/µL)	747.00 ± 2.65[a]	662.33 ± 1.45[b]	665.33 ± 2.03[b]	694.33 ± 2.03[c]	706.67 ± 3.18[d]

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

The effect of prolonged oral administration of Synriam™ on lipid profile indices: Figures 1-5 show the results of serum lipid profiles in rats. Prolonged oral administration of the Synriam™ significantly (p<0.05) decreased the concentrations of serum total cholesterol and LDL, while the concentrations of serum triglycerides, HDL and VLDL had no significant (p>0.05) alteration, when compared to their respective controls (Figures 1-5). Meanwhile, the atherogenic index level also reduced significantly (p<0.05) in a dose-dependent manner compared to control (Figure 6).

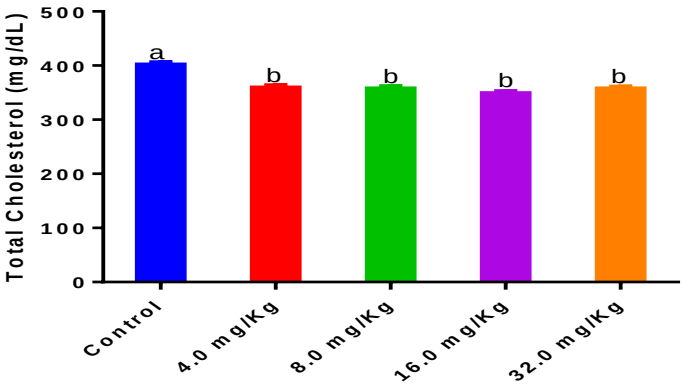

Figure 1: Serum total cholesterol concentrations of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

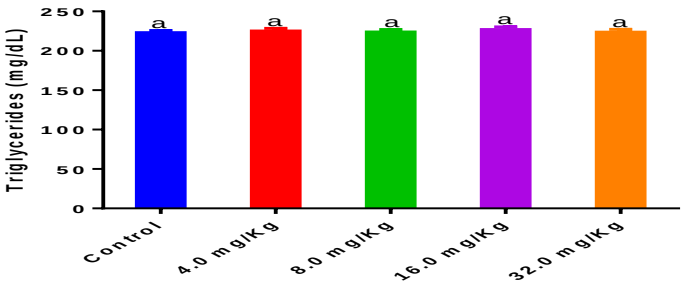

Figure 2: Serum triglycerides concentrations of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

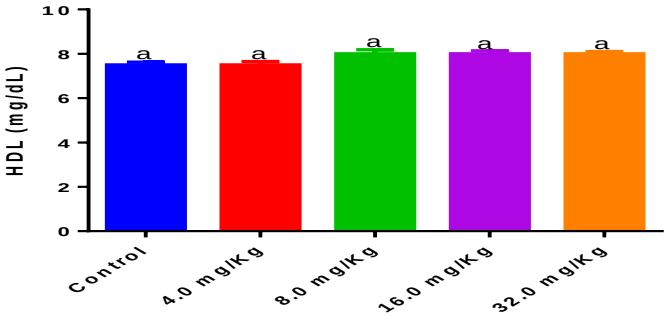

Figure 3: Serum high density lipoprotein (HDL) concentrations of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

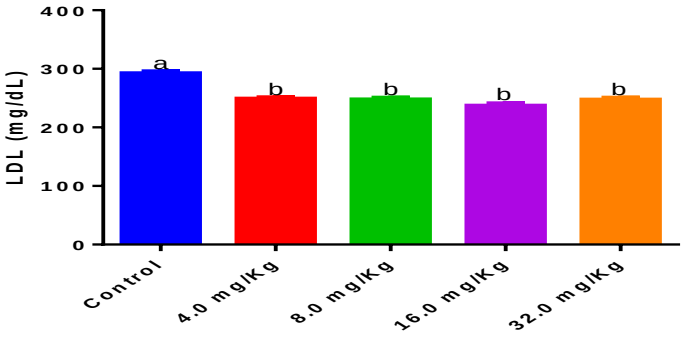

Figure 4: Serum low density lipoprotein (LDL) concentrations of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

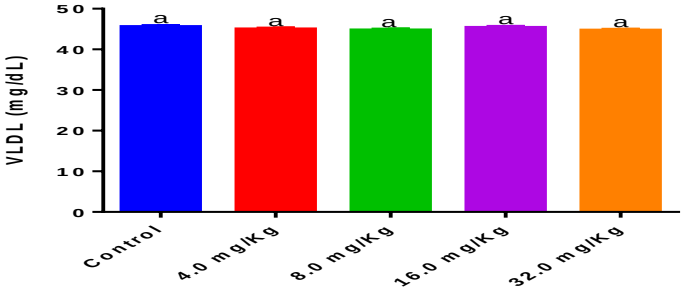

Figure 5: Serum very low density lipoprotein (VLDL) concentrations of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

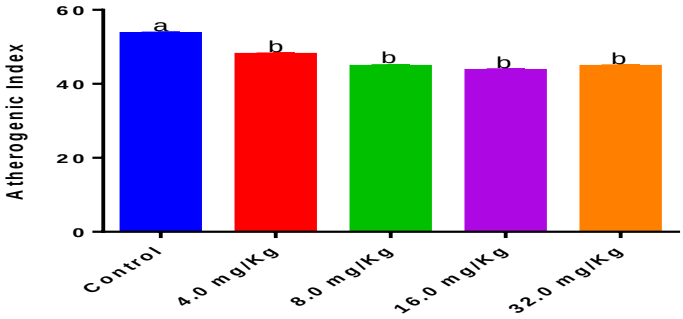

Figure 6: Serum atherogenic index levels of rats following prolonged oral administration of drug combination. Values are mean of seven determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different (p<0.05).

Discussion

Malaria is a global disease which has caused almost half of the population worldwide to be at risk of malaria, with approximately 247 million cases of malaria and 619,000 mortality in 2021, and infants, children less than 5 years of age, pregnant women and those with low immunity were the most vulnerable (Song *et al*., 2023). Its control is presently hampered not only by development of mosquitoes' resistance to the available insecticides, but also by the currently existing antimalarial agents. Therefore, it is expedient to ascertain the safety of Synriam^{TM}, a commercially available antimalarial agent in Ilorin, Kwara State, Nigeria, in rats. Fixed-dose combination of Synriam^{TM} (arterolane maleate 150 mg and piperaquine phosphate 750 mg tablet) have been established for its antimalarial efficacy, safety, tolerability, and pharmacokinetics in Phase I, II and III studies (Valecha *et al.*, 2012; Saha *et al.*, 2014; Toure *et al.*, 2015, 2016).

Cardiovascular diseases (CVDs) refer to different conditions such as cardiomyopathy, coronary heart disease, strokes, and rheumatic heart disease that affect the functions of the heart and blood vessels. Majority of CVD are due to atherosclerosis (coronary heart disease and ischemic strokes) while others caused by infections (cerebrovascular complications of malaria, rheumatic heart disease, cardiomyopathy from HIV infection) are common in developing countries (Celermajer *et al.*, 2012). Indices such as serum levels of total cholesterol, triacylglycerol, HDL-cholesterol, and LDL-cholesterol are commonly used to assess risk of CVDs. High cholesterol level in blood is linked with atherosclerosis, myocardial infarction and other CVDs (Bhatnagar *et al.*, 2008; Nelson, 2013). Since cholesterol is basically insoluble in water, it is transported bound to lipid transporters (apolipoproteins) to form lipoproteins in aqueous extracellular environment (Zhornitsky *et al.*, 2016).

The decrease in total cholesterol concentration observed throughout the period of the experiment may be attributed to the ability of the drug to increase the excretion of cholesterol thus, reducing the risk of atherosclerosis and CVDs. Similarly, decrease in LDL level observed throughout the period of the experiment may be attributed to inability of the drug to damage the arteries that carry blood from the heart to the rest of the body by preventing buildup of plaque on the arterial walls. The HDL concentrations were not altered which may indicate that the drug may not be effective in promoting risk of atherosclerosis. The decrease in atherogenic index could be an indication that the drug may prevent progression of CVDs associated with malaria, since the computed ratio is a vital pointer to CVD predisposition (Naghii *et al*., 2011; Adebayo *et al*., 2013).

The blood functions to deliver oxygen (O_2) and nutrients to tissue, remove carbon dioxide (CO_2) and metabolic waste from tissues, fight infections and cause coagulation at site of broken vessel (Nelson and Cox, 2005; Scanlon and Sanders, 2007). The biochemical, physiological and pathological status of a biological system can be analyzed through haematological parameters. The main function of the red blood cells (RBCs) is to carry O_2 to tissues and to transfer CO_2 to the lungs, and very low readings for RBC can indicate anaemia. Erythrocytes are the main site of infection in malaria because almost all the clinical manifestations are primarily due to involvement of RBCs. The multiplying parasites degrade and consume intracellular haemoglobin (Hb). The red cell membrane properties are altered and this results in increased membrane permeability (David *et al*., 2002; Scanlon and Sanders, 2007). Malaria infection is associated with reduction in Hb level resulting in haemolytic anaemia (Balogun *et al.*, 2009; Siqueira *et al.*, 2014), but dehydration and polycythemia could elevate its concentration (Hall, 2011). Packed cell volume (PCV) is a measure of the percentage of blood occupied by RBCs which is relatively constant among mammals (Everds, 2007). Mean corpuscular volume (MCV) is a

measure of the average size of RBCs (Washington and Van Hoosier, 2012), and elevated levels may indicate macrocytic anaemia possibly caused by iron deficiency (Guyton and Hall, 2006). The mean corpuscular haemoglobin (MCH) is the average quantity of Hb per erythrocyte in blood. Due to the use of RBCs in its circulation, MCH is not an accurate diagnosis of severe anaemia. Decreased MCH is however associated with microcytic anaemia (Ganong, 2001). The mean corpuscular haemoglobin concentration (MCHC), being a measure of Hb level in a given PCV value, is reduced in severe iron-deficiency anaemia or microcytic anaemia as a result of reduced Hb concentration, though falsely elevated in haemolyzed sample (Ganong, 2001; Washington and Van Hoosier, 2012). The RBC, Hb and PCV are indices associated with the total population of erythrocytes in the blood while MCHC, MCH and MCV are indices that are associated with individual erythrocyte (Adebayo *et al*., 2005).

One of the most common complications in malaria infection especially in younger children and pregnant women in high transmission areas is anaemia (Menendez *et al*., 2000). The pathogenesis of anaemia during malaria infection is thought to result from the parasite's primary target (the RBC) resulting in RBCs destruction (Kitua *et al*., 1997; Balogun *et al.,* 2009). Throughout the period of experiment, the levels of RBC count, Hb, PCV, MCV, MCH, and MCHC of the treated groups were not altered compared to untreated groups. This is an indication that the drug neither impaired the synthesis of Hb nor resulted in anaemia and did not alter the morphology and osmostic fragility of erythrocyte, and as such the respiratory gas-carrying capacity of the blood remains intact.

White blood cells (WBCs), also called leucocytes, function mainly to fight infection and defend against foreign bodies by phagocytosis and production of antibodies in the immune response. WBCs are known to increase sharply when infection occurs, as one of the first line of defense of the body (Swash and Mason, 1984; Balogun *et al.,* 2009), but in this study, the WBC parameters were not altered at all doses when compared to the control. This could imply that the production of WBCs was not impaired and the ability of the biological system to fight infections was not compromised.

Platelets (PLT) function to stop haemorrhage at the site of broken endothelium by contributing to vessel constriction, repair and host defense (Paniccia *et al*., 2015). A rise in PLT level may be caused by anaemia, inflammation, or infection, while reduction could result from inhibition of its synthesis by drugs, foods, kidney infections or dysfunction (George, 2000). PLTs are connected to the pathogenesis of inflammatory diseases such as malaria and atherosclerosis (Morrell *et al.,* 2014), and its reduction and elevation have been reported in cases of malaria (Kakar *et al.,* 1999; McMorran *et al.,* 2009; Balogun *et al.,* 2009). Since the body has a very limited PLT reserve, it therefore can be rapidly depleted, and reduction is also fairly common in malaria which may result from sequestration of PLTs in the spleen (Horstmann *et al.,* 1981; Wagner and Burger, 2003; Balogun *et al.,* 2009). The decrease observed throughout this study could indicate that prolonged oral administration of the drug may inhibit PLT formation, adversely affecting its roles in host defense which could lead to diseases such as leukaemia. More so, it may also be caused by underlying health conditions of the subjects.

Therefore, the results of the lipid profiles and blood parameters in this study revealed that, Synriam™, which is a component of arterolane malate (a short-acting drug that is effective against all parasite blood stages) and piperaquine phosphate (a slow, long-acting drug that kills residual parasites) having slightly different mechanism of action from artemisinins, has proven to be relatively safe with minimal effects at recommended doses in rats; with the decrease in PLT attributed probably to the health conditions of the subjects or the side effects of the drug. The relative safety of this drug at 4.0, 8.0, 16.0 and 32.0 mg/kg in this study supports the study of Nwikwe and Balogun (2021) who reported its safety in the hepatocytes after 28 days oral administration in rats. It also corroborates the study of Valecha *et al.* (2012) who reported its safety and efficacy in human clinical trial in patients with uncomplicated malaria at single doses of 50, 100 and 200 mg/kg.

Conclusion

Synriam™ is relatively safe at doses of 4.0-32.0 mg/kg body weight (25-200 mg; doses approved for a 70 kg man) when taken once daily with minimal effects on the lipid profiles and haematological indices of rats.

Acknowledgement

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Abbreviations:

TC=Total cholesterol, LDL=Low-density lipoprotein, TRIG=Triglycerides, HDL=High-density lipoprotein, VLDL=Very low-density lipoprotein, PCV=Packed cell volume, Hb=Haemoglobin, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentrations, WBC=White blood cells.

References

Adebayo JO, Adesokan A, Olatunji L, Buoro D, Soladoye A: Effect of ethanolic extract of *B. spectabilis* leaf on hematological and serum lipid variables in rats. Biokemistri, 17(1): 45-50. 2005.

Adebayo JO, Balogun EA, Malomo S, Soladoye A, Olatunji L, Kolawole O, Oguntoye O, Babatunde A, Akinola O, Aguiar A, Andrade I, Souza N, Krettli A: Antimalarial activity of *Cocos nucifera* husk fibre: further studies. Evid.-based Complement Altern Med, 2013: 742476. 2013.

Allison AC: Protection afforded by sickle-cell trait against sub-tertian malarial infection. Br Med J, 1: 290-4. 1954.

Amah CC, Enechi OC, Ekpo DE, Okagu IU, Ononiwu CP, Joshua PE: Safety assessment and antimalarial property of methanol extract of *Fagara zanthoxyloides* root-bark on *Plasmodium berghei*-infected mice. Comp Clin Path, 30: 217-228. 2021.

Baird JK: "Malaria caused by *Plasmodium vivax*: recurrent, difficult to treat, disabling, and threatening to life-averting the infectious bite preempts these hazards," Pathog Glob Health, 107(8): 475-9. 2013.

Balogun EA, Adebayo JO, Zailani AH, Kolawole OM, Ademowo OG: Activity of ethanolic extract of *Clerodendrum violaceum* leaves against *Plasmodium berghei* in mice. Agric Biol J N Am, 1(3): 307-12. 2009.

Bhatnagar D, Soran H, Durrington PN: Hypercholesterolaemia and its management. BMJ, 337: 503-8. 2008.

Bhattacharjee S, Coppens I, Mbengue A, Suresh N, Ghorbal M, Slouka Z, Safeukui I, Tang HY, Speicher DW, Stahelin RV, Mohandas N, Haldar K: Remodeling of the malaria parasite and host human red cell by vesicle amplification that induces artemisinin resistance. Blood, 131(11): 1234-1247. 2018.

Bunn HF: The triumph of good over evil: protection by the sickle gene against malaria. Blood, 121: 20-5. 2013.

Celermajer DS, Chow CK, Marijon E, Anstey NM, Woo KS: Cardiovascular disease in the developing world: prevalences, patterns, and the potential of early disease detection. J Am Coll Cardiol, 60(14): 1207-16. 2012.

Alessandro U: Progress in the development of piperaquine combinations for the treatment of malaria. Curr Opin Infect Dis, 22: 588-92. 2009.

David JW, Louis H, Miller DI, Baruch KM, Ogobara KD, Climent CP, David JR: Malaria and red cell. Haematol, 1: 35-57. 2002.

Dias RM, Vieira JLF, Cabral BC, da Silva IRP, Brasil LMB, Araújo EC, de Andrade MA: Lipid profile of children with malaria by *Plasmodium vivax.* J Trop Med, 2016, Article ID 9052612: 1-5. 2016.

Everds N: Hematology of laboratory mouse. In The Mouse in Biomedical Research (Fox J., Barthold, S, Davisson, M., Newcomer, C., Quimby, F., Smith, A. eds.), Academic Press, 3: 133-70. 2007.

Fielding PE, Fielding CJ: Dynamics of lipoprotein transport in the human circulatory system. New Compr Biochem, 36: 527-52. 2002.

Friedwald WT, Levey RI, Friedrickson DS: Estimation of the concentration of LDL in plasma without the use of preparative concentrating. Clin Chem, 18: 499-502. 1972.

Gallagher PG: Anemia in the pediatric patient. Blood, 140(6): 571-593. 2022.

Ganong WF: Review of Medical Physiology; 20th Edition. *Lange Medical Books McGraw-Hill Medical Publishing Division*, London. pp. 414-7. 2001.

Gautam A, Ahmed T, Sharma P, Varshney B, Kothari M, Saha N: Pharmacokinetics and pharmacodynamics of arterolane maleate following multiple oral doses in adult patients with *P. falciparum* malaria. J Clin Pharmacol, 51: 1519-28. 2011.

George JN: Platelets. Lancet, 355: 1531-9. 2000.

Guyton AC, Hall JE: Textbook of Medical Physiology (10th Edition). Harcourt International Edition. W.B. Saunders and Co., Philadelphia, USA. pp. 279-81, 782-83. 2006.

Hall JE: Guyton and Hall Textbook of Medical Physiology. 12th edition, Saunders Elsevier, Philadelphia. pp. 101-120, 303-321, 413-32. 2011.

Hernández-Castañeda MA, Lavergne M, Casanova P, Nydegger B, Merten C, Subramanian BY, Walch M: A profound membrane reorganization defines susceptibility of *Plasmodium falciparum*-infected red blood cells to lysis by granulysin and perforin. Front Immunol, 12: 643746. 2021.

Horstmann RD, Dietrich M, Bienzle U, Rasche H: Malaria-induced thrombocytopenia. Ann Haematol, 42(3): 157-64. 1981.

Jiang J, Nilsson-Ehle P, Xu N: Influence of liver cancer on lipid and lipoprotein metabolism. Lipids Health Dis, 5: 4. 2006.

Kakar A, Bhoi S, Prakash V, Kakar S: Profound thrombocytopenia in *Plasmodium vivax* malaria. Diagn. Microbiol. Infect Dis, 35(3): 243-4. 1999.

Kitua AY, Smith TA, Alonso PL, Urassa H, Masanja H, Kimario J, Tanner M: The role of low level *Plasmodium falciparum* parasitaemia in anemia among infants living in an area of intense and perennial transmission. Trop Med Int Health, 2: 325-33. 1997.

Kreidenweiss A, Mordmüller B, Krishna S, Kremsner PG: Antimalarial activity of a synthetic endoperoxide (RBx-11160/OZ277) against *Plasmodium falciparum* isolates from Gabon. Antimicrob Agents Chemother, 50: 1535-7. 2006.

Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, Coppens I: *Plasmodium* salvages cholesterol internalized by LDL and synthesized *de novo* in the liver. Cell Microbiol, 13(4): 569-86. 2011.

Lamarche B, Després JP, Moorjani S, Cantin B, Dagenais GR, Lupien PJ: Triglycerides and HDL-cholesterol as risk factors for ischemic heart disease. Results from the Quebec cardiovascular study. Atherosclerosis, 119(2): 235-45. 1996.

Maerki S, Brun R, Charman S, Dorn A, Matile H, Wittlin S: *In vitro* assessment of the pharmacodynamic properties and the partitioning of OZ277/RBx-11160 in cultures of *Plasmodium falciparum*. J Antimicrob Chemother, 58: 52-8. 2006.

McMorran B, Marshall V, deGraaf C, Shabbar M, Smyth G, Corbin J, Alexander W, Foote S: Platelets kill intraerythrocytic parasites and mediate survival to infection. Science, 323(5915): 797-800. 2009.

Megabiaw F, Eshetu T, Kassahun Z, Aemero M: Liver enzymes and lipid profile of malaria patients before and after antimalarial drug treatment at Dembia Primary Hospital and Teda Health Center, Northwest, Ethiopia. Res. Rep Trop Med, 11-23. 2022.

Menendez C, Fleming AF, Alonso PL: Malaria-related anaemia. Parasitol Today, 16: 469-76. 2000.

Morrell CN, Aggrey AA, Chapman LM, Modjeski KL: Emerging roles for platelets as immune and inflammatory cells. Blood, 123(18): 2759-67. 2014.

Naghii MR, Almadadi M, Zarchi AA: Regular physical activity as a basic component of lifestyle modification reduces major cardiovascular risk factors among male armored force personnel of Shabestar army installation in Iran. Work, 40(2): 217-27. 2011.

Nelson DL, Cox MM: Amino acid oxidation and production of urea; Lipid Biosynthesis. In: Lehninger Principles of Biochemistry. 4th ed. W. H. Freeman and Company, England. pp. 664. 2005.

Nelson RH: Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care-Clin Off Pract, 40(1): 195-211. 2013.

Njoku OU, Ononogbu IC, Nwachukwu DE: Plasma cholesterol, b-carotene and ascorbic acid changes in human malaria. J Commun Dis, 27(3): 186-90. 1995.

Nureye D, Assefa S: Old and recent advances in life cycle, pathogenesis, diagnosis, prevention, and treatment of malaria including perspectives in Ethiopia. Sci World J, 2020, Article ID 1295381: 1-17. 2020.

Nwikwe DC, Balogun EA: Assessment of hepatotoxicity effects of long term administration of Synriam™ in rats. Biokemistri, 33(4): 235-248. 2021.

Paniccia R, Priora R, Liotta AA, Abbate R: Platelet function tests: a comparative review. Vasc Health Risk Manag, 11: 133. 2015.

Ridley RG: Medical need, scientific opportunity and the drive for antimalarial drugs. Nature, 415: 686-93. 2002.

Robbins PD, Morelli AE: Regulation of immune responses by extracellular vesicles. Nat Rev Immunol, 14: 195-208. 2014.

Ruwende C, Hill A: Glucose-6-phosphate dehydrogenase deficiency and malaria. J Mol Med, 76: 581-8. 1998.

Saha N, Moehrle JJ, Zutshi A, Sharma P, Kaur P, Iyer SS: Safety, tolerability and pharmacokinetic profile of single and multiple oral doses of arterolane (RBx11160) maleate in healthy subjects. J Clin Pharmacol, 54(4): 386-93. 2014.

Scanlon VC, Sanders T: Essentials of Anatomy and Physiology. 5th edition, F.A. Davis Company, Philadelphia. pp. 163-93, 251-87, 379, 421. 2007.

Shanks GD: Treatment of *falciparum* malaria in the age of drug resistance. J Postgrad Med, 52: 277-80. 2006.

Siqueira AM, Cavalcante JA, Vítor-Silva S, Reyes-Lecca RC, Alencar AC, Monteiro WM, Alexandre MA, Maria Paula GM, Guinovart C, Bassat Q, Alecrim MD: Influence of age on the hemoglobin concentration of malaria-infected patients in a reference centre in the Brazilian Amazon. Mem Inst Oswaldo Cruz, 109(5): 569-76. 2014.

Sirak S, Fola AA, Worku L, Biadgo B: Malaria parasitemia and its association with lipid and hematological parameters among malaria-infected patients attending at Metema Hospital, Northwest Ethiopia. Pathol Lab Med. 8: 43-50. 2016.

Song X, Cheng W, Zhu H, Li Y, Li J: Additive Therapy of *Plasmodium berghei*-induced experimental cerebral malaria via dihydroartemisinin combined with Rapamycin and Atorvastatin. Microbiol Spectr, 11(2): e02317-22. 2023.

Swash M, Mason S: Hutchison Clinical Methods. 18th ed. East Sussex Bailliere Tindall. pp. 34. 1984.

Tietz NW: Clinical Guide to Laboratory Tests, WB Saunders, Philadelphia, Pa, USA, 3rd edition. pp. 22-23. 1995.

Toure OA, Rulisa S, Anvikar A, Rao B, Mishra P, Jalali R, Arora S, Roy A, Saha N, Iyer S, Sharma P, Valecha N: 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, 14: 469. 2015.

Toure OA, Valecha N, Tshefu A, Thompson R, Krudsood R, Gaye O, Rao BHK, Sagara I, Bose T, Mohanty S, Rao BS, Anvikar A, Mwapasa V, Noedl H, Arora S, Roy A, Iyer S, Sharma P, Saha N, Jalali R: 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. Clin Infect Dis, 1-8. 2016.

Valecha N, Krudsood S, Tangpukdee N, Mohanty S, Kumar S, Tyagi P, Anvikar A, Mohanty R, Rao B, Jha A, Shahi B, Singh J, Roy A, Kaur P, Kothari M, Mehta S, Gautam A, Paliwal J, Arora S, Saha N: Arterolane maleate plus piperaquine phosphate for treatment of uncomplicated *Plasmodium falciparum* malaria: A comparative, multicenter, randomized clinical trial. Clin Infect Dis, 55(5): 663–71. 2012.

Valecha N, Looareesuwan S, Martensson A, Abdulla S, Krudsood S, Tangpukdee N, Mohanty S, Mishra S, Tyagi P, Moehrie J, Gautam A, Paliwal J, Roy A, Kothari M, Saha N, Dash A, Bjorkman A: Arterolane, a new synthetic trioxolane for treatment of uncomplicated *Plasmodium falciparum* malaria: A phase ii, multicenter, randomized, dose-finding clinical trial. Clin Infect Dis, 51(6): 684-91. 2010.

Valecha N, Savargaonkar D, Srivastava B, Rao BHK, Tripathi S, Gogtay N, Kochar S, Kumar N, Rajadhyaksha LJ, Solanki B, Jalali R, Arora S, Roy A, Saha N, Iyer S, Sharma P, Anvikar A: 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, 15: 42. 2016.

Vennerstrom JL, Arbe-Barnes S, Brun R, Charman SA, Chiu FC, Chollet J, Dong Y, Dorns A, Hunziker D, Matile H, McIntosh K, Padmanilayam M, Tomas J, Scheurer C, Scorneaux B, Tang Y, Urwyler H, Wittlin S, Charman W: Identification of an antimalarial synthetic trioxolane drug development candidate. Nature, 430(7002): 900-4. 2004.

Wagner DD, Burger PC: Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol, 23; 2131-7. 2003.

Wang Z, Xi J, Hao X, Deng W, Liu J, Wei C, Gao Y, Zhang L, Wang H: Red blood cells release microparticles containing human argonaute 2 and miRNAs to target genes of *Plasmodium falciparum*. Emerg Microbes Infect, 6(8): e75. 2017.

Washington IM, Van Hoosier G: Clinical Biochemistry and Hematology. In: Suckow, M.A., Stevens, K.A. and Wilson, R.P. (eds.), The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents, Academic Press, San Diego. pp. 57-116. 2012.

WHO. World Health Organization; World Malaria Report 2015. Geneva: WHO reports; 2015, pp. 243.

Zhornitsky S, McKay K, Metz L, Rangachari M: Cholesterol and markers of cholesterol turn-over in multiple sclerosis: relationship with disease outcomes. Mult. Scler. Relat. Disord., 5: 53-65. 2016.