Effect of Artesunate on Serum Bilirubin and Albumin in Swiss Wistar Rats

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ABSTRACT

Patients often take antimalarial drugs indiscriminately without a doctor's prescription as treatment for uncomplicated malaria in the tropics, especially in Africa, where malaria is endemic. If these patients eventually visit clinicians during complications, the clinicians request liver functions tests, which include Serum bilirubin and albumin, from the medical laboratories before making clinical decisions. The study was done to evaluate the effects of artesunate (antimalarial) on serum bilirubin and albumin of Wistar rats. The study was conducted on a total of 30 rats (5 groups of 6 rats each). Rats in the control group were given distilled water only while those in test groups were administered with 2.0mg/kg, 4.0mg/kg, 8.0mg/kg, and 16.0mg/kg of artesunate, respectively. Bilirubin was estimated using Jendrassik and Grof method while Albumin was estimated using Bromocresol green binding method (BCG). The results and statistical analysis indicated remarkable differences ($P<0.05$) in total bilirubin (TB), unconjugated bilirubin (UB), and albumin between the test and control groups but not in conjugated bilirubin (CB) ($P> 0.05$) between the test and control groups. The marked increase in total bilirubin was dose-dependent and attributed to unconjugated hyperbilirubinaemia resulting from increased lysis of red blood cells initiated by the drug. People in malaria-endemic areas and who take antimalarials often should evaluate their liver functions at least once a year.

Key Words: *Artesunate, Antimalarial drugs, Bilirubin, Albumin, Liver function, Malaria*

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INTRODUCTION

Malaria is a parasitic infectious disease, caused by *Plasmodium species* that infect the red blood cell (RBC) when an infected female anopheles mosquito inoculate them into the human host during a blood meal [1]. Transfusion of infected blood or its products can also transmit the parasite to the human host [2]. Five (5) Plasmodium species can infect and cause malaria in humans. They can be transmitted from one human host to another. These include *Plasmodium falciparum, P. ovale, P. knowlesi, P. vivax,* and *P. malariae* [3].

If the female anopheles mosquito ingests the sexual stages of plasmodium while feeding on infected blood, she becomes infected. The plasmodium develops in the mosquito and is passed into another human host during the next blood meal. In humans, the parasite first stage develops in the liver, from where more developed plasmodia are released into the bloodstream to infect RBCs, reproduce and cause RBCs to lyse and release another set of parasites [4]. If many cells lyse, it could lead to jaundice from the build-up of bilirubin from the hemoglobin (Hb) in the dead red cells [5].

P. *falciparum* is more virulent among the plasmodium species that cause malaria in man and it causes more severe disease. It accounts for the majority of malarial deaths [6]. Malaria is among the commonest and clinically important parasitic diseases in the world. The parasites cause illness in about 200 to 500 x 10^6 persons yearly [7] and account for about 500,000 deaths worldwide annually, mostly in

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children [8]. Most infections and deaths caused by malaria are in Asia and Africa [9]. Patients who have been properly diagnosed of being infected with malaria are administered antimalarial drugs for treatment [10].

The therapeutic aims are to completely cure uncomplicated malaria as soon as possible and to limit it from worsening to severe disease. Uncomplicated malaria is defined as when a patient shows symptoms of malaria and positive parasitological test (microscopy or Rapid Diagnostic Test) but shows no signs of severe malaria [3].

Antimalarial drugs, for example, Artesunate, are also prescribed for prophylaxis to people traveling to areas where malaria is prevalent. The type of drugs prescribed is dependent on the drug resistance in the areas [11].

Among the most common antimalarial drugs in use for malaria treatment are artemisinin derivatives, amodiaquine, chloroquine, atovaquone/proguanil, quinine, mefloquine, mepacrine or quinacrine, sulfadoxine/pyrimethamine, and primaquine [8]. Among them, Artemisinin is the most rapid-acting class, acting against the asexual stage gametocytes and blocking the sexual stage for both uncomplicated and severe malaria.

Artemisinin is a natural antimalarial drug derived from the plant *Artemisia annua.* Artemether and artesunate are members of the artemisinin antimalarials (sesquiterpene lactone compounds), developed to improve the bioavailability of artemisinin [3]. They are used to treat severe malaria for speedy clearance of all asexual stages of P. *falciparum,* with faster fever resolution than with other antimalarial drugs [12, 13].

Artesunate is a water-soluble, hemisuccinate derivative of dihydroartemisinin. It can be administered through parenteral (IM, IV) and enteral (oral) routes. For severe malaria, parenteral administration is recommended for initial treatment [3, 14].

The World Health Organization (WHO) recently approved Artemisinin-based Combination Therapy (ACT) as a first-line drug to treat uncomplicated falciparum malaria [6, 15] due to the increasing rate of malaria parasites resistance to quinine and other antimalarial drugs.

The ACT combines artemisinin derivative with another antimalarial drug with a different mode of action and longer-acting [3]. It is the recommended standard for the treatment of malaria in Nigeria [16]. It involves the simultaneous use of two or more drugs with separate modes of action and different targets in the parasite to kill the blood schizonts.

ACT regimens are strongly recommended, based on high-quality evidence, to provide treatment for 3 days with artemisinin derivative [3]. If properly followed, this gives efficient clearance of asexual and early transmission stages of parasites, while the infectious mature sexual stages are unaffected. To halt transmission, the ACT is often combined with Primaquine [15, 16]. The following artemisinin derivatives have been developed and distributed worldwide to combine with other drugs for treating malaria. Viz artemether, artesunate, dihydroartemisinin, artemisinin, and arteether. Many of them are associated with cases of idiosyncratic liver injury [8].

Upon administration, artesunate is hydrolyzed to Dihydroartemisinin (DHA), which accumulates in the *P. falciparum*-infected cells. DHA is the active metabolite and lasts longer than the artesunate in the infected RBCs. The plasma half-life of artesunate is between 3-29 minutes while that of DHA is between 40-95 minutes [15]. DHA binds to the plasma membrane of the infected RBC and suppresses the sarcoplasmic/endoplasmic reticulum calcium pump of *P. falciparum* [16].

Among the artemisinin-based drugs, artesunate is the most commonly used today. It mediates its effect by reducing the transmission of sexual stages (gametocyte) of the parasite. WHO recommends a complete course of 5 or 7 days, starting with 4.0mg per kg (body weight) for the first three days (combined with mefloquine), followed by 2.0mg per kg for the remaining days [15].

During infection of the RBCs, the plasmodium degrades Hb to get essential amino acids (aas) needed by the parasite for energy and to synthesize its protein. Haem is released from the iron-porphyrin complex of the degraded Hb. The two major pathways utilized in the mechanism of action of artemisinin involve inhibition of calcium transporters (PfATP618) of the parasite [16] and the breakdown of iron (or haem) [15].

Reactive oxygen species formed from the free ferrous ion (Fe^{2+}) generated from haem breakdown inhibit parasite's cell division [14] by cleaving the endoperoxide bridge.

The antimalarial drug inhibits replication of the parasite and formation of food vacuole by diffusion in the infected RBCs and the drug becomes protonated. Since the food vacuole has a PH of about 4.7 (acidic), the drug cannot diffuse out. It caps the hemozoin molecule and prevents more haem biocrystallization and consequently leads to accumulation of haem. The drug then binds to haem to form haem-drug complex, which is toxic to the cell and interrupts the function of the cell membrane. The action of the toxic complex ultimately leads to RBC lysis and autodigestion of parasite cells [4]. Haem build-up leads to hyperbilirubinaemia due to the release of excess bilirubin from RBC lysis [5].

The liver plays vital biochemical roles in several synthetic, digestive, detoxifying, metabolic, and excretory functions in the body. It has some reserve capability, being a large organ while performing its biochemical functions. The liver can prevent the decrease in protein concentrations, except there is pronounced liver damage [17]. In many individuals that have liver diseases, there can be normal liver functions, though there is extensive damage. Liver

disease in cases like that may be detected only by tests that detect liver injury and this is commonly accomplished by estimating the plasma concentrations of liver enzymes, that are released to the blood in a somewhat pulsatile fashion from hepatocytes, depending on the degree of liver injury. Liver functions can also be assessed by the measurement of serum bilirubin and albumin concentrations in blood. The biosynthetic function involves the regulation of protein, carbohydrate, and lipid metabolism. Albumin is synthesized only by the liver and it's commonly estimated in serum to assess the function of the liver [18].

The excretory function can be assessed by measuring the serum levels of bilirubin. Bilirubin is a byproduct of haem turnover from RBC lysis. It is excreted in bile and urine. If the rate of RBCs lysis is faster than normal, there is a tendency for bilirubin to accumulate in the blood and may cause jaundice [5]. Metabolism of bilirubin starts with the lysis of RBCs in the body. Hb is degraded to haem and globin. Bilirubin is formed from the haem fraction and is bound to albumin in the blood, transported to the liver to be conjugated with glucuronic acid, forming the conjugated (direct) bilirubin, and is released into bile that is stored in the gall bladder or taken directly to the small intestines where it is acted upon by intestinal bacteria. A small fraction of the end products undergo hepatic-duodenal re-uptake and later reappears in urine [5].

Under normal circumstances, the process of conjugation is very efficient and ensures a low amount of unconjugated bilirubin in plasma. In certain diseases with either an increased rate of bilirubin turnover or reduced conjugation of bilirubin, unconjugated hyperbilirubinaemia occurs. Conjugated hyperbilirubinaemia may result from reduced secretion of direct bilirubin into bile (as seen in hepatitis), or from diminished bile movement into the intestine (as seen in obstruction of bile ducts) [15]. Haemolysis can be seen in several medical procedures and diseases, such as malaria and the administration of certain drugs. Among the drugs are antimalarial medications [14].

Accumulation of bilirubin may also result from inadequate or impaired removal by the liver or leaking into the bloodstream after removal [18]. When the level rises to 3mg/dl or higher, it discolors other tissues, white parts of the eye, and skin yellow, a condition known as jaundice. Serum bilirubin estimation involves measurement of both total and direct bilirubin in the screening or monitoring of diseases of the liver or gall bladder [16]. In chronic liver diseases, the patients may exhibit high levels of bilirubin after blood transfusion, due to the high turnover of the transfused RBCs [14]. Unconjugated hyperbilirubinaemia may be due to unrestrained formation of bilirubin or reduced metabolic process by the liver [18].

Albumin is a small spherical protein (mw 66.3KDa). It accounts for about 50% of total plasma protein by mass. It has relatively high concentrations in plasma and due to its small size, it is the main protein constituent in extravascular fluids of the body [19]. Albumin is produced only in the liver. Measurement of albumin in the plasma is used as a good marker to evaluate its synthesis by the liver [20]. In apparently healthy individuals, about 0.2g to 0.3g of serum albumin is formed daily per kg body weight. Its serum concentration is preserved by the rate of synthetic and catabolic processes and its distribution between extravascular and vascular compartments. In inflammatory conditions or insufficient protein consumption, albumin synthesis may be suppressed. A decrease in albumin synthesis may lead to a serum albumin decrease of greater than 3.0g/L [20, 21].

Any chemical substance (drugs or toxins) that has either slight or pronounced hepatotoxic effects on the liver may affect its synthetic ability. The majority of the drugs bind to albumin and are taken to the liver for detoxification. Some drugs also bind to globulin [21]. For the fact that albumin is synthesized by the liver, a decrease of albumin concentration in serum may be indicative of liver injury or disease. Decreased albumin concentration may also come from comprehensive protein wasting, malnutrition, and extensive leakage in urine resulting from renal impairment [22].

Many substances like drugs, toxins, lipids, and hormones are transported in the blood, bound to albumin, and taken to the liver where they are detoxified by being converted to water-soluble forms for excretion [23]. Decreased albumin concentrations lead to lowered plasma oncotic pressure, which lets fluid out from interstitial spaces to the peritoneum, and produces ascites [21]. Acute liver damage does not usually cause low albumin concentrations in serum. It usually takes many weeks of consistent decrease of albumin synthesis to observe a drop in the amount of albumin in serum. Cirrhosis causes chronic liver damage and is the commonest reason for low albumin concentration. In chronic liver disease, before remarkable liver disease and cirrhosis set in, the albumin concentration in serum can remain normal. In late-stage liver injury, the serum albumin concentration may be <35.0g/L [21, 22]. Measurement of the amount of albumin in serum can give a clue of a patient's status of a diseased liver or the body's inability to absorb adequate protein in diets [22].

This research was designed and performed to evaluate the effects of artesunate (antimalarial drug) on some liver function parameters, namely bilirubin and albumin, in rats treated with the drugs, and to determine if high bilirubin levels would affect albumin estimation and to assess if serum albumin estimation is a reliable marker for liver function.

MATERIALS AND METHODS

Test animals

The study was conducted on thirty (30) Swiss Wistar rats of 3 to 5 months old, of both genders, and having body weights ranging from 100-360g. The rats were commercially procured and housed/caged in wire gauzed cages and they were fed on finisher mash and tap water for 7 days under atmospheric conditions.

Drug preparation and dosing

On the 8th day, the rats were separated into 5 groups of 6 rats each. Rats in the first group [group one (1)] were used as control. Rats in the other groups (2-5) were placed on oral administration (by compulsion) of artesunate. 100mg of artesunate tablets were dissolved in 100ml of distilled water and administered to the rats based on their body weights as as shown in **Table 1** for 7 days and they were also fed on the same finisher mash throughout the period. On the 15th day, no drug was administered and the blood samples were collected.

Table 1. Drug dosage administered to the rats

Group		**Drug Dosage** (per kg body weight)
Group 1	-	2.0mL of Distilled water
Group 2	-	2.0mg (2.0mL)
Group 3	-	4.0mg (4.0mL)
Group 4	-	8.0mg (8.0mL)
Group 5	-	16.0mg (16.0mL)

Collection of blood

After 7 days of artesunate administration, each rat was euthanized with chloroform, pinned to the dissecting board, and dissected open along the ventral sagittal axis and the blood was quickly collected from the heart by aortic puncture (using a syringe with a sharp needle) while the animal was still breathing. 3ml - 5ml of blood was collected from each rat into a plain glass sample bottle and allowed to clot before separation, avoiding haemolysis that could interfere with the tests. The cells (residues) were discarded while the sera (supernatant) were used for the test - to estimate the biochemical parameters, namely albumin, and bilirubin.

Estimation of bilirubin

Randox reagents from Randox laboratories Ltd, Antrim, United Kingdom, were purchased & used for the biochemical assays in line with Cheesbrough [23]. The serum bilirubin was estimated using the Jendrassik and Grof method, based on the principle that bilirubin reacts with diazotized sulphanilic acid in the presence of hydrochloric acid and sodium nitrite to form a pink-colored azobilirubin complex. The conjugated (direct) bilirubin reacts directly with the diazotized sulphanilic acid, while the unconjugated (indirect) bilirubin requires an accelerator (caffeine) to form the azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline (sodium) tartrate reagent. The optical density of the colored complex was read spectrophotometrically at 580nm (for total bilirubin) and 540nm (for conjugated bilirubin) [23, 24].

Estimation of albumin

Serum albumin was estimated using the bromocresol green (BCG) binding method. BCG is an indicator that is yellow under acidic conditions (pH 3.5 - 4.2), at which albumin specifically binds with BCG to form a blue-green colored complex, which absorbance is read in the spectrophotometer at 580nm [24].

RESULTS AND DISCUSSION

The results are presented in the table below.

Table 2. The Mean ± SD of Serum Bilirubin and Albumin Concentrations in the Different Groups of Rats Administered with Varying Doses of Artesunate

	Group 1 (No drug)	**Group 2 (2mg/kg)**	**Group 3 (4mg/kg)**	**Group 4 (8mg/kg)**	**Group 5 (16mg/kg)**
Total bilirubin (TB), µmol/L	5.819±0.606	6.580±1.401	14.022±1.725	20.282±2.695	28.222±3.01
Conjugated bilirubin (CB), µmol/L	0.103±0.004	0.204±0.024	0.197±0.028	0.163±0.007	0.234±0.023
Unconjugated bilirubin (UB), µmol/L	5.716±0.606	6.376±1.416	13.825±1.725	20.119±2.699	27.988±3.02
Albumin, g/L	38.60±3.52	36.99±1.21	34.85±1.62	30.91±1.69	30.29±1.83

From the above **Table 2**, there was a remarkable increase ($P<0.05$) in concentrations of total bilirubin and unconjugated bilirubin following increasing doses of artesunate. No significant difference ($P>0.05$) in the concentrations of conjugated bilirubin in serum between different groups administered with different doses of the drug was observed. Also, from the **Table 2**, there was a significantly decreased ($P<0.05$) albumin concentrations as the drug dosage increased in correlation with the increased concentration of unconjugated bilirubin.

A significant decrease ($P<0.05$) between the test groups and the control group for albumin level was also observed. The decrease was more marked with increased drug doses. However, no significant difference ($P>0.05$) in serum bilirubin pattern was observed between group 1 and group 2. A significant increase ($P<0.05$) in TB and unconjugated bilirubin concentrations was observed between groups 3,

4, 5, and the control group, respectively. No difference in conjugated bilirubin concentrations between the test and the control groups was observed.

The results proved that administration of artesunate caused increased lysis of RBCs, which in turn led to excessive production of bilirubin (mainly the unconjugated fraction). The increased levels of unconjugated bilirubin with no significant increase in the level of conjugated bilirubin was attributable to increased Hb breakdown from RBC lysis, releasing bilirubin into blood, which exceeded the amount the liver could conjugate.

The presentation of artesunate in 50.0 mg tablets and the recommended effective dose of 6.0-8.0 mg/kg for adults, once daily for 3 days [3] gave insight into formulating the doses (2.0-16.0 mg/kg) for rats in this study. The dosage and duration of drug administration provided an ample opportunity to effectively study and evaluate the drug effects at varying doses.

From this study, the significant increase in unconjugated bilirubin and total bilirubin was attributed to increased lysis of RBCs initiated by the drug, which led to haem build-up and unconjugated hyperbilirubinaemia, making the bilirubin load too much for the liver to conjugate. The dose-dependent increase in serum bilirubin was suggestive of liver impairment. This agreed with Onovo *et al*. [11] and Rifai *et al.* [17], that "high indirect bilirubin concentration suggests the excessive formation of bilirubin or decreased conjugation in the liver".

The significant decrease in albumin concentration is attributable to the high level of indirect bilirubin which got bound to albumin and interfered with its reactive sites in solution. This equally agreed with Rifai *et al.,* [17], that "bilirubin is loosely bound to albumin and transported to the liver in the unconjugated form".

The decrease in albumin concentration may also be attributed to the toxicity of artesunate to the rats' livers. Reports by Rifai *et al.* [17] agreed that "decreased level of albumin seen in hepatocellular disease results from alcohol and toxins direct suppression of albumin synthesis by liver". Benoit *et al*. [22] also reported that "low albumin in serum suggests that function of the liver is compromised". Previous reports showed that prolonged administration of artequin (also artemisinin derivative) could predispose to low serum proteins and globulin with accompanied elevations in liver enzymes activity, signifying hepatocellular damage [24].

The hepatotoxicity might be caused by the reactive oxygen species produced by the artesunate as part of its antimalarial action. This agreed with the work of Onovo *et al*. [11], that "the artesunate dose-dependent elevation of serum bilirubin and increases in liver enzymes (ALT, AST) activity in serum suggest hepatotoxic effects on the liver and leads to disturbance of normal functions of the liver".

This disturbance in the liver function, may not be attributed to marked damage to the liver, even though it shows some degrees of significance. More research and studies are needed. Earlier reports claimed that artesunate has adverse effects on digestive tracts and neuronal effects that are short-lived. Such effects include diarrhea, headache, nausea/vomiting, loss of appetite, dizziness, stomach cramps, insomnia, and itching [25]. However, the above signs were not observed in this study, except the usual initial hesitation to swallow the drug solution. Also, we did not observe any significant change ($P>0.05$) in the weights of the rats during our study. Onovo *et al*. [11] had reported earlier that taking artesunate could cause loss of body weight in the animals at all doses of drugs tested. Our report disagrees with this claim.

The study does not support earlier reports by Reuling *et al*., [26] that "there were no distinctive differences between drugs and severity or occurrence of LFT abnormalities. Previous malaria drug studies had also reported drug-induced liver injury [27, 28].

Bigoniya *et al.* [29] reported that short-term artesunate administration of 8.0mg per kg per day caused damage to the liver, coupled with abnormal hematological parameters, that calls for concern for patients' safety. Their reports equally stated that 45 days regimen of artesunate at a dosage of 8.0 mg per kg per day caused a significant increase of total bilirubin and liver enzymes (ALT, ALP, AST) concentrations in serum. However, our results disagree with theirs that artesunate treatment did not have a significant effect on serum albumin and total protein. Our study, therefore, agrees with Omotosho *et al*. [30] in their research that reported a decrease in total protein concentrations following treatment with artesunate.

CONCLUSION

Administration of artesunate (antimalarial) caused a significant increase in serum unconjugated bilirubin levels and the significant difference, highest concentrations at the highest dose and lowest concentrations at the lowest dose of the drug, showed a correlation between the drug dosage and action. It can also be concluded that high levels of unconjugated bilirubin in serum interfered with albumin estimation. Hence, the liver may be damaged by elevated doses of artesunate as shown by increased serum bilirubin levels and decreased albumin.

Administration of Artesunate (antimalarial) at the right dose of 6 - 8mg/Kg body weight and duration (3 days) is relatively safe. But higher doses and/or prolonged administration longer than the recommended duration of 3 days could cause liver cell damage and predispose the body to low serum albumin and elevated bilirubin levels.

Our findings suggest a need to re-evaluate and understand LFT dysfunctions in malaria treatment. This will have a

significant impact on clinical decision-making and antimalarial drug development.

The observed effects of artesunate (antimalarial) in the rats may also be observed in humans, maybe at minimal or slower rates. It is, therefore, recommended that patients whose serum bilirubin levels and albumin are to be estimated should not be on any blood-lysing drug that could affect their estimation, at least 14 days before the test. Also, there should not be over-dependence on bilirubin and albumin estimations alone for the assessment of liver functions. The LFT parameters should not be interpreted in isolation of the others and should be correlated with the clinical presentation. Caution should be exercised in drug prescription, especially antimalarial drugs, to patients with liver disease.

This study shows that liver function test abnormalities seem overlooked but common features of uncomplicated falciparum malaria treatment. This should be put into consideration in antimalarial drug development.

Patients living in malaria-endemic areas and who take antimalarials often should investigate their liver functions at least once annually.

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REFERENCES

[1] Selimov MA, Nagdalian AA, Povetkin SN, Statsenko EN, Kulumbekova IR, Kulumbekov GR, et al. Investigation of $CdCl_2$ Influence on Red Blood Cell Morphology. Int J Pharm Phytopharmacol Res. 2019;9(5):8-13.

[2] Kiani AA, Kazemi AR, Mahmoudvand H, Fathi N, Mahaki S, Ezzatkhah F. Prevalence and Associated Risk Factors of Soil-Transmitted Helminthic Parasites in Iranian Children with Hypereosinophilia. Entomol Appl Sci Lett. 2020;7(2):20-5.

[3] World Health Organization. Guidelines for the Treatment of Malaria - Third Edition. 2015.

[4] Meshnick SR. Artemisinin: Mechanism of action, resistance, and toxicity. Int J Parasitol. 2002;32(13):1655-60.

[5] Meibalan E, Marti M. Biology of malaria transmission. Cold Spring Harb Perspect Med. 2017;7(3):a025452. doi:10.1101/cshperspect.a025452

[6] LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2017. Available from: https://www.ncbi.nlm.nih.gov/books/

[7] Bremen JG. Manifestations, Determinants, and Estimates of the malaria burden. Am J Trop Med Hyg. 2001;64:1-11.

[8] Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for vivax malaria. Lancet Infect Dis. 2010;10(6):405-16.

[9] Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet. 2010;376(9753):1647-57.

[10] Sinclair D, Donegan S, Isba R, Lalloo DG. Artesunate versus quinine for treating severe malaria. Cochrane Database Syst Rev. 2012;(6):CD005967.

[11] Onovo A, Madusoromuo MA, Nta IE. Effect of artesunate on liver functions of the wister rat. Int J Biochem, Bioinform Biotechnol Stud. 2016;1(1):19-29.

[12] World Health Organization. New Malaria Guidelines for Treatment and Procurement of Medicines. Geneva. 2010.

[13] Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature. 2015;520(7549):683-7.

[14] Straimer J, Gnädig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science. 2015;347(6220):428-31.

[15] Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, et al. Artemisinins target the SERCA of Plasmodium falciparum. Nature. 2003;424(6951):957-61.

[16] Gopalakrishnan AM, Kumar N. Antimalarial action of artesunate involves DNA damage mediated by reactive oxygen species. Antimicrob Agents Chemother. 2015;59(1):317-25.

[17] Simundic AM, Rifai N, Horvath AR, Wittwer CT. Preanalytical variation. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics, 8th ed. St. Louis (MI): Elsevier; 2019. pp.39-50.

[18] Fevery J, Muraca M, Mesa V, Van Steenbergen W, Blanckaert N. Plasma bilirubin pigments in health and disease. Mol Aspects Med. 1987;9(5):391-404.

[19] Pincus MR, Tierno P, Dafour R. Evaluation of Liver Function, Henry's clinical diagnosis and management by laboratory methods. 2nd ed. Philadelphia, Saunders Elsevier; 2006. pp.521-6.
[20] Jendrassik L, Grof P. Simplified Photometric methods for the determination of bilirubin. Biochemistry. 1938:81-9.
[21] Martin AC. Clinical Chemistry and metabolic medicine. 7th ed. Book power publishing; 2006. pp. 283-6.
[22] Ruot B, Breuillé D, Rambourdin F, Bayle G, Capitan P, Obled C. Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. Am J Physiol Endocrinol Metab. 2000;279(2):E244-51.
[23] Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed. Part 1. Cambridge University Press; 2005. pp. 355-8.
[24] Ugwu PI, Obiechina MC, Anyaehie USB, Ofem OE, Elendu UM. Comparative evaluation of the effect of two anti-malaria drugs (chloroquine and artequin) on some serum biochemical parameters in rats. Int J Med Sci Appl Biosci. 2020;5(2). Available from: http://www.casirmediapublishing.com
[25] Navaratnam V, Mansor SM, Sit NW, Grace J, Li Q, Olliaro P. Pharmacokinetics of artemisinin-type compounds. Clin Pharmacokinet. 2000;39(4):255-70.
[26] Reuling IJ, de Jong GM, Yap XZ, Asghar M, Walk J, van de Schans LA, et al. Liver injury in uncomplicated malaria is an overlooked phenomenon: an observational study. EBioMed. 2018;36:131-9.
[27] Silva-Pinto A, Ruas R, Almeida F, Duro R, Silva A, Abreu C, et al. Artemether-lumefantrine and liver enzyme abnormalities in non-severe Plasmodium falciparum malaria in returned travellers: a retrospective comparative study with quinine-doxycycline in a Portuguese centre. Malar J. 2017;16(1):43.
[28] Duparc S, Borghini-Fuhrer I, Craft CJ, Arbe-Barnes S, Miller RM, Shin CS, et al. Safety and efficacy of pyronaridine-artesunate in uncomplicated acute malaria: an integrated analysis of individual patient data from six randomized clinical trials. Malar J. 2013;12(1):70.
[29] Bigoniya P, Sahu T, Tiwari V. Hematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicol Rep. 2015;2:280-8. Available from: www.elsevier.com/locate/toxrep
[30] Omotosho OO, Adebiyi MA, Oyeyemi MO. Comparative study of the haematology and serum biochemistry of male wistar rats treated with chloroquine and artesunate. J Physiol Pharmacol Adv. 2014;4(8):413-9.