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Structure-Activity-Relationship (SAR) of Artemisinins on some Biological Systems in Male Guinea Pigs

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Abstract: Background: Artemisinin-based Combination Therapies (ACTs) are currently the first-line drugs used in the treatment of uncomplicated malaria. Consequently, the use of artemisinins is particularly high in areas with malaria endemicity like the sub Sahara. The present study investigated the effects of 3 frequently used artemisinin derivatives: artesunate, artemether and dihydroartemisinin (DHA) on biochemical, hematological and semen parameters in the male guinea pig, using different doses (half, normal and double therapeutic doses) of each drug. Artesunate and DHA were administered by oral gavage, while artemether was given by i.m. injection. **Result:** Serum prostatic acid phosphatase (ACPP) and glutamate-oxaloacetate transaminase (SGOT) were significantly ($p<0.05$) and dose-dependently increased in artesunate (186 and 48.2%) and DHA (233.5 and 68.2%) exposed animals. In addition, DHA also caused significant dose-dependent elevations in Serum Glutamate-Pyruvate Transaminase (SGPT) and triglyceride levels, while only triglycerides and both parameters were elevated significantly only in the double clinical dose animal groups of artemether and artesunate respectively. Furthermore, WBC counts in artesunate-, DHA- and artemether-treated animals were significantly and dose-dependently increased, compared to the control animals. The drugs caused 60, 78.2 and 50% increases respectively. Lymphocytes count was also significantly increased by 16.8, 21.7 and 20.5% by the drugs respectively, while neutrophils count was decreased by artemether (10.7%) and DHA (10.4%) at the double therapeutic dose. Furthermore, while there were no significant changes in the semen parameters of artemether-treated animals, there was significant ($p<0.05$) reductions in the epididymal sperm concentration and motility in artesunate- and DHA-treated animals, compared to the controls. These parameters were decreased by 24.4 and 23%, respectively in artesunate-treated animals and 48.9 and 55.1%, respectively in DHA-exposed animals. **Conclusions:** Artemisinin derivatives are capable of causing alterations in biochemical, hematological and reproductive functions, which may be due to increased oxidative stress in the liver, blood cells and the testis. Furthermore, the toxicological effects of DHA>artesunate>artemether, which is attributed to differences in their chemical structures.

Key words: Dihydroartemisinin, hepatotoxicity, neutropenia, spermatogenesis

INTRODUCTION

Malaria is one of the most deadly diseases in Africa, causing high morbidity and mortality with their attendant adverse economic consequences (WHO, 1998; Sachs and Malaney, 2002). Effective treatment of malaria has been a great challenge due to resistance of the malaria parasite to antimalaria drugs. Currently, artemisinin compounds are advocated for the treatment of the disease in Africa due to their high efficacies (Greenwood *et al*., 2005; Maiteki-Sebuguzi *et al*., 2008).

Artemisinin is the active component of the Chinese medicinal herb called *Artemisia annua*. It is a peroxide-bridged sesquiterpene lactone compound. Semi-synthetic derivatives of artemisinin, obtained by structural modifications of artemisinin are also available. These derivatives of artemisinin are more frequently used in malaria chemotherapy, because of their better pharmacokinetic properties and higher efficacies than the parent artemisinin compound. The most widely used artemisinin derivatives are artesunate, artemether and dihydroartemisinin (artenimol, DHA). Others include arteether (artemotil) and artelinic acid. Although artemisinin derivatives are effective against the Plasmodium parasite (as monotherapies), combination therapies consisting of artemisinins and other standard antimalaria drugs have been demonstrated to have better parasite clearance and efficacies (Olliaro and Taylor, 2004;

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Adjuik *et al*., 2004; Nosten and White, 2007). These artemisinin-based combination therapies (ACTs) include: artesunate+amodiaquine, artemether+lumefantrine, artesunate+sulfadoxine+pyrimethamine and artesunate+ mefloquine (WHO, 1995; Nosten and White, 2007).

Furthermore, previous reports have described artemisinin derivatives to be generally safe and well-tolerated (Nosten and White, 2007; Maiteki-Sebuguzi *et al*., 2008). However, there are concerns about their potentials for neuro- and reproductive toxicities (Nontprasert *et al*., 2002; WHO, 2003; Raji *et al*., 2005; Nwanjo *et al*., 2007; Obianime and Aprioku, 2009). In addition, previous toxicological studies on artemisinins evaluated their effects on specific systems, limiting such studies to make qualitative safety evaluations of these drugs (Raji *et al*., 2005; Nwanjo *et al*., 2007). Also, most of the previous studies were carried out in other animal models and not much is documented on the guinea pig system, which has better drug metabolism, compared to most of the other animal models used (Gregus *et al*., 1988).

In the present study, the comparative dose-dependent responses of artesunate, artemether and DHA on biochemical, hematological and semen parameters were investigated in the male guinea pig. Three doses were used for each drug, corresponding to half of the therapeutic dose, the therapeutic dose and double the therapeutic dose of each drug.

MATERIALS AND METHODS

Materials: Artesunate (Artesunat) tablets (Mekophar Chemical Pharmaceutical Joint-stock Company, Vietnam); artemether (Paluther) injection (Aventis InterContinental, France) and dihydroartemisinin (Alaxin) tablets (Bliss GVS Pharma Ltd., India) were obtained from the Pharmacy Department of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

Artesunate and DHA tablets were powdered separately in a glass mortar, mixed with distilled water and were administered as aqueous suspensions by oral gavage at 0.9 mL kg^{-1} b.wt. The drug suspensions were continuously agitated during administration in order to deliver the drugs homogeneously to the animals. Artemether was dissolved in dimethyl sulphoxide (DMSO) and given at a dose of 0.25 mL kg^{-1} intramuscularly.

Animals: Outbred strains of adult male guinea-pigs (*Cavia porcellus*) weighing between 650-670 g, aged 20-21 weeks were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were allowed to acclimatize for 14 days in a well ventilated room at a room temperature of 28.0±2.0°C under natural lighting condition. They were housed in shoebox cages provided with beddings to allow absorption of urine. The animals were fed with standard rodent chow (Topfeeds Ltd., Sapele, Nigeria) and allowed free access to tap water *ad libitum*. The animals were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care (2009).

Methods: A total number of 55 guinea pigs were used in the experiment: 45 were randomly distributed into 3 major groups labeled as 'artesunate', 'DHA' and 'artemether', each containing 3 subgroups (n = 5), while the remaining10 pigs were divided into two 'control' groups (D and W). The animals in 'artesunate' subgroups were labeled as: A, B and C; 'DHA' subgroups as: E, F and G and 'artemether' subgroups as: X, Y and Z.

The animals in 'artesunate' group (subgroups A, B and C) were orally administered with artesunate daily for 5 consecutive days at different dose levels. Subgroup A was given 4, 2, 2, 2 and 2 mg kg^{-1}, equivalent to the therapeutic dose of artesunate for the treatment of uncomplicated malaria (Angus *et al*., 2002). Subgroup B animals received 2, 1, 1, 1 and 1 mg kg^{-1}, equivalent to half of the therapeutic dose. Subgroup C was given 8, 4, 4, 4 and 4 mg kg^{-1} (double the therapeutic dose).

Animals in 'DHA' group (subgroups E, F and G) received oral administration of DHA daily for 7 consecutive days at different dose levels. Subgroup E was given: 2.2, 1.1, 1.1, 1.1, 1.1 and 1.1 mg kg^{-1}, which is equivalent to the therapeutic dose of DHA for the treatment of uncomplicated malaria (Looareesuwan *et al*., 1996). Subgroup F received 1.1, 0.55, 0.55, 0.55, 0.55 and 0.55 mg kg^{-1}, equivalent to half of the therapeutic dose; while subgroup G had 4.4, 2.2, 2.2, 2.2, 2.2 and 2.2 mg kg^{-1} (double therapeutic dose).

Artemether was applied intramuscularly in an oil solution to the animals in 'artemether' group (i.e., subgroups X, Y and Z) for 6 consecutive days at different daily concentrations. Subgroup X animals received 3.2, 1.6, 1.6, 1.6, 1.6 and 1.6 mg kg^{-1}, which is equivalent to the therapeutic dose of artemether for the treatment of uncomplicated malaria (Khan *et al*., 2006). Subgroup Y animals had 1.6, 0.8, 0.8, 0.8, 0.8 and 0.8 mg kg^{-1}, equivalent to half of the therapeutic dose. Subgroup Z was given 6.4, 3.2, 3.2, 3.2, 3.2 and 3.2 mg kg^{-1} (double therapeutic dose).

'Group D' animals were injected DMSO (0.1 mL) intramuscularly for 6 days, while 'group W' animals were given distilled water (0.5 mL) orally for 7 days. 'Group D' animals represented control for artemether experimental group, while 'group W' served as the control for artesunate and DHA experimental groups.

All animals were sacrificed under pentobarbitone anaesthesia at 37 mg kg^{-1} i.p. (Flecknell, 1996) at the end of each treatment period. Blood samples were collected and processed for biochemical and hematological assays. Epididymal sperm was also collected for semen analysis.

Biochemical parameters: Blood sample was centrifuged for 15 min at 3,000 rpm and clear serum was separated from the cells and stored at -80°C and assayed for biochemical parameters. Alkaline phosphatase was assayed by the phenolphthalein method (Babson *et al*., 1966); total and prostatic acid phosphatases by colorimetric method (Fishman and Davidson, 2006); urea by Urease-Berthelot method (Kaplan, 2006); total cholesterol using the enzymatic endpoint method (Kayamori *et al*., 1999); albumin by bromocresol green method (Doumas *et al*., 1997); triglycerides by simple colorimetric method described by Sugiura *et al*. (1977) and serum transaminases (SGOT and SGPT) levels were measured according to the method described by Reitman and Frankel (1957).

Hematological parameters: Whole blood was collected from the animals into EDTA bottles and assayed for RBC, WBC, differential cell counts of WBC, PCV and Hb, using standard laboratory techniques.

Measurement of semen parameters: The caudal epididymis was carefully isolated and then placed in a Petri dish containing 3.0 mL of $NaHCO_3$ buffered Tyrodes's Lactate solution. Several incisions (1 mm) were made on it and semen was gently drawn into plastic transfer pipette and transferred into 5 mL test tubes for analysis. Semen analysis was carried out immediately using the new improved Neubauer counting chamber for determination of the concentration of spermatozoa, sperm motility, percentages of abnormal sperm cells (sperm morphology) and debris using standard laboratory techniques (Baker, 2007).

Statistical analysis: Data were expressed as Means±standard errors of mean. Comparisons between control values and the values obtained in treated groups of guinea-pigs were performed with one-way analysis of variance (ANOVA). Statistical significance was set at $p<0.05$.

RESULTS

Biochemical parameters: In the present study, while no significant ($p<0.05$) changes were observed in most of the biochemical parameters in artemether exposed pigs, artesunate and DHA caused significant and dose-dependent elevations in the levels of serum prostatic acid phosphatase (ACPP) and glutamate-oxaloacetate transaminase (SGOT), compared to the control animals (Fig. 1a-d). The basal serum ACPP level obtained in the control animals was 2.0±0.58 IU L^{-1}, while its levels in

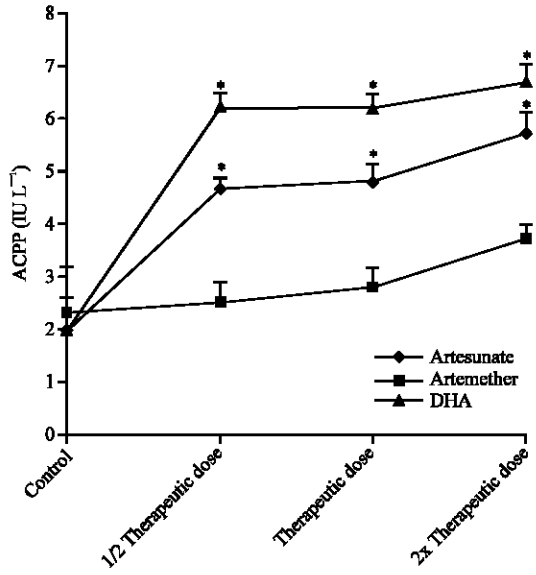

Fig. 1a: Effects of artemisinin derivatives on the serum level of prostatic acid phosphatase (ACPP) in the male guinea pig. Data are expressed as Mean±SEM. *Significant at $p<0.05$

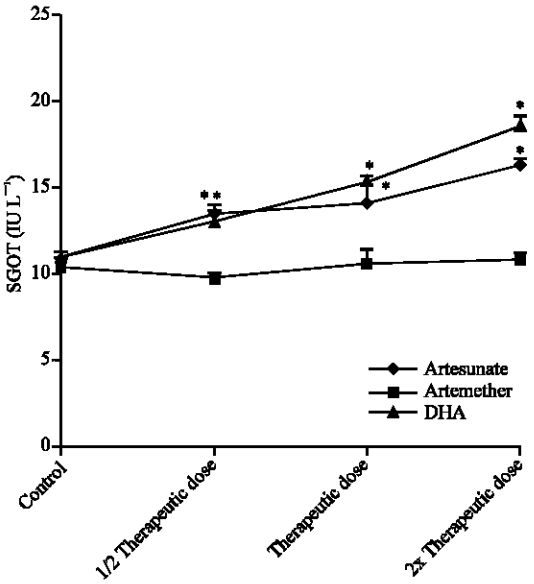

Fig. 1b: Effects of artemisinin derivatives on serum glutamic oxaloacetic transaminase (SGOT) level in the male guinea pig. Data are expressed as Mean±SEM. *Significant at $p<0.05$

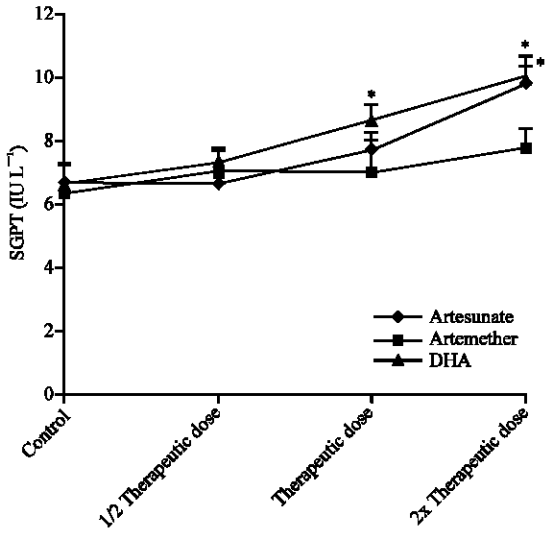

Fig. 1c: Effects of artemisinin derivatives on serum glutamic pyruvic transaminase (SGPT) level in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

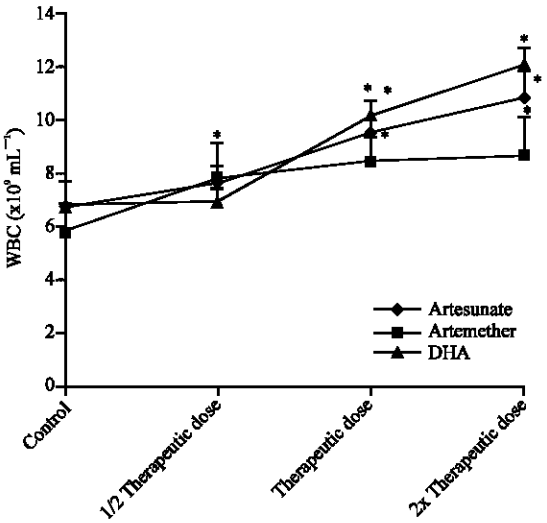

Fig. 2a: Effects of artemisinin derivatives on white blood cells (WBC) counts in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

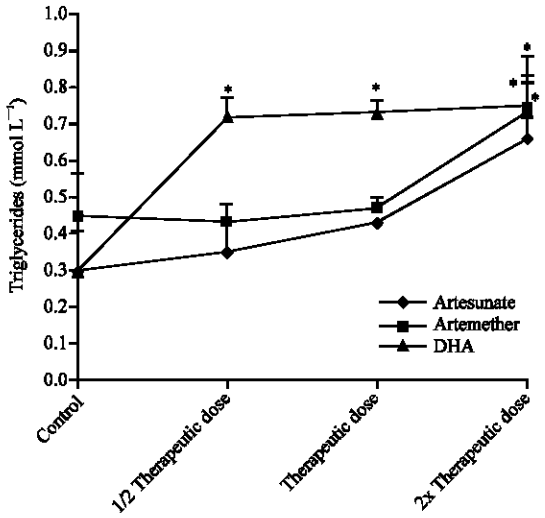

Fig. 1d: Effects of artemisinin derivatives on the serum level of triglycerides in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

artesunate- and DHA-treated animals were 5.72±0.41 and 6.67±0.33 IU L^{-1}, respectively (Fig. 1a). These correspond to 186 and 233.5% increases by artesunate and DHA respectively. Also, the maximum levels of SGOT obtained in artesunate- and DHA-treated animals were: 16.3±0.33 and 18.5±0.65 IU L^{-1}, respectively. These values represent 48.2 and 68.2% increases respectively, when compared to its value (11.0±0.58 IU L^{-1}) in the control animals (Fig. 1b). Furthermore, DHA also caused significant (p<0.05) and dose-dependent increase in the Serum Glutamate-Pyruvate Transaminase (SGPT) and triglyceride levels, but significant increases for both were observed only in the artesunate subgroup that received double therapeutic dose, when compared to the control animals (Fig. 1c, d). The double therapeutic dose of artemether also caused significant increase in serum triglycerides. However, there were no significant (p<0.05) changes in the serum levels of other biochemical parameters in experimental animals, compared to control animal groups (Not shown).

Hematological parameters: The blood levels of White Blood Cells (WBC) and lymphocytes were significantly (p<0.05) and dose-dependently increased in artesunate-, DHA- and artemether-treated groups of animals, while neutrophils count was significantly decreased, compared to control animals counts (Fig. 2a-c).

The WBC counts in animals that were given double therapeutic doses of artesunate and DHA were: 10.8±0.98×10^9 mL^{-1} and 12.03±0.62×10^9 mL^{-1}, respectively, compared to 6.75±0.9×10^9 mL^{-1} in animals given only distilled water (Fig. 2a). These values were equivalent to 60 and 78.2% increases respectively. Furthermore, the WBC count in artemether was 8.7±1.39×10^9 mL^{-1}, while WBC count in the control animals group was 5.8±0.95×10^9 mL^{-1} (Fig. 2a),which is equivalent to 50% increase. In addition, while lymphocytes count was significantly (p<0.05) increased, neutrophils count was decreased in the double therapeutic dose artemether- and DHA-treated animal groups, compared to the control

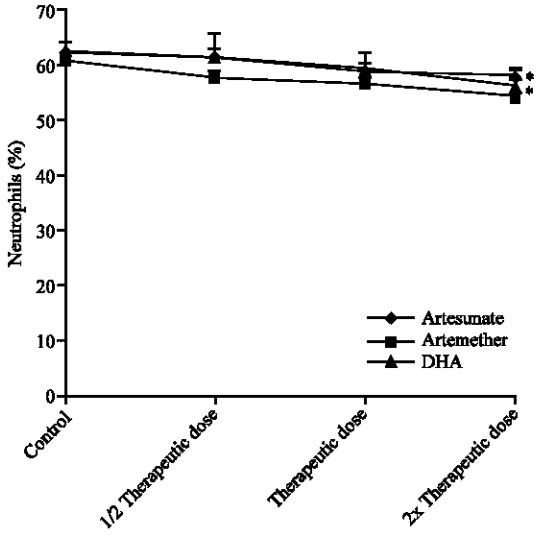

Fig. 2b: Effects of artemisinin derivatives on neutrophils count in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

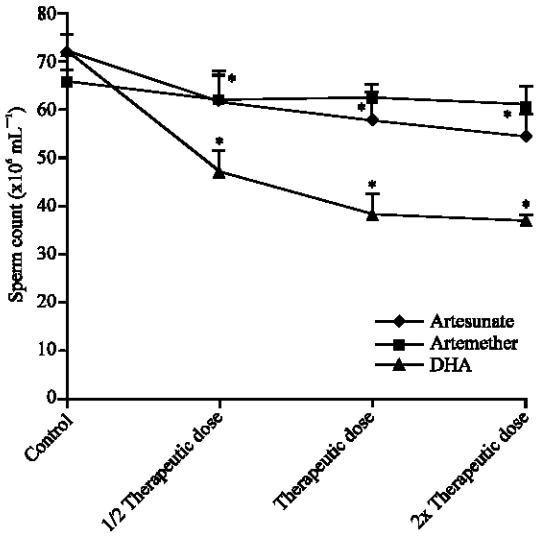

Fig. 3a: Effects of artemisinin derivatives on sperm count in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

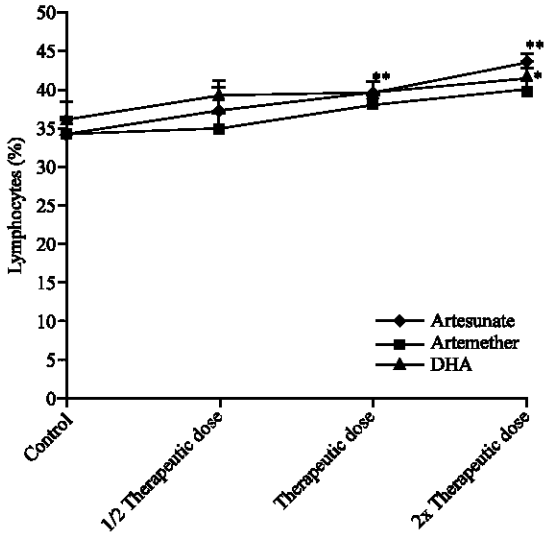

Fig. 2c: Effects of artemisinin derivatives on lymphocytes count in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

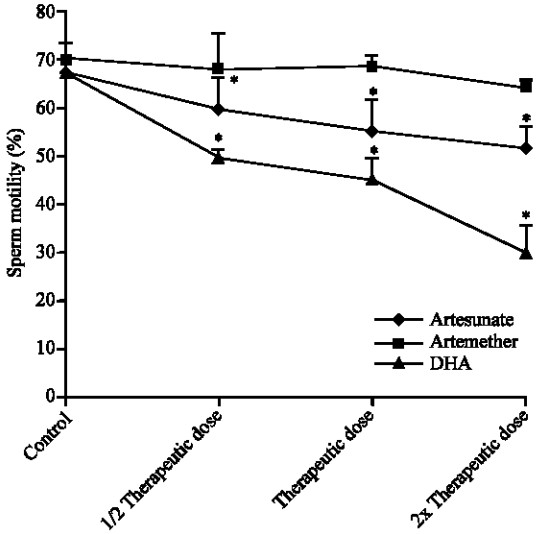

Fig. 3b: Effects of artemisinin derivatives on sperm motility in the male guinea pig. Data are expressed as Mean±SEM. *Significant at p<0.05

animal groups (Fig. 2b, c). Furthermore, the drugs caused no significant changes in the blood levels of the other hematological parameters (i.e., RBC, Hb and PCV) in experimental animals, compared to the control animals (Not shown).

Semen parameters: Epididymal sperm concentration and motility in artesunate and DHA exposed animals were significantly ($p<0.05$) lower than the values obtained in animals that were given only distilled water. The effects were also dose-dependent. The lowest sperm counts in artesunate- and DHA-treated animals were $54.3\pm4.41\times10^{6}$ mL^{-1} and $36.7\pm4.01\times10^{6}$ mL^{-1}, respectively, compared to $71.8\pm3.15\times10^{6}$ mL^{-1} obtained in the control animals (Fig. 3a). These values were equivalent to 24.4 and 48.9% reductions respectively. In addition, the lowest sperm motility values in artesunate- and DHA-treated animals were 52.0±4.4% and 30.3±5.56%, respectively, compared to 67.5±2.89% obtained in the control animals (Fig. 3b). These values were equivalent to 23 and 55.1% reductions respectively.

Table 1a: Effects of artesunate on percentages of abnormal sperm cells and debris in male guinea pigs

Dose	Abnormal sperm cells (Morphology) (%)	Sperm debris (%)
Control (Distilled H_2O)	10.0±2.04	12.5±1.44
½ Therapeutic dose	10.0±1.64	15.0±2.04
Therapeutic dose	11.3±2.39	16.3±2.33
2x Therapeutic dose	18.7±4.31	20.7±1.67*

Data are expressed as Mean±SEM. *Significant at $p<0.05$

Table 1b: Effects of artemether on percentages of abnormal sperm cells and debris in male guinea pigs

Dose	Abnormal sperm cells (Morphology) (%)	Sperm debris (%)
Control (DMSO)	11.3±1.25	13.8±2.39
½ Therapeutic dose	11.5±1.40	16.3±4.27
Therapeutic dose	12.5±1.44	21.7±8.82
2x Therapeutic dose	18.3±1.67*	18.8±1.25

Data are expressed as Mean±SEM. *Significant at $p<0.05$

Table 1c: Effects of DHA on percentages of abnormal sperm cells and debris in male guinea pigs

Dose	Abnormal sperm cells (Morphology) (%)	Sperm debris (%)
Control (Distilled H_2O)	10.0±2.04	12.5±1.44
½ Therapeutic dose	18.3±3.67	23.8±4.54*
Therapeutic dose	21.7±2.41*	26.7±3.10*
2x Therapeutic dose	25.0±3.54*	30.0±5.00*

Data are expressed as Mean±SEM. *Significant at $p<0.05$

Furthermore, the percentages of abnormal sperm cells (morphology) and debris were significantly increased in DHA-treated animals (Table 1c), while there were no significant changes in artesunate- and DHA-exposed animals, when compared to the control animals (Table 1a, b). Also, there were no significant changes in the values of sperm count and motility in artemether-injected animals, compared to DMSO-injected animals (Fig. 3a, b).

DISCUSSION

In this study, the comparative effects of three commonly used artemisinin derivatives on biochemical (phosphatase enzymes, serum transaminases, albumin, triglycerides, urea and cholesterol), hematological (PCV, Hb, RBC, WBC and white blood cell differential counts) and semen parameters (total sperm count, sperm motility, abnormal sperm cells and debris) were investigated in the male guinea pig. The artemisinin derivatives used were: artesunate, artemether and dihydroartemisinin (DHA). DHA caused significant ($p<0.05$) dose-dependent increase in the serum levels of triglycerides, while triglyceride level was increased only in artesunate and artemether animal groups that were exposed to double therapeutic dose. This observation suggests that administration of DHA or its accumulation from the metabolism of other artemisinin derivatives in the body may have adverse effects on the cardiovascular system (Austin, 1989; Nakanishi *et al*., 2002). This is consistent with previous reports on the potential of artemisinins to cause prolongation of QT-interval in the heart (Qiao *et al*., 2003).

Furthermore, while artemether had no significant ($p<0.05$) effects on the other biochemical parameters, artesunate and DHA caused significant ($p<0.05$) and dose-dependent increases in the levels of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and serum prostatic acid phosphatase (ACPP). The basal SGOT and SGPT levels were increased by 48.2 and 47%, respectively in artesunate-treated animals and 68.2 and 50%, respectively in DHA-treated animals. In addition, serum ACPP level was increased by 186 and 233.5% in artesunate- and DHA-treated animals respectively. These findings are similar to the results of previous studies which had shown that artemisinin derivatives cause elevations in the serum levels of hepatic enzymes in other animal models (Ngokere *et al*., 2004; Nwanjo and Oze, 2007). However, those results were obtained in separate studies involving a single artemisinin derivative which makes the present study a suitable bench mark to evaluate the structure-activity-relationship of artemisinin derivatives on biochemical function in animal models.

SGOT or aspartate aminotransferase (AST) enzyme is present in liver, heart, kidney and other cells, while SGPT or alanine aminotransferase (ALT) is a more specific enzyme that is synthesized by the liver (Black, 1980). The serum levels of the two enzymes are used as surrogate markers of hepatic toxicity (Achliya *et al*., 2004; Vahdati-Mashhadian *et al*., 2005; Ewaraiah and Satyanarayana, 2010). In the present study, an elevation of SGOT and SGPT by artesunate and DHA is highly indicative of hepatic toxicity, which is consistent with previous studies (Ngokere *et al*., 2004; Tabassum and Agrawal, 2003). Furthermore, increase in the serum ACPP levels may underlie adverse effects on the testicular structures (Lin *et al*., 1980; Chu and Lin, 1998). This is corroborated by significant dose-dependent reductions in the epididymal sperm concentration and motility observed in artesunate- and DHA-treated animals.

With the basal sperm count and motility values decreased by 24.4 and 23%, respectively, in artesunate-treated animals and 55.1 and 48.9%, respectively, in DHA-treated animals, it is evident that DHA may be a more toxic drug than the other artemisinin derivatives on the testis, over the dose ranges used in this experiment. In addition, the percentages of abnormal sperm cells (morphology) and debris were significantly increased in DHA-exposed animals, which further reveal the adverse testicular effects of DHA.

Sperm production (spermatogenesis) occurs primarily in the seminiferous tubules. The process is regulated by testosterone, secreted by the Leydig cells of the testis and gonadotropins (LH and FSH), secreted by the anterior pituitary; while the Sertoli cells in the testis helps in the nourishment of growing spermatozoa (Elkington and Blackshaw, 1974; Ganong, 2001). Recent studies had revealed that artemisinin derivatives have no significant ($p<0.05$) effects on the hypothalamo-pituitary axis in the testis (Raji *et al*., 2005; Nwanjo *et al*., 2007), suggesting that the testicular effects of artesunate and DHA in this study may be due to direct deleterious effects (necrosis) on the seminiferous epithelium, Leydig cells and Sertoli cells through increased oxidative stress.

Furthermore, the hematology data showed that the artemisinin derivatives had significant ($p<0.05$) effects on the blood levels of WBC, neutrophils and lymphocytes, without significant effects on the other hematological parameters measured. Basal WBC count was increased dose-dependently by artesunate, DHA and artemether, with maximum percentage increases of 69.2, 74.4 and 50, respectively. Lymphocytes count was also increased in artesunate-, DHA- and artemether-treated animal groups respectively, while mild neutropenia was observed in only animals exposed to artemether and DHA. White blood cells in the body constitute a special system for combating infectious and toxic agents. The increase in WBC counts (leucocytosis) and lymphocytes (lymphocytosis) is highly suggestive of an immunological response to trauma induced by the drugs (Guyton and Hall, 2006). Our findings also showed that artemisinins, given at the therapeutic doses may not cause neutropenia, which is consistent with prior studies (Maiteki-Sebuguzi *et al*., 2008), but concentrations higher than the therapeutic doses of artemisinins may induce neutropenia, which corroborates concerns of neutropenia observed in malaria treatment with ACTs in immune compromised individuals (Gasasira *et al*., 2008). The mild neutropenia observed in animals exposed to high doses of artemether and DHA may be due to injurious effects on hepatic cells (Juarez-Navarro *et al*., 2005). This is also consistent with our biochemical results above. Furthermore, the agents caused no significant effects on the other hematological parameters, which indicate clearly that, over the dose ranges used in this experiment, the artemisinin derivatives may have no significant effects on the number and function of red blood cells, but have significant effects on immunological cells.

From the data of this study, it shows that the parameters (biochemical, hematological and reproductive) investigated were differently affected by the drugs in the order of DHA> artesunate > artemether. These differences may lie in the structural differences of the drugs. DHA is a reduced product of artemisinin and it is the primary metabolite of artemisinin and its derivatives (Svensson *et al*., 2003). DHA is water soluble, relatively unstable and very prone to oxidative reactions because of the lactol moiety in its structure (Fig. 4a, b). Artesunate is a hemisuccinate ester of dihydroartemisinin (Fig. 4b, c). It is polar and has poor stability in aqueous solution, because facile hydrolysis of its ester linkage occurs readily, which makes the drug to easily undergo oxidative reactions. Artemether is a methyl ether of artemisinin (Fig. 4a, d). The methyl substituent in artemether makes the compound chemically stable and more lipophillic. Thus, our results followed the structurally-dependent chemical reactivity profile of the drugs, DHA causing the most significant effects and artemether producing the least effects. Furthermore, since the effects on the parameters investigated were most pronounced with

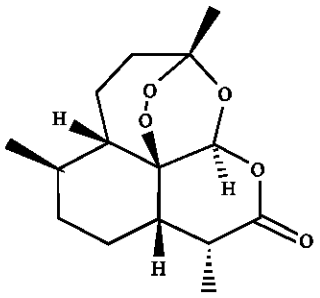

Fig. 4a: Structure of artemisinin

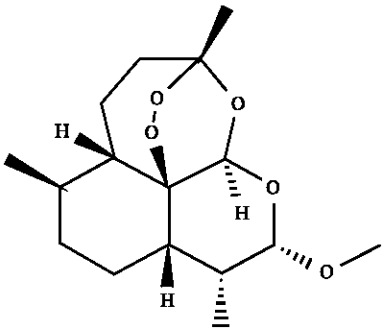

Fig. 4b: Structure of Dihydroartemisinin (DHA)

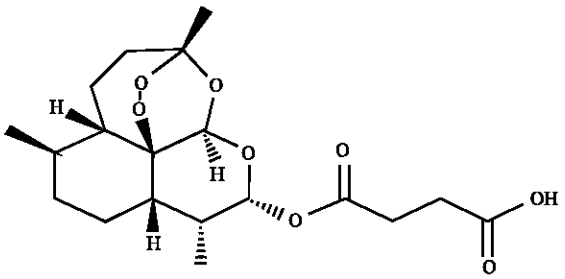

Fig. 4c: Structure of artesunate

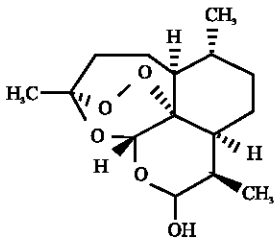

Fig. 4d: Structure of artemether

DHA, the toxicity of artesunate and artemether and probably other artemisinin derivatives may depend on the rate and extent of their metabolism to DHA (the active metabolite). This may be determined by both the drug and patient characteristics. Being a simple ester, artesunate undergoes first-pass metabolism in the plasma by the action of esterases before being metabolized in the liver. This makes it more rapidly and completely converted to DHA and this may account for its observed greater level of toxicity than artemether, which is only metabolized by hepatic cytochrome P450 enzymes in the liver (Svensson *et al*., 2003; Simonsson *et al*., 2003). Additionally, the observed lower artemether toxicity may be due to limited distribution into body tissues because of its high lipophilicity, as a result of the methyl ether substituent in its chemical structure. This is consistent with the observations of previous similar studies (Li *et al*., 1998; Svensson *et al*., 2003). Furthermore, individuals with effective P450 metabolic enzymes system may suffer more adverse effects from artemisinins, since artemisinins are metabolized by hepatic cytochrome P450 enzymes (Svensson *et al*., 2003; Simonsson *et al*., 2003).

CONCLUSIONS

The study shows that the artemisinin derivatives are capable of causing hepatotoxicity and leucocytosis. Although clinical doses of the drugs may have no significant ($p<0.05$) effects on neutrophils, higher doses may induce neutopenia. Furthermore, the drugs cause male reproductive dysfunction, probably due to damages to the seminiferous tubules, Leydig cells and Sertoli cells. In addition, the study shows that the drugs investigated have structure-activity-relationship in the order: DHA> artesunate > artemether, indicating that there may be need for dosage adjustment when artemisinin derivatives are administered concurrently with hepatic P450 enzyme inducers, as this may result in accumulation of DHA and increase toxicity.

From the findings of the present study, there is need for a closer and more qualitative safety evaluation of artemisinins. This is particularly important because of the fact that artemisinin derivatives are used very frequently in clinical and non clinical settings, where the drugs are obtained over the counter without prescriptions in most African countries including Nigeria.

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REFERENCES

Achliya, G.S., S.G. Wadodkar and A.K. Dorle, 2004. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. J. Ethnopharmacol., 90: 229-232.

Adjuik, M., A. Babiker, P. Garner, P. Olliaro, W. Taylor and N. White, 2004. Artesunate combinations for treatment of malaria: Meta-analysis. Lancet, 363: 9-17.

Angus, B.J., I. Thaiaporn, K. Chanthapadith, Y. Suputtamongkol and N.J. White, 2002. Oral artesunate dose-response relationship in acute Falciparum malaria. Antimicrob. Agents Chemother., 46: 778-782.

Austin, M.A., 1989. Plasma triglyceride as a risk factor for coronary heart disease. The epidemiologic evidence and beyond. Am. J. Epidemiol., 129: 249-259.

Babson, L.A., S.J. Greeley, C.M. Coleman and G.D. Phillips, 1966. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. Clin. Chem., 12: 482-490.

Baker, D.J., 2007. Semen analysis. Clin. Lab. Sci., 20: 172-187.

Black, M., 1980. Acetaminophen hepatotoxicity. Gastroentology, 78: 82-92.

Canadian Council On Animal Care, 2009. The Care and Use of Farm Animals in Research, Teaching and Testing. CCAC., Ottawa, ON., pp: 12-15.

Chu, T.M. and M.F. Lin, 1998. PSA and acid phosphatase in the diagnosis of prostate cancer. J. Clin. Ligand Assay., 21: 24-34.

Doumas, B.T., W.A. Watson and H.G. Biggs, 1997. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chimica Acta, 258: 21-30.

Elkington, J.S.H. and A.W. Blackshaw, 1974. Studies in testicular function. I. Quantitative effects of FSH, LH, testosterone and dihydrotestosterone on restoration and maintenance of spermatogenesis in the hypophysectomized rat. Aus. J. Biol. Sci., 27: 47-57.

Ewaraiah, M.C. and T. Satyanarayana, 2010. Hepatoprotective activity of extracts from stem of Mussaenda erythrophylla Lam. Against carbon tetrachloride-induced toxicity in rats. JPRHC, 2: 23-31.

Fishman, H.W. and M.H. Davidson, 2006. Determination of serum acid phosphatases method. Biochem. Anal., 4: 257-284.

Flecknell, P.A., 1996. Laboratory Animal Anaesthesia. 2nd Edn., Academic Press Inc., USA, pp: 170.

Ganong, W.F., 2001. Review of Medical Physiology. 20th Edn., Lange Medical Books/McGraw-Hill Medical Publishing Division, London, ISBN: 0-8385-8282-6, pp: 414-417.

Gasasira, A.F., M.R. Kamya, J. Achan, T. Mebrahtu and J.N. Kalyango et al., 2008. High risk of neutropenia in HIV-infected children following treatment with artesunate plus amodiaquine for uncomplicated malaria in Uganda. Clin. Infect. Dis., 46: 985-991.

Greenwood, B.M., K. Bojang, C.J. Whitty and G.A. Targett, 2005. Malaria. The Lancet, 365: 1487-1498.

Gregus, Z., C. Madhu and C.D. Klaassen, 1988. Species variation in toxication and detoxication of acetaminophen in vivo: A comparative study of biliary and urinary excretion of acetaminophen metabolites. J. Pharmacol. Exp. Ther., 244: 91-99.

Guyton, A.C. and J.E. Hall, 2006. Text Book of Medical Physiology. 11th Edn., Elsevier Saunder, Philadelphia.

Juarez-Navarro, A., L. Vera-de-Leon, J.M. Navarro, R. Chirino-Sprung, M. Diaz-Hernandez, L. Casillas-Davila and M. Dehesa-Violante, 2005. Incidence and severity of infections according to the development of neutropenia during combined therapy with pegylated interferon-alpha2a plus ribavirin in chronic hepatitis C infection. Methods Findings Exp. Clin. Pharmacol., 27: 317-322.

Kaplan, A., 2006. The Determination of Urea, Ammonia and Urease. In: Methods of Biochemical Analysis, Glick, D. (Ed.). John Wiley and Sons, California.

Kayamori, Y., H. Hatsuyama, T. Tsujioka, M. Nasu and Y. Katayama, 1999. Endpoint colorimetric method for assaying total cholesterol in serum with cholesterol dehydrogenase. Clin. Chem., 45: 2158-2163.

Khan, M.A., R.A. Jr. Smego, S.T. Razi and S.T. Beg, 2006. Emerging drug resistance and guidelines for treatment of malaria. Med. Today, 4: 81-87.

Li, Q.G., J.O. Peggins, L.L. Fleckenstein, K. Masonic, M.H. Heiffer and T.G. Brewer, 1998. The pharmacokinetics and bioavailability of dihydroartemisinin, arteether, artemether, artesunic acid and artelinic acid in rats. J. Pharmacy Pharmacol., 50: 173-182.

Lin, M.F., C.L. Lee, J.W. Wojcieszyn, M.C. Wang, L.A. Valenzuela, G.P. Murphy and T.M. Chu, 1980. Fundamental biochemical and immunological aspects of prostatic acid phosphatase. Prostate, 1: 415-425.

Looareesuwan, S., P. Wilairatana, S. Vanijanonta, P. Pitisuttithum, C. Viravan and K. Kraisintu, 1996. Treatment of acute, uncomplicated, falciparium malaria with oral dihydroartemisinin. Ann. Trop. Med. Parasitol., 90: 21-28.

Maiteki-Sebuguzi, C., P. Jagannathan, V.M. Yau, T.D. Clark and D. Njama-Meya et al., 2008. Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children. Malaria J., 7: 106-109.

Nakanishi, N., M. Okamota, K. Makino, K. Suzuki and K. Tatara, 2002. Distribution and cardiovascular risk correlates of serum triglycerides in young Japanese adults. Industrial Health, 40: 28-35.

Ngokere, A.A., T.C. Ngokere and A.P. Ikwudinma, 2004. Acute study of histomorphological and biochemical changes caused by artesunate in visceral organs of the rabbit. J. Exp. Clin. Anat., 3: 11-16.

Nontprasert, A., S. Pukrittayakamee, A.M. Dondorp, R. Clemens, S. Looareesuvan and N.J. White, 2002. Neuropathologic toxicity of artemisinin derivatives in a mouse model. Am. J. Trop. Med. Hyg., 67: 423-429.

Nosten, F. and N.J. White, 2007. Artemisinin-based combination treatment of falciparum malaria. Am. J. Trop. Med. Hyg., 77: 181-192.

Nwanjo, H.U. and G. Oze, 2007. Acute hepatotocixity following administration of artesunate in guinea pigs. Internet J. Toxicol., Vol. 4, No. 1.

Nwanjo, H.U., I.I. Iroagba, I.N. natuanya and N.A. Eze, 2007. Antifertility activity of dihydroartemisinin in male albino rats. Internet J. Endocrin, 4: 1-5.

Obianime, A.W. and J.S. Aprioku, 2009. Comparative testicular histopathological effects of artemisinin derivatives and some ACTS in the guinea-pig. J. Exp. Clin. Anatomy, 8: 31-40.

Olliaro, P.L. and W.R. Taylor, 2004. Developing artemisinin-based drug combinations for the treatment of drug-resistant falciparum malaria: A review. J. Postgrad. Med., 50: 40-44.

Qiao, G.F., B.F. Yang, W.H. Li and B.Y. Li, 2003. Effects of artemisinin on action potentials from C-type nodose ganglion neurons. Acta Pharmacologica Sinica, 24: 937-942.

Raji, Y., T.O. Osonuga, O.S. Akinsomisoye, O.A. Osonuga and O.O. Mewoyeka, 2005. Gonadotoxicity evaluation of oral artemisinin derivatives in male rats. J. Med. Sci., 5: 303-306.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.

Sachs, J. and P. Malaney, 2002. The economic and social burden of malaria. Nature, 415: 680-685.

Simonsson, U.S.H., B. Jansson, T.N. Hai, D.X. Huong, G. Tybring and M. Ashton, 2003. *Artemisinin autoinduction* is caused by involvement of cytochrome P450 2B6 but not 2C9. Clin. Pharmacol. Thererapeutics, 74: 32-43.

Sugiura, M., T. Oikawa, K. Hirano, H. Maeda, H. Yoshimura, M. Sugiyama and T. Kuratsu, 1977. A simple colorimetric method for determination of serum triglycerides with lipoprotein lipase and glycerol dehydrogenas. Clinica Chimica Acta, 81: 125-130.

Svensson, U.S., M. Maki-Jouppila, K.J. Hoffmann and M. Ashton, 2003. Characterisation of the human liver *in vitro* metabolic pattern of artemisinin and auto-induction in the rat by use of nonlinear mixed effects modelling. Biopharmaceutics Drug Disposition, 24: 71-85.

Tabassum, N. and S.S. Agrawal, 2003. Hepatoprotective of activity of *Embelia ribes* against paracetamol induced hepatocellular damage in mice. Exp. Med., 10: 43-44.

Vahdati-Mashhadian, N., H. Rakhshandeh and A. Omidi, 2005. An investigation on LD50 and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie, 60: 544-547.

WHO, 2003. Assessment of the Safety of Artemisinin Compounds in Pregnancy. World Health Organization, Geneva.

WHO, 1995. The Role of Artemisinin and its Derivatives in the Current Treatment of Malaria. World Health Organization, Geneva.

WHO, 1998. Malaria Chemotherapy. World Health Organization, Geneva.