International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 7 (1): 84-95, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.84.95
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Mechanism of Action of Artemisinins on Biochemical, Hematological and Reproductive Parameters in Male Guinea Pigs

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Abstract: The present study intends to investigate the effects/mechanisms of frequently used artemisinin derivatives: artesunate and dihydroartemisinin (DHA) on biochemical, hematological and spermatic parameters in the male guinea pig. Half, normal and double therapeutic doses of each drug were given to different groups of animals (n = 5) by oral gavage. Similar treatments were given to other animal groups after pretreatments with vitamins C (1.5 mg kg^{-1}), E (50 mg kg^{-1}) and C+E; while only distilled water was given to a control group. Animals were sacrificed at the end of drug treatments and blood samples and semen were analyzed. Artesunate and DHA caused significant ($p<0.05$) and dose-dependent increase in the serum levels of prostatic acid phosphatase, aspartate aminotransferase and alanine aminotransferase. Also, serum creatinine level was significantly increased, while uric acid level was decreased by both drugs. Furthermore, while having no significant effects on RBC, hemoglobin and hematocrit, artesunate and DHA caused dose-dependent increase in WBC and lymphocyte counts and decrease in neutrophils. Sperm count and motility were also significantly decreased by both drugs; however pretreatments with vitamins C and E blocked all the individual effects of artesunate and DHA. The results show that artesunate and DHA may be toxic to the liver, testis and hematopoietic cells resulting from increase in oxidative stress via stimulation of protein kinase C activity.

Key words: Artemisinins, biochemical, hematopoetic, oxidative stress, PKC and semen

INTRODUCTION

Malaria, a mosquito-borne disease is a serious health challenge to mankind. Human malaria results from infection with *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* or *Plasmodium malariae*, but a large majority of the clinical cases and mortalities is caused by *Plasmodium falciparum* (Bozdech *et al*., 2003). The Plasmodium parasite (a protozoan) is transmitted to humans by mosquitoes of the genus Anopheles. *P. falciparum* is the leading cause of disease in most parts of the world, producing over 500 million new infections, with a mortality estimate of about 3 million deaths each year (Snow *et al*., 2005). The disease is endemic in sub-Saharan Africa and children below the age of five suffer the greatest burden of the disease (Aultman *et al*., 2002). Despite efforts to control the disease, malaria is among the top three deadly communicable diseases and the most deadly tropical disease (Sachs and Malaney, 2002). The consequences of the disease are further compounded by extremely low living conditions in poor nations.

Control measures of the disease include preventive (which involves control of the vector of the malaria parasite by use of insecticides, mosquito nets and maintenance of good sanitary conditions) and treatment of the disease by the use of drugs. The earliest drugs used were the aminoquinolines, among which chloroquine was the mainstay for nearly 60 years. Since, its synthesis in 1940s, chloroquine has been the first line of drug in malaria treatment because of its unique properties: quick onset of action, high efficacy, low cost and tolerable adverse effects. However, due to the emergence of resistance to chloroquine and other drugs, newer anti-malarial drugs have been developed including the artemisinins. Artemisinin is presently considered as perfect replacement for chloroquine, because it has all the above qualities of chloroquine and probably more (WHO, 1995; Katzung, 2007).

Artemisinin, also called qinghaosu in China, was discovered in 1972 by a Chinese scientist as the active principle of the leaves of a Chinese medicinal plant known as Artemisia annua, which has been used in Chinese traditional medicine for more than a thousand years to

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treat malaria and other skin diseases (Klayman, 1985). It is a potent and rapidly acting blood schizonticide, eliciting shorter parasite clearance times and rapid symptomatic responses than chloroquine and many other anti-malarials. It acts against the asexual stages and gametocytes and also blocks sporogony, thereby affecting all stages or sites of the parasite's life cycle. Semi synthetic derivatives of artemisinin are also available, which include artesunate, artemether, dihydroartemisinin (DHA), arteether and artelinic acid. These derivatives are structurally modified forms of artemisinin, with better pharmacokinetic properties and higher efficacy than the parent artemisinin compound. DHA, which is water soluble, is the active metabolite of all artemisinin compounds. Artesunate is also water soluble, while artemether and the other derivatives are lipid soluble. Currently, artemisinin based combination therapies (ACTs) consisting of artemisinin or its derivatives and other anti-malarial drugs are employed as the first line anti-malarial agents in malaria chemotherapy (Olliaro and Taylor, 2004; Nosten and White, 2007).

Artemisinins are considered to have high safety margins (Nosten and White, 2007); however they may be toxic under certain conditions (Arab *et al*., 2009). Artemisinins are selectively distributed into *P. falciparum* infected erythrocytes, where they cause malaria parasite's death through generation of free radicals (Vyas *et al*., 2002; Woodrow *et al*., 2005; Little *et al*., 2009). However, these drugs are also distributed in other organs including the liver, CNS, plasma, lungs, etc (Zhao and Song, 1989; Vyas *et al*., 2002; Davis *et al*., 2003) making such organs possible targets of toxicity. Increased production of free radicals such as Reactive Oxygen Species (ROS) can induce oxidative stress which causes damage to important cellular components such as cell membranes, proteins, DNA etc and impair normal biochemical functions. To prevent oxidative stress and maintain redox homeostasis, the body has evolved a number of interrelated antioxidant mechanisms which includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), gluthathione peroxidase (GSH-Px) and heme oygenase; and vitamins (Sies, 1997; Toyokuni, 1999). The antioxidative vitamins include vitamins C and E which are potent antioxidants that are useful in the body to maintain redox homeostasis (Herrera and Barbas, 2001; Padayatty *et al*., 2003). Furthermore, the control of redox balance is critical to cellular development, differentiation and homeostasis and when there is a disturbance in the balance between the production of ROS or free radicals and antioxidant defense, oxidative stress occurs (Sies, 1997). Oxidative stress is implicated in various pathological conditions including: arteriosclerosis, Parkinson's disease, heart disease, aging, arthritis, diabetes, cancer, Alzheimer's disease etc. (Floyd, 1990; Kohen and Nyska, 2002). It also affects male reproductive function adversely (Aitken *et al*., 2003; Turner and Lysiak, 2008).

The present study investigated the influences of vitamins C and E on the effects of artesunate and DHA on some biochemical, hematological and semen parameters of the male guinea pig.

MATERIALS AND METHODS

Materials: Artesunate (Artesunat) tablets (Mekophar Chemical Pharmaceutical Joint-stock Company, Vietnam); dihydroartemisinin (Alaxin) tablets (Bliss GVS Pharma Ltd., India); vitamin C tablets (Emzor Pharmaceutical Ltd., Nigeria) and vitamin E gelules (Strides-Colab Ltd., India) were obtained from the Pharmacy Department of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria in November, 2009. Artesunate, DHA and vitamin C tablets were powdered separately in a glass mortar, mixed with distilled water and 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. Vitamin E was also given by oral gavage in its oily formulation at a dose of 0.2 mL kg^{-1}.

Animals: Outbred strains of adult male guinea-pigs (*Cavia porcellus*) weighing between 650-670 g and 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 with wire bar lids. Bedding was provided 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*. All the animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by Canadian Council of Animal Care (2009).

Methods: A total number of 125 guinea pigs were used for the experiment. One hundred and twenty animals were randomly distributed into 8 major groups: artesunate, DHA, vitamin C+artesunate, vitamin E+artesunate, vitamin C+vitamin E+artesunate, vitamin C+DHA, vitamin E+ DHA and vitamin C + vitamin E + DHA, each containing

3 subgroups of 5 pigs/group. A ninth group consisting of only 5 animals was used as the control.

The animals in the first subgroup of artesunate group were administered artesunate for 5 consecutive days at a daily dose of 4, 2, 2, 2 and 2 mg kg^{-1}. This is equivalent to the clinical dose of artesunate for the treatment of uncomplicated malaria (Angus *et al*., 2002) and is referred to in this experiment as mid dose of artesunate. The second subgroup received 2, 1, 1, 1 and 1 mg kg^{-1} of artesunate, which is equivalent to half the clinical dose (low dose). The third subgroup was given 8, 4, 4, 4 and 4 mg kg^{-1} of artesunate, equivalent to double the clinical dose (high dose).

The first subgroup in DHA group was given 2.2, 1.1, 1.1, 1.1, 1.1 and 1.1 mg kg^{-1} dose levels of DHA for 7 consecutive days, which is equivalent to its recommended clinical dose for the treatment of uncomplicated malaria (Looareesuwan *et al*., 1996). This dose is also referred to in this experiment as mid dose of DHA. The second subgroup received 1.1, 0.55, 0.55, 0.55, 0.55 and 0.55 mg kg^{-1} of DHA, equivalent to half the clinical dose (low dose). The third subgroup had 4.4, 2.2, 2.2, 2.2, 2.2 and 2.2 mg kg^{-1} of DHA, equivalent to double the clinical dose (high dose).

The vitamin C + artesunate animals were pretreated with 1.5 mg kg^{-1} of vitamin C orally for 1 h before low dose, mid dose and high dose of artesunate was administered as described earlier. Vitamin E + artesunate group was pretreated with 50 mg kg^{-1} of vitamin E orally for 1 h before low dose, mid dose and high dose of artesunate was administered as described earlier. Vitamin C + vitamin E + artesunate group was pretreated with a combination of vitamins C (1.5 mg kg^{-1}) and E (50 mg kg^{-1}) for 1 h before low dose, mid dose and high dose of artesunate was administered as described earlier.

The vitamin C+ DHA animals were pretreated with 1.5 mg kg^{-1} of vitamin C orally for 1 h before low dose, mid dose and high dose of DHA was administered as described earlier.

Vitamin E+ DHA group was pretreated with 50 mg kg^{-1} of vitamin E orally for 1hr before low dose, mid dose and high dose of DHA was administered as described earlier. Vitamin C + vitamin E + DHA' group was pretreated with a combination of vitamins C (1.5 mg kg^{-1}) and E (50 mg kg^{-1}) for 1 h before low dose, mid dose and high dose of DHA was administered as described earlier.

The control group was given distilled water (0.5 mL) daily for 7 days.

All animals were sacrificed by decapitation under pentobarbitone anaesthesia, 37 mg kg^{-1} i.p. (Flecknell, 2009) 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.

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

Biochemical parameters: Blood sample collected was centrifuged for 15 min at 3,000 rpm and clear serum was then separated from the cells and stored at -80°C and assayed for alkaline phosphatase (ALP), total acid phosphatase (ACPT), prostatic acid phosphatase (ACPP), aspartate transaminase (AST), alanine transaminase (ALT), urea, uric acid, cholesterol and total protein. ALP was assayed by the phenolphthalein method (Babson *et al*., 1966); ACPT and ACPP by colorimetric method (Fishman and Davidson, 2004) and the transaminases (AST and ALT) were measured according to the method of Reitman and Frankel (1957). Furthermore, urea was assayed by Urease-Berthelot method (Weatherburn, 1967); uric acid by Fossati *et al*. (1980), total cholesterol by the enzymatic endpoint method (Kayamori *et al*., 1999) and total protein was measured by biuret method as described by Henry *et al*. (1974).

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 are expressed as Means±standard errors of mean. Comparisons between control values and values of treated animal groups were performed with one-way Analysis of Variance (ANOVA). Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Effects of vitamins C and E pretreatments on artesunate and DHA-induced serum levels of biochemical parameters: The serum levels of prostatic acid phosphatase (ACPP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and were

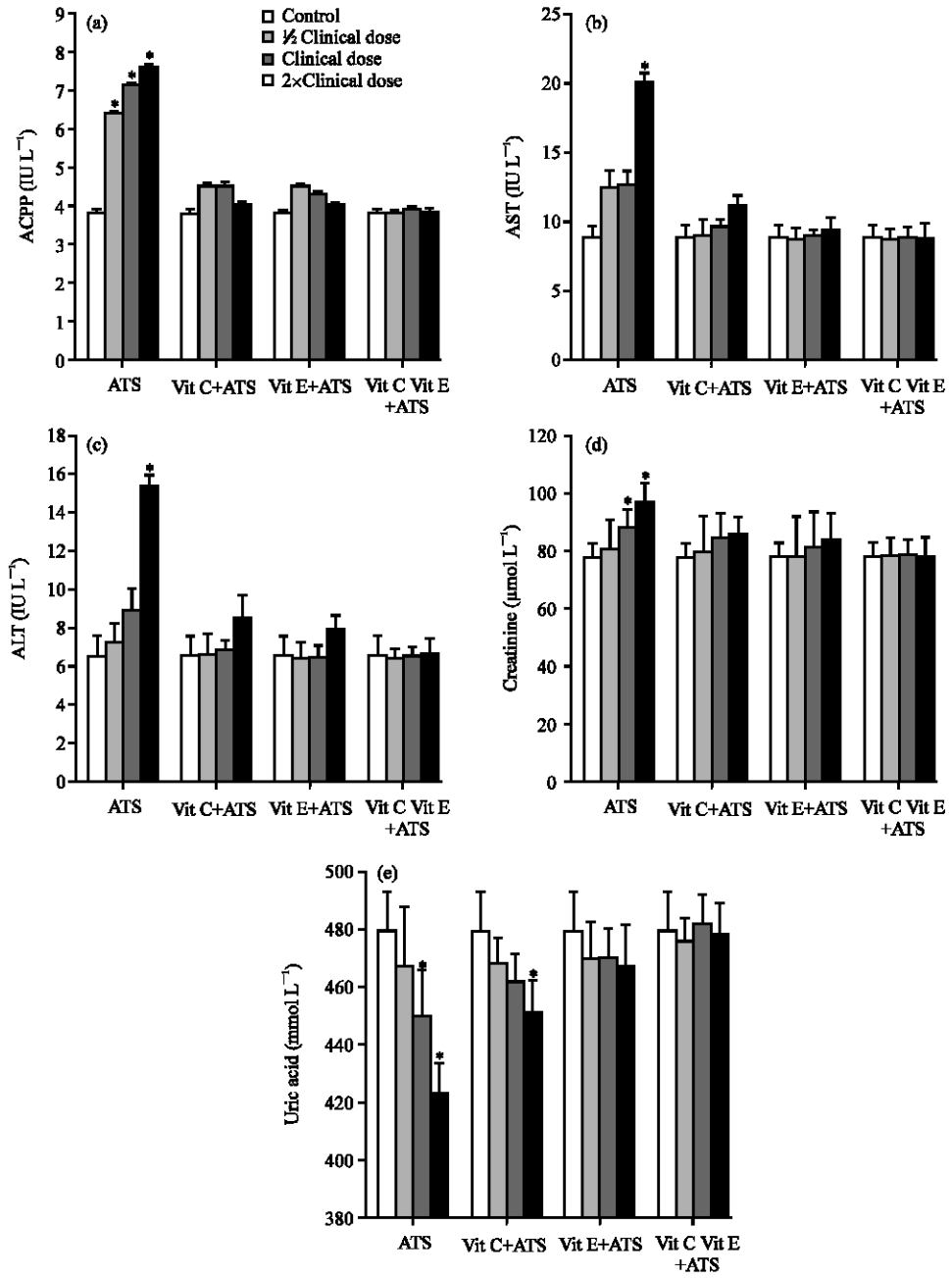

Fig. 1: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on artesunate-induced serum levels of: (a) prostatic acid phosphatase (ACPP), (b) aspartate aminotransferase (AST), (c) alanine aminotransferase (ALT), (d) creatinine and (e) uric acid in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (ATS: Artesunate; Vit C: Vitamin C; Vit E: Vitamin E)

significantly ($p<0.05$) and dose-dependently increased in animal groups that were administered with artesunate and DHA, compared to control animal group. The maximum serum levels of ACPP, AST and ALT obtained in artesunate-treated animals were: 7.6±0.09, 20.0±2.35 and 15.25±0.6 IU L^{-1}, respectively (Fig. 1a-c), while 8.4±1.22, 24.75±2.2 and 18.0±1.47 IU L^{-1} were obtained, respectively in DHA-treated animals (Fig. 2a-c). Compared to the control serum values for ACPP (3.80±0.07 IU L^{-1}), AST (8.83±1.22 IU L^{-1}) and ALT (6.50±1.0 IU L^{-1}), the above values of artesunate represent 100, 126.5 and 134.6% increases in the parameters, respectively and the DHA

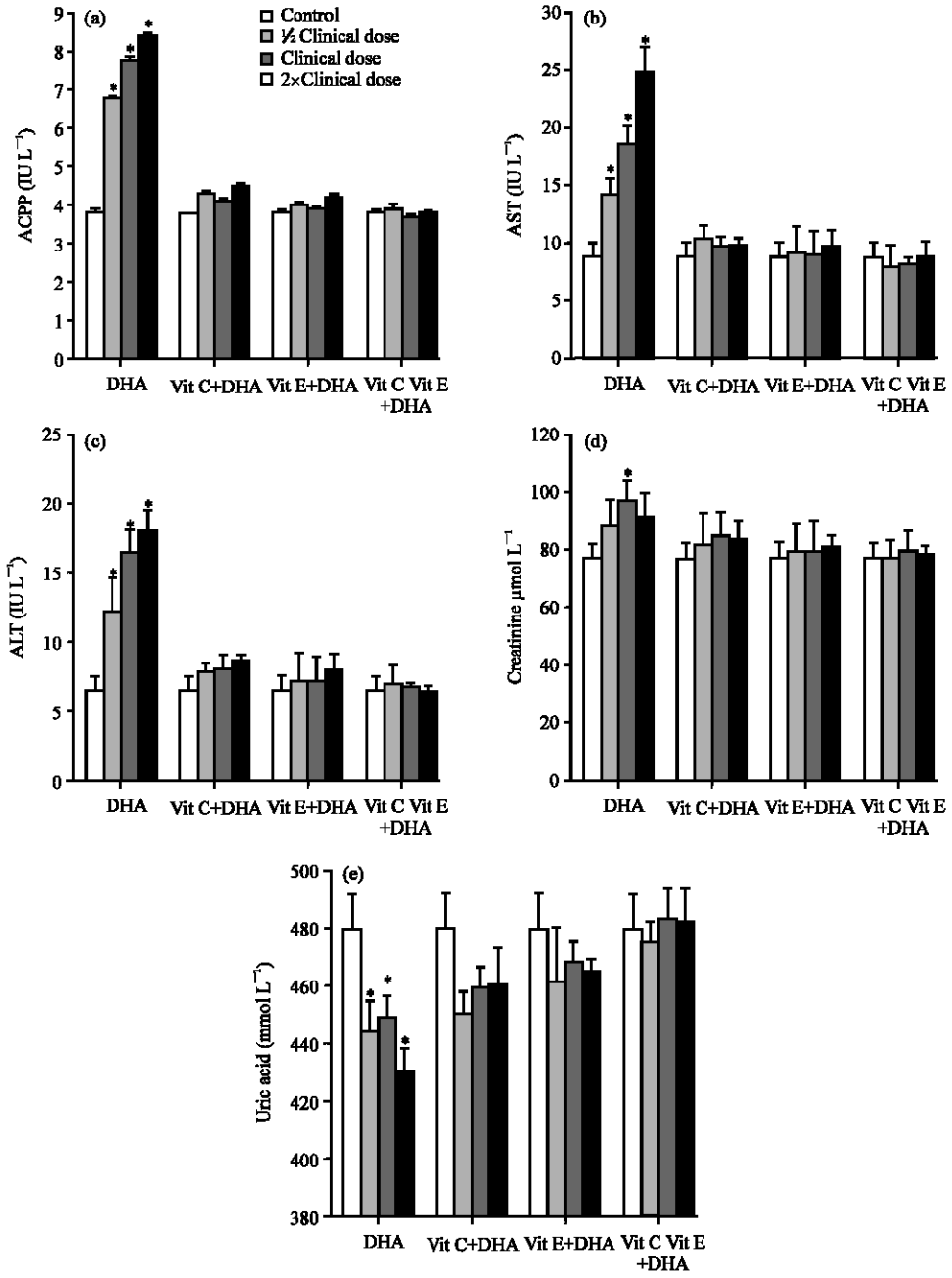

Fig. 2: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on dihydroartemisinin-induced serum levels of: (a) prostatic acid phosphatase (ACPP), (b) aspartate aminotransferase (AST), (c) alanine aminotransferase (ALT), (d) creatinine and (e) uric acid in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (DHA: Dihydroartemisinin; Vit C: Vitamin C; Vit E: Vitamin E)

values represent 121, 180 and 177% increases, respectively. In addition, while the serum creatinine levels obtained in animal groups that were given artesunate (97.0±5.76 µmol L^{-1}) and DHA (92.0±7.7 µmol L^{-1}) were significantly ($p<0.05$) higher than the control value (77.50±5.0 µmol L^{-1}), the serum uric acid levels in artesunate-treated (423.5±9.2 mmol L^{-1}) and DHA-treated animals (430.8±7.6 mmol L^{-1}) were significantly lower, when compared to the baseline data of uric acid (480.0±12.4 mmol L^{-1}) obtained in control animal group (Fig. 1d-e, 2d-e). The serum levels of ACPP, AST, ALT, uric acid and creatinine in animal groups that were

pretreated with vitamins C, E and a combination of vitamins C and E before administration of artesunate or DHA were not significantly different from the corresponding values of control animals. However, the values obtained in animals pretreated with a combination of both vitamins were nearer to the control values for these biochemical parameters, compared to the values obtained in animals pretreated with a single vitamin, either C or E (Fig. 1, 2). Furthermore, there were no significant differences between the experimental and control animal groups in the serum levels of the other biochemical parameters measured (Not shown).

Effects of vitamins C and E pretreatments on artesunate and DHA-induced blood levels of hematological parameters in male guinea pigs: Artesunate and DHA caused significant ($p<0.05$) dose-dependent increase in WBC counts. The WBC counts in animal groups given high doses of artesunate ($15.8\pm1.36\times10^9$ mL^{-1}) and DHA ($18.08\pm0.8\times10^9$ mL^{-1}) were higher than the WBC count ($8.52\pm0.50\times10^9$ mL^{-1}) in control animals (Fig. 3a, 4a). These values correspond to 85.4 and 112% increases respectively. Furthermore, neutrophil count was decreased, while lymphocyte count was increased dose-dependently in artesunate and DHA-treated animals but only the values of high dose animal groups were significant ($p<0.05$), compared to the control values. Neutrophil counts in control animal group, high dose artesunate-treated animal group and high dose DHA-treated animal group were: 65.67 ± 1.31, 59.5 ± 1.03, and 56.0 ± 1.41%, respectively (Fig. 3b, 4b), representing 9.4 and 14.7% decreases by artesunate and DHA, respectively. Also, lymphocyte counts in control animal group, high dose artesunate-treated animal group and DHA-treated animal group were: 31.17 ± 1.22, 37.5 ± 0.96 and 42.0 ± 1.4% (Fig. 3c, 4c). These values are equivalent to 20.3 and 34.7% increases in lymphocyte level by artesunate and DHA, respectively. The blood levels of WBC, neutrophils and lymphocytes in animal groups that were pretreated with vitamins C, E and a combination of both vitamins before administration of artesunate or DHA were not significantly different from the values obtained

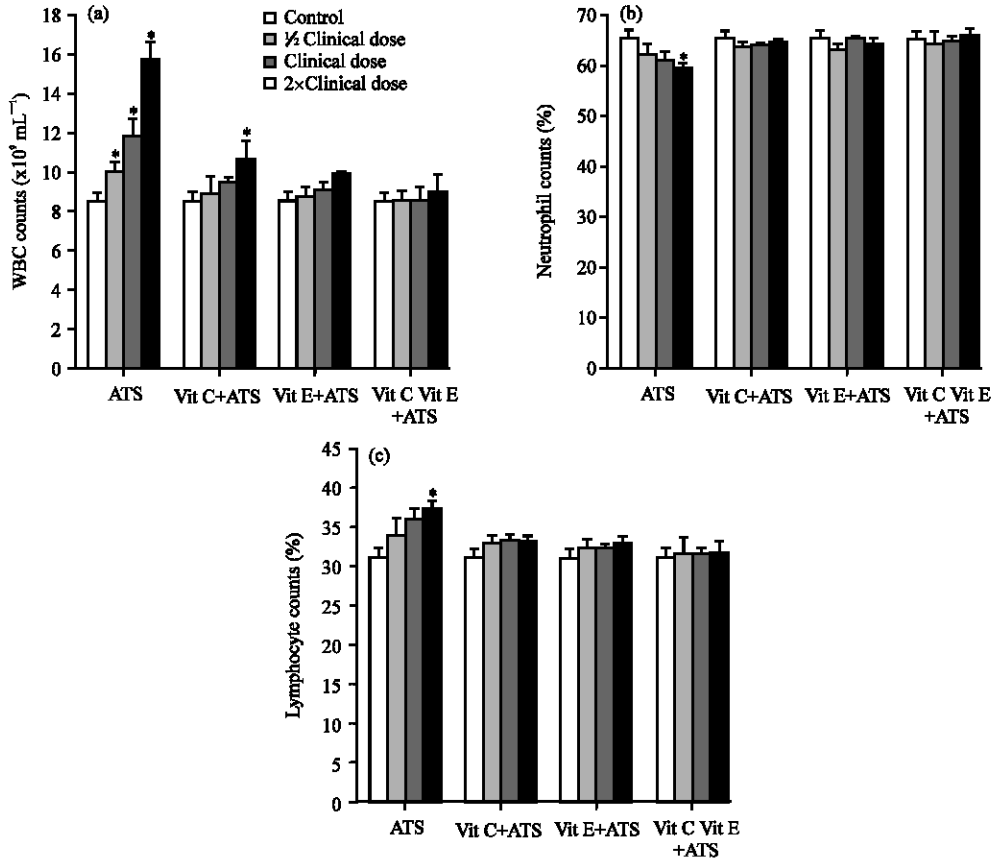

Fig. 3: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on artesunate-induced blood levels of: (a) White Blood Cell (WBC) counts, (b) neutrophil counts and (c) lymphocyte counts in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (ATS: Artesunate; Vit C: Vitamin C; Vit E: Vitamin E)

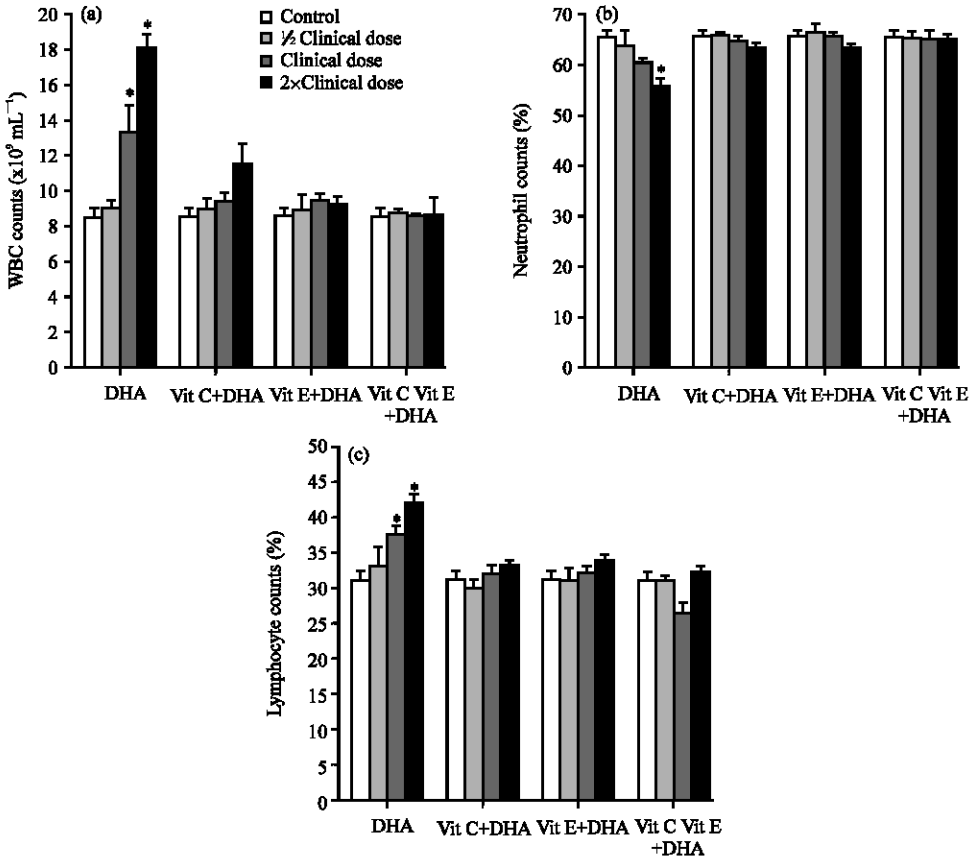

Fig. 4: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on Dihydroartemisinin-induced blood levels of: (a) white blood cell (WBC) counts, (b) neutrophil counts and (c) lymphocyte counts in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (DHA: Dihydroartemisinin; Vit C: Vitamin C; Vit E: Vitamin E)

in the control animals (Fig. 3 and 4). However, the values obtained in animals pretreated with combination of both vitamins were nearer to the control values for these hematological parameters, compared to the values obtained in animals pretreated with a single vitamin, either C or E (Fig. 3, 4). Furthermore, there were no significant changes in the blood levels of the other hematological parameters (PCV, Hb, RBC, monocytes, eosinophils and basophils) between experimental and control animal groups (Not shown).

Effects of vitamins C and E pretreatments on artesunate and DHA-induced toxicological effects on semen parameters: The sperm counts and motility of animals given artesunate and DHA were significantly ($p<0.05$) decreased, compared to the control values. The lowest sperm counts ($56.5\pm5.2\times10^6$ and $51.6\pm4.0\times10^6$ mL^{-1}) were obtained in animal groups that were given low dose artesunate and high dose DHA, respectively, compared to ($81.14\pm2.34\times10^6$ mL^{-1}) in control animals (Fig. 5a, 6a). These values are equivalent to 30.4 and 36.4% reductions, respectively in the control sperm count value. The sperm motility values in artesunate and DHA-treated animal groups were also significantly reduced compared to the control value. The results were dose-dependent in the DHA-treated animal groups and biphasic in artesunate-treated animals, with lowest sperm motility values of 53.7±6.25 and 40.00±4.4%, respectively, compared to 81.57±2.64% obtained in the control animals (Figs 5b, 6b). These values correspond to 34.2 and 51% reductions respectively. Furthermore, the percentages of abnormal sperm cells (morphology) and debris were significantly higher in the animal groups that received mid dose of DHA, while morphology values were higher in animals that received low and mid doses artesunate, compared to the control animal values (Table 1, 2). Furthermore, the sperm count, motility, morphology and debris of most animal groups that were pretreated with

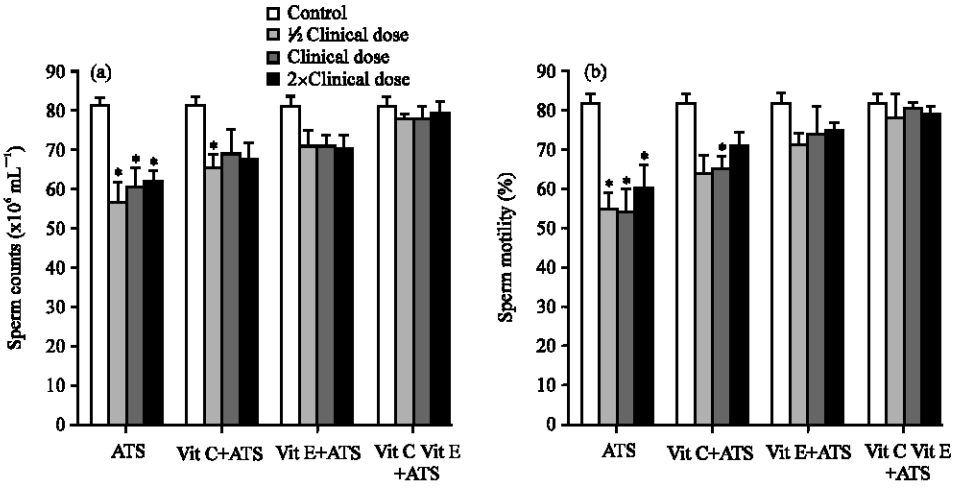

Fig. 5: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on artesunate-induced toxicological effects on: (a) sperm count and (b) sperm motility in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (ATS: Artesunate; Vit C: Vitamin C; Vit E: Vitamin E)

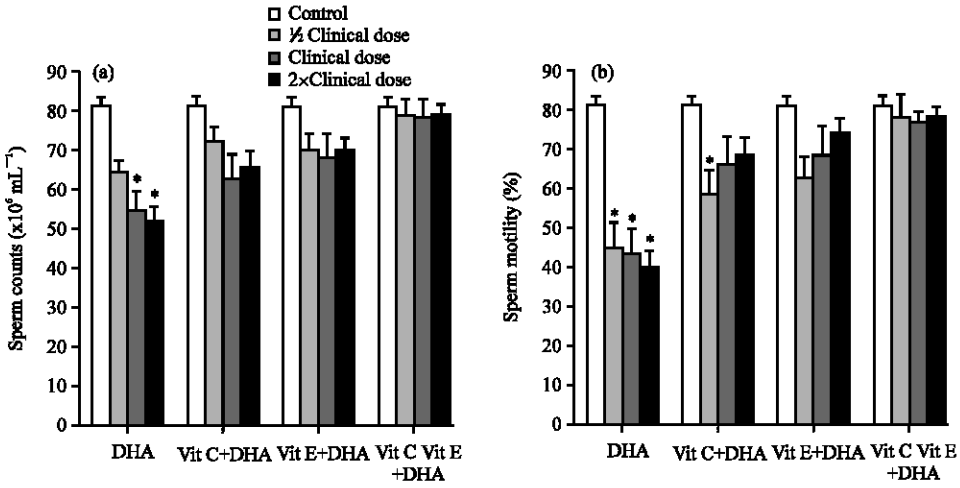

Fig. 6: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on dihydroartemisinin-induced toxicological effects on: (a) sperm count and (b) sperm motility in the male guinea pig. Data are expressed as Means±SEM. *Significant at $p<0.05$. (DHA: Dihydroartemisinin; Vit C: Vitamin C; Vit E: Vitamin E)

vitamins C, E and a combination of vitamins C and E were not significantly different from the values obtained in the control animal group (Table 1, 2 and Fig 5, 6). However, the values obtained in animals pretreated with a combination of both vitamins were nearer to the control values for all the semen parameters, compared to the values obtained in animals pretreated with a single vitamin, either C or E (Table 1, 2, Fig. 5, 6).

In the present study, the influences of vitamins C, E and a combination of both vitamins on the effects of artesunate and DHA on some biochemical, hematological and semen parameters of the male guinea pig were investigated. Although, there are reports on the effects of artemisinins on some of these parameters in earlier studies, most of such studies were carried out in other animal models and under different experimental conditions (Ngokere *et al*., 2004; Raji *et al*., 2005; Adaramoye *et al*., 2008). The guinea pig was used in this study because of its better drug metabolic system (Gregus *et al*., 1988). Also, the mechanism of these drugs on the investigated systems had mostly been speculative prior to this study.

The biochemical result shows that artesunate and DHA caused significant ($p<0.05$) and dose-dependent increase in the serum levels of prostatic acid phosphatase

Table 1: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on artesunate-induced effects on some semen parameters in male guinea pigs. Data are expressed as Means±SEM. *Significant at p<0.05. (ATS: artesunate; Vit C: Vitamin C; Vit E: Vitamin E)

Dose	Abnormal sperm cells (Morphology) (%)	Sperm debris (%)
Control	15.00±2.44	15.71±3.99
½ Clinical dose		
ATS	28.25±1.30*	16.50±1.44
Vit C+ATS	18.25±2.41	15.25±1.25
Vit E+ATS	15.50±2.03	16.50±3.87
Vit C+Vit E+ATS	15.25±2.20	15.50±2.50
Clinical dose		
ATS	25.10±2.50*	22.50±3.26
Vit C+ATS	20.25±1.25	20.00±2.00
Vit E+ATS	16.50±3.00	17.50±1.25
Vit C+Vit E+ATS	15.25±2.51	16.50±2.45
2xClinical dose		
ATS	21.25±2.25	18.75±4.29
Vit C+ATS	16.25±2.39	18.50±2.50
Vit E+ATS	17.25±1.25	16.60±0.00
Vit C+Vit E+ATS	15.50±1.44	16.00±0.00

Table 2: Effects of vitamins C, E and a combination of vitamins C and E pretreatments on dihydroartemisinin-induced effects on some semen parameters in male guinea pigs. Data are expressed as means ± SEM. * Significant at p<0.05. (DHA: Dihydroartemisinin; Vit C: Vitamin C; Vit E: Vitamin E)

Dose	Abnormal sperm cells (Morphology) (%)	Sperm debris(%)
Control	15.00±2.44	15.71±3.99
½ Clinical dose		
DHA	22.00±4.39	22.50±1.44
Vit C+DHA	17.50±2.25	15.25±1.25
Vit E+DHA	17.00±2.33	16.50±0.87
Vit C+Vit E +DHA	15.00±1.25	15.50±2.50
Clinical dose		
DHA	32.20±2.3*	30.5±2.09*
Vit C+DHA	25.0±2.25*	21.20±2.50*
Vit E+DHA	18.5±4.33	20.00±1.25
Vit C+Vit E +DHA	16.20±3.0	15.82±1.44
2xClinical dose		
DHA	29.90±1.25*	25.75±4.29
Vit C+DHA	27.25±2.39	18.50±5.50
Vit E+DHA	17.90±3.25	16.51±1.90
Vit C+Vit E +DHA	15.50±1.74	16.10±2.00

(ACPP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). AST and ALT are hepatic enzymes and their serum levels are used as surrogate markers of hepatic toxicity. In the present study, elevation in the serum levels of AST and ALT by artesunate and DHA is highly indicative of hepatic toxicity (Vahdati-Mashhadian *et al*., 2005; Ewaraiah and Satyanarayana, 2010). 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 (Ngokere *et al*., 2004; Nwanjo and Oze, 2007; Udobre *et al*., 2009). Additionally, the increase in serum ACPP levels may be due to adverse effects on the testicular structures, especially the prostate (Lin *et al*., 1980; Chu and Lin, 1998). With the serum levels of ACPP, AST and ALT increased by 100, 126.5 and 134.6%, respectively by artesunate and 121, 180 and 177%, respectively by DHA, it shows that DHA may be more toxic to the liver than artesunate. Furthermore, consistent with the findings of Adaramoye *et al*. (2008), artesunate and DHA caused significant increase in the serum creatinine level. The drugs also decreased serum uric acid level, without significant effects on alkaline phosphatase (ALP), total acid phosphatase (ACPT), urea, cholesterol and total protein. Elevation in the serum levels of creatinine is suggestive of renal toxicity (Perrone *et al*., 1992; Traynor *et al*., 2006), while reduction in the serum uric acid concentration is an indication of reduced plasma antioxidative potential (Hooper *et al*., 2000; Knapp *et al*., 2004).

Furthermore, while artesunate and DHA had no significant effects on red blood cell counts, hemoglobin and hematocrit, the drugs caused significant dose-dependent increase in WBC and lymphocyte counts, with a decrease in neutrophil counts (neutropenia), showing clear indications of trauma in the animals (Guyton, 2006). In addition, sperm counts and motility were significantly decreased by both drugs, which are consistent with previous studies (Raji *et al*., 2005; Nwanjo *et al*., 2007; Obianime and Aprioku, 2009). Maximal effects on the sperm counts and motility were observed at the low dose of artesunate and high dose of DHA. Furthermore, the percentages of abnormal sperm cells (morphology) and debris were significantly increased at the mid dose of DHA, while only morphology was increased by artesunate at the low and mid doses. These results show that the testicular tissue may be very sensitive to artesunate, making low concentrations (sub clinical doses) of the drug to cause toxicity to the testis.

Pretreatment of animals with vitamins C, E and a combination of the two vitamins prevented or blocked the individual effects of artesunate and DHA on the biochemical parameters (ACPP, AST, ALT, creatinine and uric acid); hematological parameters (WBC, lymphocytes and neutrophil counts) and semen parameters (sperm count, motility, morphology and debris). Vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol) are potent and effective hydrophilic and lipophilic antioxidants, respectively (Niki *et al*., 1995; Padayatty *et al*., 2003). The vitamins stabilize cell membranes by preventing lipid and fatty acid peroxidation through the scavenging or quenching of free radicals (ROS), thereby protecting the cell membranes from oxidative damage (Niki *et al*., 1995; Traber and Atkinson, 2007). The results thus indicate strongly that the mechanism of the observed effects of artesunate and DHA in this study may be via increase in oxidative stress.

This mechanism may be related to the anti-malarial action of the drugs. The characteristic peroxide lactone structure in the artemisinins is indispensable for their anti-malarial activity. Splitting of this endoperoxide bridge by heme iron species results in the release of Reactive Oxygen Species (ROS) that eventually cause parasite's death (Woodrow *et al*., 2005; Little *et al*., 2009). Although, this process takes place within plasmodium-infected erythrocytes, artemisinins distributed in other parts of the body could also be oxidized to generate ROS that will induce oxidative stress and cause toxicity as observed in this study. In the liver, the metabolism of artemisinins by cytochrome P450 causes production of ROS by inducing superoxide dismutase and O-xanthine oxidase enzymes (Robert *et al*., 2000). The latter causes mobilization of iron from ferritin, which splits the endoperoxide bridge of artemisinin compounds to release ROS. This may account for the serum elevation of the hepatic enzymes (AST and ALT). Also, increase in oxidative stress by the artemisinins in the testis may be responsible for the adverse effects on the semen parameters. This is in agreement with previous reports that have shown that testicular function is sensitive to oxidative stress and ROS are highly implicated in the etiology of male infertility in experimental animals and man (Aitken *et al*., 2003; Agarwal *et al*., 2005; Turner and Lysiak, 2008). Previous studies with artemisinins had also shown similar observations of increased oxidative stress in embryonic stem cells (Wartenberg *et al*., 2003) and human leukemia cells (Kim *et al*., 2003).

Furthermore, the values obtained in the animal groups that were pretreated with a combination of vitamins C and E were nearer to the control values than the values obtained in only vitamin C or vitamin E pretreated animal groups. This observed complimentary anti-oxidative action of the vitamins is consistent with previous studies (Niki *et al*., 1995; Tanaka *et al*., 1997). Vitamins C and E have different mechanisms in signal transduction. While vitamin E is a known inhibitor of protein Kinase C (PKC) activity (Freedman *et al*., 1996), the enhancement of endothelial Nitric Oxide (NO) synthesis by vitamin C is partly considered to be via stimulation cGMP (Huang *et al*., 2000; Heller *et al*., 2001). Stimulation of cGMP causes extrusion of intracellular calcium ions from its stores and will therefore affect calcium metabolism/utility. These actions (which may also contribute to their antioxidant effects) make vitamins C and E beneficial in diseases that result from increased oxidative stress (Freedman *et al*., 1996; Maxwell, 2000). In addition, protein kinase C activity is dependent on calcium (Gregory, 1997), thus a simultaneous inhibition of calcium and PKC pathways may result in the potentiation of the inhibitory responses of the two vitamins and this may account for the enhancement of their individual anti-oxidative effects.

We conclude that artesunate and DHA may be toxic to the liver, causing elevation in the serum levels of hepatic enzymes. Both agents may not cause anemia, but they may cause leucocytosis and at high doses neutropenia. Furthermore, artesunate and DHA may also affect testicular function, by interfering with spermatogenesis to cause reduced sperm counts and motility. The mechanism of these actions is proposed to be mediated via stimulation of protein kinase C activity, causing increased oxidative stress within the affected cells and tissues.

ACKNOWLEDGMENTS

Authors thank Mr. Joshua Isirima and Mrs. Matilda Deeko both of the Department of Pharmacology and Mr. M.D. Gwotmot of the Department of Human Physiology in University of Port Harcourt, Nigeria for their technical assistance.

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