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Full Length Research Paper

Comparative study of artesunate, ACTs and their combinants on the biochemical parameters of male guinea-pigs

A. W. Obianime and J. S. Aprioku*

Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.

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In this study, the effects of different doses of artesunate, artesunate/sulfadoxine/ pyrimethamine, artesunate/amodiaquine and their combinants (sulfadoxine/pyrimethamine and artesunate/ amodiaquine) on the biochemical parameters- alkaline phosphatase (ALP), total acid phosphatase (ACPT), prostatic acid phosphatase (ACPP), urea, creatinine, uric acid and total cholesterol of the male guinea-pig were investigated. Basal serum ACPP value was increased from 3.50 ± 0.42 to 4.75 ± 0.85 and 4.75 ± 0.48 IU/L by amodiaquine and sulfadoxine/pyrimethamine respectively at their subclinical doses. These values were significant at $p < 0.05$. Furthermore, the agents caused no significant effects ($p < 0.05$) on serum ALP and ACPT. Artesunate/sulfadoxine/pyrimethamine significantly increased ($p < 0.05$) serum urea at the 3 doses, while the effects of artesunate and artesunate/amodiaquine were biphasic, decreasing basal serum urea level at lower doses and increasing at higher doses. However, basal serum creatinine level was increased from 62.5 ± 6.7 to 89.8 ± 4.8 µmol/L by sulfadoxine/ pyrimethamine and decreased to 53.0 ± 0 µmol/L by artesunate/amodiaquine at their subclinical doses, while the other agents caused no significant ($p < 0.05$) effects. Furthermore, control serum uric acid level was significantly decreased from 368.8 ± 10.0 to 318.3 ± 5.8, 306.0 ± 1.2 and 312.3 ± 2.6 mmol/L by artesunate, amodiaquine and artesunate/amodiaquine, after administration of half their clinical doses, respectively. Total blood cholesterol was also significantly reduced from 2.43 ± 0.13 to 2.1 ± 0 and 1.9 ± 0 mmol/L after administration of clinical doses of amodiaquine and artesunate/amodiaquine, respectively. These biochemical changes may be due to oxidative effects induced by these agents.

Key words: Biochemical, phosphatase enzymes, prostatic acid phosphatase, ACT, combinants.

INTRODUCTION

Malaria is an endemic disease caused by different species of the genus Plasmodium which include *P. falciparium, P. vivax, P. ovale,* and *P. malariae.* The development of the parasite passes through an asexual phase in man and a sexual phase in the mosquitoe (the vector of the parasite). Malaria parasite exists in various forms-sporozoites, merozoites, schizonts, etc (Kyes et al., 2001). Blood schizonticides which suppress malaria by destroying the asexual blood forms of the parasites are the mainstay of acute malaria treatment and some are used for prophylaxis. They include 4-aminoquinolines (amodiaquine and chloroquine), the related aryl aminoalcohols (mefloquine and quinine) and artemisinin and its derivatives (artemeter and artesunate). Others include halofantrine, lumefantrine and antimetabolites like pyrimethamine which act synergistically when combined with the sulfaonamide-sulfadoxine (WHO, 2000; Katzung, 2004).

The treatment of malaria has posed great challenge to medicine and development of effective antimalarial drugs. This is due to the development of resistance of parasite to most antimalarial agents (Djimdé et al., 2001; Wongsrichanalai et al., 2002; Sharma, 2005) resulting in immense impact on the socio-economy of man (WHO,

*Corresponding author. E-mail: sydaprio@yahoo.com. Tel.: +2348035082379.

Abbreviations: ACT, Artemisinin-based combination therapy; **ALP,** alkaline phosphatase; **ACT,** total acid phosphatase; **ACPP,** prostatic acid phosphatase; **GP,** guinea-pig.

1998; Sachs and Malaney, 2002).

The choice of treatment for malaria has therefore metamorphosed from the inexpensive, effective and orally administered chloroquine to artemisinin and its derivatives (White, 1999). The artemisinins are highly efficacious against multi-drug resistant *P. falciparum* and capable of producing up to 10,000 fold reductions in parasite biomass per asexual cycle (Haynes, 2001; Nosten and White, 2007). However, it has been reported that due to treatment failures associated with monotherapy and artemisinins (Price et al., 1998; McIntosh and Olliaro, 2000), combined therapy of artemisinin with other antimalarial agents known as the artemisinin-based combination treatments (ACTs) have been recommended (White, 1997). The world health organization has recommended these regimens/combinations as-artesunate/ sulfadoxine/pyrimethamine (SP), artesunate/ amodiaquine, artemether/lumefantrine and artesunate/ mefloquine. The ACTs are particularly effective because of their high killing rates and they have become the main chemotherapeutic agents for malaria (Haynes, 2001).

The high prevalence of plasmodium infection, particularly in the tropics, easy accessibility of drugs (including antimalarials) over the counter and drug resistance, had lead to a widespread and indiscriminate use of antimalarials with their attendant consequences of drug toxicity. Nosten and White (2007) had reported that artemisinin and its derivatives on their own have relatively low toxicological effects and that any toxicity observed in artemisinin combination treatments may be due to the partner agents. However, studies with animal and cell-line had shown that artemisinin and its analogues cause growth inhibition and apoptosis of oral cancer cells (Nam et al., 2006), stimulation of differentiation of promyelocytic leukaemia cell line (Kim et al., 2003), acute hepatotoxicity in guinea-pigs (Nwanjo and Oze, 2007) and acute morphological and biochemical changes in visceral organs of rabbits (Ngokere et al., 2004) through an action on PKC and an increase in protein kinase C isoforms. These result in the production of oxygen free radicals and peroxide formation which will lead to the deleterious effects of these agents (Meshnick, 1994; Robert et al., 2000).

It is, therefore, in that light that we decided to investigate the effects of artemisinin-based combinations (arte-sunate/amodiaquine and artesunate/sulphadoxine/ pyrimethamine) on the biochemical parameters of the male guinea-pig in comparison with the effects of the individual partner agents (artesunate, amodiaquine, and sulphadoxine/pyrimethamine).

MATERIALS AND METHODS

Drugs

All the drugs used were obtained from the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt. The artemisinin derivative used was artesunate (Arinate), manufactured by ERFA, Rue des Cultivateurs 25, 1040 Brussels. The ACTs were-artesunate/sulphadoxine/pyrimethamine (Farenax) and artesunate/ amodiaquine (Dart) manufactured by Swiss Pharma Nigeria Ltd, Lagos, Nigeria; while the combinant drugs were amodiaquine (Camoquine) manufactured by Pfizer Afrique de l'Quest BP 3857-Dakar R.P. Senegal and sulfadoxine/ pyrimethamine (Fansidar) by Swiss Pharma Nigeria Ltd, Lagos, Nigeria. The agents were administered in distilled water obtained from the Department of Chemistry, Faculty of Sciences, University of Port Harcourt, Nigeria.

Animals

Outbred strains of adult male guinea-pigs (GPs) of average weight 450 ± 5 g were obtained from the animal house of the University of Port Harcourt, Nigeria and allowed to acclimatize for 14 days. The guinea-pigs were fed with alfalfa feeds daily *ad libitum* at a room temperature of 28±2°C with 12 h light/dark cycle.

The effects on biochemical parameters

The animals were divided into six (6) groups A, B, C, D, E and F of 5 animals each and orally administered with the following normal clinical doses of the agents. The animals in group A (the control) were administered with distilled water, while those in group B were administered with 2 mg/kg body weight of artesunate 12 h for 3 days. Animals in group C were given 10 mg base/kg body weight of amodiaquine once daily for 3 days, while those in group D received a single dose of 1.25/25 mg base/kg body weight of sulfadoxine/ pyrimethamine. The animals in group E were dosed with 4 mg/kg body weight of artesunate and 10 mg base/kg body weight of amodiaquine once a day for 3 days. Finally, the animals in group F received 4 mg/kg body weight of artesunate, given once a day for 3 days and a single administration of 1.25/25 mg base/kg of sulfadoxine/pyrimethamine. These experiments with the antimalarial agents were carried out simultaneously with other sets of animals using half and double the above doses of the antimalarial agents.

At the end of the series of experiments (that is, each treatment course), the animals were sacrificed and blood samples were collected, centrifuged for 15 min at 3,000 rpm and clear serum was then separated from the cells and stored at -80°C. The serum samples were assayed for alkaline phosphatase using the phenolphthalein method (Babson et al., 1966), total and prostatic acid phosphatases, using colourimetric method (Fishman and Davidson, 2006) and uric acid using enzymatic colourimetric method (Fossati et al., 1980). Also, urea was assayed using urease-Berthelot method (Weatherbum, 1967), total cholesterol using the enzymatic endpoint method (Trinder, 1969; Roeschlau et al., 1974) and creatinine assay was done by alkaline picrate method (Tietz et al., 1986).

Statistical analysis

Data were expressed as means ± standard errors of mean. Comparisons between control and treated groups of guinea-pigs were performed with one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test. Statistical significance was set at $P < 0.05$

RESULTS

In n = 5 experiments, the antimalarial agents used had no significant effects on ALP and ACPT, but caused moderate increases in the serum level of ACPP (Figures

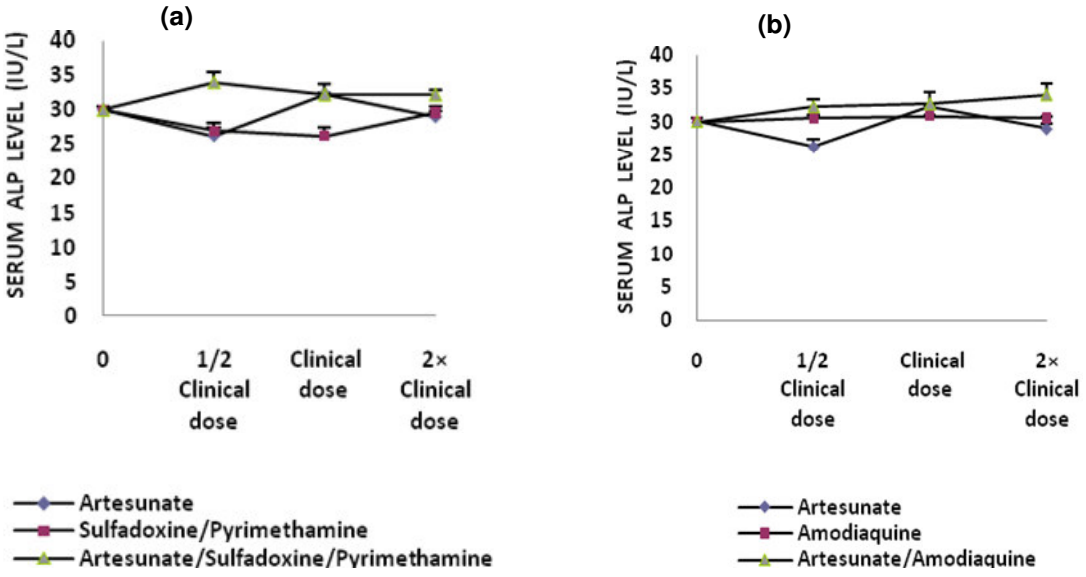

Figure 1. The effects of antimalarial agents on basal serum ALP levels of the male guinea-pig, following administration of half, normal and double clinical doses of the agents. Data are mean ± SEM, n = 5; *Indicates significant differences from control at p<0.05 ANOVA.

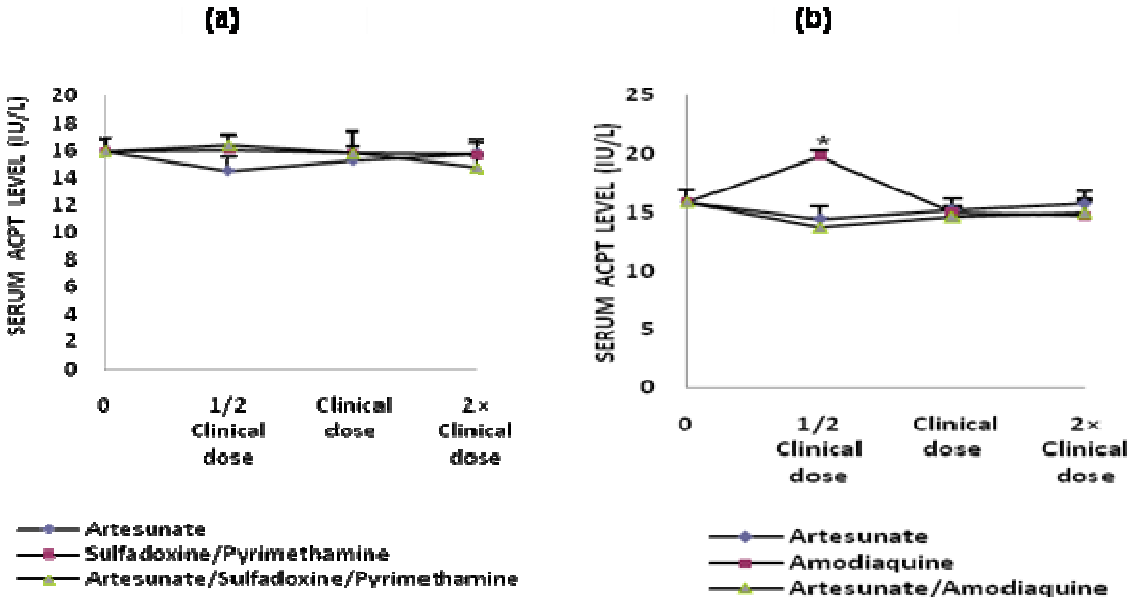

Figure 2. The effects of antimalarial agents on basal serum ACPT levels of the male guinea-pig, following administration of half, normal and double clinical doses of the agents. Data are mean ± SEM, n = 5; *Indicates significant differences from control at p<0.05 ANOVA

1a and b, 2a and b and 3). These effects were mostly observed at half the clinical doses of these agents (Figures 3a and b). Compared to the basal prostatic acid serum value of 3.50 ± 0.42 IU/L, the values obtained with artesunate, amodiaquine and artesunate/amodiaquine were- 2.50 ± 0.5, 4.75 ± 0.85, 3.50 ± 0.65 units, while serum levels in sulfadoxine/pyrimethamine- and artesunate/sulfadoxine/pyrimethamine-treated animals were 4.75 ± 0.48 and 3.00 ± 0.58 IU/L respectively. However, only the effects of amodiaquine and sulfadoxine/pyrimethamine were significant at $p < 0.05$. The effects of the agents at the normal and double clinical doses were similar and were also not significantly different ($p < 0.05$) from the control serum level (Figures 3a and b).

In this study, artesunate, sulfadoxine/pyrimethamine and the ACTs caused different effects on basal serum

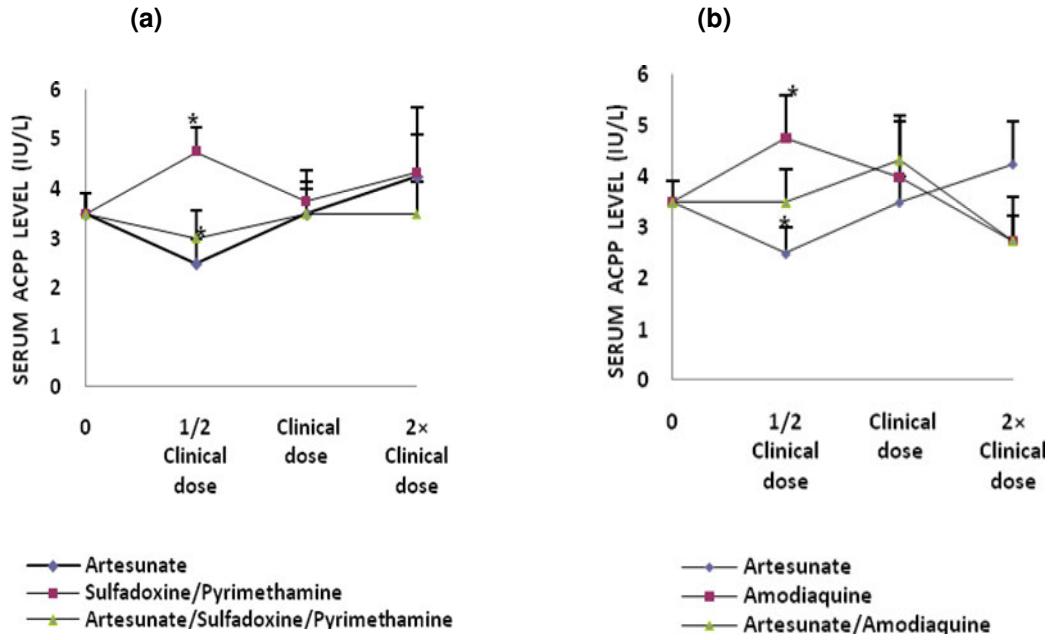

Figure 3. The effects of antimalarial agents on basal serum ACPP levels of the male guinea-pig, following administration of half, normal and double clinical doses of the agents. Data are mean ± SEM, n = 5; *Indicates significant differences from control at p<0.05 ANOVA.

urea. Analysis of the urea data shows that, at the sub clinical dose, artesunate, amodiaquine and their combination product (the ACT) respectively decreased the serum urea level by 3.4, 0 and 5.1 units. At the normal clinical dose, the individual agents had no significant effect, however the ACT caused a 4.1 units increase, which shows synergistic responses by the ACT. At the double clinical dose, artesunate and amodiaquine caused 3.3 and 0 units increases, while the ACT caused no effect (Table 1). Furthermore, artesunate and sulfadoxine/ pyrimethamine decreased the basal serum urea level by 3.4 and 4.6 units respectively at the sub clinical dose, while the ACT (artesunate/sulfadoxine/pyrimethamine) caused a 5.4 units increase of the basal value, which indicates a reversal effect of the individual agents. At the normal clinical dose, whereas the individual agents caused no significant effects on urea, the ACT increased serum urea level by 0.4 units. Finally, at the double clinical dose, the individual agents caused 3.3 and 0 units increases respectively, while the ACT caused 0.5 unit increase (Table 1). These results show that the effects of artesunate and artesunate/amodiaquine were biphasic, having antioxidative actions at the lower doses and oxidative actions at the higher doses. However, artesunate/ sulfadoxine/pyrimethamine significantly increased ($p < 0.05$) serum urea at the 3 doses, reversing the antioxidative effects of the individual agents at the lower doses and potentiating their oxidative actions at the higher doses (Table 1).

The analysis of uric acid data shows that administration of sub, normal and double clinical doses of artesunate, amodiaquine and artesunate/amodiaquine decreased the basal serum uric acid level by 50.5, 62.8 and 56.5 units, 63.8, 45.8 and 0, 48.8 and 44.8, 68.8 units respectively (Table 1). Furthermore, while sulfadoxine/ pyrimethamine caused no significant effects on uric acid over the dose range, artesunate/sulfadoxine/pyrimethamine decreased its basal value by 48.3 at the normal clinical dose, without significant effects at the other doses (Table 1).

Furthermore, analysis of the creatinine data shows that the artesunate and amodiaquine had no significant effects on basal creatinine level at the different doses, while the ACT (that is, artesunate/amodiaquine) decreased its serum value by 9.5, 19.5 and 0 units at the sub, normal and double clinical doses respectively, indicating a relative freedom of renal toxicity by these agents (Table 1). Furthermore, sulfadoxine/pyrimethamine increased the basal serum creatinine level by 27.3, 10.5 and 0 units when administered with their sub, normal and double clinical doses respectively, whereas artesunate and the ACT caused no significant effects at $p < 0.05$ (Table 1). These results show that sulfadoxine/pyrimethamine may be toxic to renal function, especially at subclinical concentrations. However, the other agents may relatively have little or no renal toxicity over the dose range used in this study. Also, it shows that the ACTs may improve adverse effects of the individual agents on renal function.

Finally, total blood cholesterol was significantly reduced from 2.43 ± 0.13 to 2.1 ± 0 and 1.9 ± 0 mmol/L after administration of clinical doses of amodiaquine and artesunate/amodiaquine respectively, without significant effects by the other agents (Table 1).

Table 1. The effects of antimalarial agents on the basal serum levels of urea, uric acid, creatinine and total cholesterol of the male guinea-pig, following administration of half, normal and double clinical doses of the agents . Data are mean ± SEM, n = 5; * Indicates significant differences from control at p<0.05 ANOVA.

Dose	Urea (g/L)	Uric acid (mmol/L)	Creatinine (µmol/L)	Total cholesterol (mmol/L)
Control	7.7 ± 1.13	368.8 ± 10.0	62.5 ± 6.7	2.43 ± 0.13
Artesunate				
½ Clinical dose	4.27 ± 0.9*	318.3 ± 5.8*	69.0 ± 5.29	2.23 ± 0.13
Normal Clinical dose	7.5 ± 0.43	305.0 ± 1.9*	64.0 ± 3.89	2.28 ± 0.1
2 × clinical dose	10.97 ± 1.59*	320.0 ± 1.53*	65.0 ± 5.29	2.23 ± 0.09
Amodiaquine				
½ Clinical dose	10.67 ± 3.8	306.0 ± 1.2*	79.0 ± 3.5	2.1 ± 0*
Normal Clinical dose	11.23 ± 2.8	323.0 ± 9.3*	71.33 ± 10.9	2.1 ± 0*
2 × clinical dose	6.8 ± 1.5	324.0 ± 1.7*	71.0 ± 2.0	2.2 ± 0.06
Sulfadoxine/pyrimethamine				
½ Clinical dose	3.13 ± 7.4*	342.5 ± 14.9	89.8 ± 4.8*	2.38 ± 0.5
Normal Clinical dose	3.4 ± 7.0	340.3 ± 1.9	73.0 ± 6.0*	2.23 ± 0.09
2 × clinical dose	5.53 ± 2.1	343.5 ± 11.3	63.75 ± 9.2*	2.25 ± 0.05
Artesunate/Amodiaquine				
½ Clinical dose	2.57 ± 0.5*	312.3 ± 2.6*	53.0 ± 0	2.3 ± 0.06
Normal Clinical dose	11.8 ± 0*	347.0 ± 0	43.0 ± 0*	1.9 ± 0*
2 × clinical dose	6.13 ± 2.96	300.0 ± 4.36*	65.0 ± 4.0	2.43 ± 0.07
Artesunate/ Sulfadoxine/pyrimethamine				
½ Clinical dose	13.1 ± 1.66*	336.0 ± 15.6	52.0 ± 7.6	2.26 ± 0.07
Normal Clinical dose	8.1 ± 0.1*	320.5 ± 8.5*	67.0 ± 6.0	2.2 ± 0.1
2 × clinical dose	8.2 ± 0.9*	355.0 ± 1.73	51.0 ± 5.3	2.27 ± 0.2

DISCUSSION

In this study, the effects of half, normal and double clinical concentrations of ACTs (artesunate/amodiaquine and artesunate/sulfadoxine/pyrimethamine) and their combinants (artesunate, amodiaquine and sulfadoxine/ pyrimethamine) on ALP. ACPT, ACPP, urea, creatinine, uric acid and total cholesterol on male guinea-pigs were investigated.

Phosphatase enzymes are produced by specific cells/ tissues and organs. Human prostatic acid phosphatase is a major phosphatase and a differentiation marker in normal, well-differentiated prostate epithelial cells (Yam, 1974; Lin et al., 1980). Alkaline and total acid phosphatases are produced by several parts of the body especially in the liver. The serum levels of these enzymes are used as surrogate markers for toxicities of the appropriate tissues/organs (Wang et al., 1981; Chu and Lin, 1998). In this study, the agents caused no significant effect ($p < 0.05$) on serum alkaline and total acid phosphatases, however the sub clinical doses of amodiaquine and sulfadoxine/pyrimethamine caused moderate significant increases in prostatic acid phosphatase, showing that these agents may not be free from toxicity, especially on the prostatic/testicular structures of the guinea-pig.

Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys (Gaspari et al., 1998) and their serum concentrations are commonly used to screen for renal or cardiovascular diseases (Perrone et al., 1992; Wannamethee et al., 1997; Nankivell, 2001; Traynor et al., 2006). Serum creatinine is mainly produced by the metabolism of creatine or creatine phosphate in skeletal muscle, but also originates from dietary sources of creatinine such as cooked meat (Nankivell, 2001), while urea is a metabolic product of protein metabolism. Furthermore, the biochemical processes involved in the biosynthesis of these metabolic wastes are mediated via oxidation-reduction (redox) processes (Bloch and Schoenheimer, 1939; Mollica et al., 1998; Wyss and Kaddurah-Daouk, 2000). Elevation of the serum levels of creatinine and urea is an indication of abnormal renal function (Perrone et al., 1992; Mouton and Holder, 2006). In this study, amodiaquine had no significant effects on urea and creatinine ($p < 0.05$). However, while the lower doses of artesunate and artesunate/amodiaquine significantly decreased serum urea levels, they caused significant increases at the double clinical dose, showing that these agents may have antioxidative actions at the lower doses and oxidative actions at the higher doses. In addition, artesunate/sulfa-

doxine/pyrimethamine significantly increased ($p < 0.05$) serum urea level at the 3 doses, reversing the decreases induced by the partner agents at their sub clinical doses to an increase and potentiating the induced serum elevations of urea at the other doses. Artesunate/ amodiaquine also potentiated the effects of the individual agents at the higher doses. Thus, the single agents may have antioxidative and oxidative effects depending on the concentration. Furthermore, their combinations converted antioxidative responses to oxidative. The results of this study also showed that sulfadoxine/ pyrimethamine significantly increased ($p < 0.05$) the basal serum level of creatinine and may be toxic to renal function. This effect was most potent at the subclinical concentration of the agent. However, the other agents either decreased or had no significant effect ($p < 0.05$) on serum creatinine level. Also, adverse effects of the individual agents on renal function may be improved. In this study, artesunate combination with sulfadoxine/pyrimethamine (ACT) ameliorated sulfadoxine/ pyrimethamine-induced toxicity on serum creatinine level. Thus, these agents may relatively have little or no renal toxicity over the dose range used in this study.

In this study, the basal serum uric acid level was significantly decreased by most of the agents at $p < 0.05$. Uric acid, a metabolic product of purines is an antioxidant which scanvenges reactive oxygen radicals in the blood (Hooper et al., 2000; Knapp et al., 2004). Increase in the serum level of uric acid is an indication of oxidative stress in the body, while a decrease suggests a reduction of purine metabolism and antioxidative actions of blood. Thus, this result suggests that these agents may decrease purine metabolism, resulting in low production of uric acid, thereby exposing the system to oxidative stress/damage.

Finally, amodiaquine and artesunate/amodiaquine decreased basal serum total cholesterol concentration, thus having cholesterol-lowering effects at their clinical doses. Lipid profile, particularly with blood cholesterol level has been linked strongly with blood pressure and coronary heart disease (Cotran, 1999). These agents may therefore be beneficial in these pathological conditions.

The results of this study may be explained by the oxidative effects of these agents. The results obtained suggest that the agents may have antioxidative and oxidative actions depending on the concentration. However, the oxidative effects of the agents appear to be more dominant in the combined therapies. These results are important because most antimalarial drugs are bought over the counter and used indiscriminately through self-medication, resulting in the use of subclinical and higher than clinical doses of these agents. Furthermore, the resistance of the *Plasmodium* parasite has caused the use of multiple antimalarial agents within a short period of time which often results in high concentrations of different antimalarial agents in the plasma.

The antiplasmodial action of the artemisinins has been attributed to the generation of reactive oxygen species (ROS), through the activation of the drug peroxide bond by iron (II)-heme produced during hemoglobin degradation in the erythrocyte (Jefford, 2001). Similarly, studies of artemisinins on embryonic stem-cells in mice have shown that these compounds raised intracellular levels of ROS (Wartenberg et al., 2003). Furthermore, Kim et al. (2003) had shown that artemisinins have an effect on PKC and PKC isoforms at the transductional level of cellular activity. Also known is the effect of some of these antimalarial agents on Ca^{2+} metabolism in smooth muscle activity (Guerrero and Zacharías, 1986). Therefore, these ACTs may be potentiating oxidative radical generation through an effect on PKC and Ca^{2+} metabolism, thus creating a synergy (Nishizuka, 1984) in toxicological production/generation of ROS which cause the damage.

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