

## Coartemether in Dietary Oil Induces Oxidative Stress and Hepatotoxicity in Albino Rat

\*Anyasor God'swill Nduka, Odunsanya Olutayo Tolulope

Biochemistry Unit, Department of Chemical and Environmental Sciences, School of Science and Technology, Babcock University, Ilisan-Remo, Ogun State, P.M.B. 21244 Ikeja, Lagos, Nigeria.

[anyasorgodswill@yahoo.co.uk](mailto:anyasorgodswill@yahoo.co.uk); [anyasorg@babcockuni.edu.ng](mailto:anyasorg@babcockuni.edu.ng)

**Abstract:** Lipid rich diet enhances the bioavailability and therapeutic potency of coartemether (artemether-lumefantrine), an ACT antimalarial drug. However, its impact on the coartemether cytotoxicity has stimulated this investigation. Thus, this study evaluated the *in vivo* effect of coartemether in the presence of oil on the antioxidant and hepatotoxic biomarkers in albino rats for 3 days. Twenty (20) rats were randomly divided into four groups of five rats per group. Group I (control) received normal placebo saline (0.9% NaCl), group II (C) was treated with coartemether (4 mg artemether: 24 mg lumefantrine), group III (O) received sunflower oil (1.0 ml) and group IV (C-O) was co-administered coartemether (4 mg artemether: 24 mg lumefantrine) and (1.0 ml sunflower oil). Result showed no significant difference ( $P>0.05$ ) in SOD and GSH levels in all treated groups compared to control while there was a significant ( $P<0.05$ ) elevated catalase activity in the liver and heart of C-O and coartemether treated animals compared to other treatment groups. The C-O treated animals had significantly ( $P<0.05$ ) high kidney and heart GST activity compared with other groups. Coartemether treated animals had an elevated plasma malondialdehyde content which was reversed in C-O group. Furthermore, coartemether and C-O treated groups had elevated alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase levels compared with the control animals. However, bilirubin levels were high and low in coartemether and C-O treated groups respectively. Therefore, investigation from this study shows that coartemether in an oil medium could induce oxidative stress and hepatotoxic biomarkers.

[Anyasor God'swill Nduka, Odunsanya Olutayo Tolulope. Coartemether in Dietary Oil Induces Oxidative Stress and Hepatotoxicity in Albino Rat. Researcher. 2011;3(7):35-41]. (ISSN: 1553-9865). <http://www.sciencepub.net>.

**Key words:** coartemether; hepatotoxicity; oil; oxidative stress

### 1.0 Introduction

The World Health Organization officially reported in its 2008 World Malaria Report that an estimated 247 million confirmed clinical cases of malaria were documented globally in 2006 (Ehrhardt & Meyer, 2009). The deleterious consequences of malaria continue to increase in endemic areas as a result of the emergence and widespread dissemination of drug resistant *plasmodium falciparum*. Malaria is the most important parasitic infection in people and has become a priority for the international health community as is also, the focus of several new initiatives. One of such new initiatives includes the development of artemisinin combination therapies (ACTs) proven to be the most effective first-line strategy for the treatment of uncomplicated *P. falciparum* malaria, presently eliciting a high degree of resistance to conventional antimalarial drugs (Wongrischanalai *et al.*, 2002; Breman *et al.*, 2004).

Coartemether (Artemether-Lumefantrine) is an effective ACT known popularly under the brand names COARTEM<sup>®</sup> or RIAMET<sup>®</sup> and is used majorly in Africa, due to its high efficacy against the parasite. The standard recommended dosage regimen for the drug is a fixed 6-dose regimen (24 tablets; 480

mg artemether and 2880 mg lumefantrine, given over 3 days) as the oral first line treatment with the substance to be administered at 0, 8, 24, 36, 48 and 60 h of the treatment course (Van Vugt, 1999).

Food (especially oily diet) enhances the bioavailability and increased efficacy of artemether-lumefantrine against *P. falciparum* parasite. Although, this effect is more apparent for lumefantrine, probably because of the presence of butyl groups as evident in its structure, contributing to its lipophilicity (Ezzet *et al.*, 2000). Administration of artemether-lumefantrine to healthy volunteers at the same time with a high-fat meal increases the bioavailability of artemether and lumefantrine by two and sixteen fold respectively as compared with the administration during the fasted state (White *et al.*, 1999). Co-administration of coartemether with grape fruits also increases its bioavailability (van Agtmael *et al.*, 1999).

The components of coartemether have dissimilar modes of action providing synergistic activity against *P. falciparum* (Cousin *et al.*, 2008). Upon administration of coartemether, artemether is presumed to have a rapid onset of action and elimination, whereas lumefantrine has a slower onset of action and elimination and it is expected to provide

long-term cure rate after a short treatment course. The combination thus provides rapid clearance of parasitemia and most malaria-related symptoms, coupled with prevention of recrudescence. The site of antiparasitic action of both components is the food vacuole of the malaria parasite, where they are thought to interfere with the conversion of heme, a toxic intermediate produced during hemoglobin breakdown, to non-toxic hemozoin (Pilar *et al.*, 2008). Lumefantrine is thought to interfere with the polymerisation process, while artemether generates reactive metabolites (dihydroxyartemisinin) through its conversion by CYP3A4/5 mostly at the liver which could damage both the host liver as well as the parasites (Anyasor *et al.*, 2009; Mwesiwa *et al.*, 2010). Therefore, this study investigated the potentials of oily food to exacerbate the toxic effects of coartemether on antioxidant system and liver function enzymes.

## 2.0 Materials and Methods

### 2.1 Animals

Twenty male albino rats (Wister strain) were purchased from the Preclinical Animal House, Physiology Department, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. Animals were transported to the Animal House in the Department of Chemical and Environmental Sciences, School of Science and Technology, Babcock University, Ogun State, Nigeria. Animals were maintained in an environment with a standard temperature condition  $25 \pm 2$  °C. Standard laboratory chow and water were provided *ad libitum*. A period of 14 days was allowed for animals to be acclimatized before any experimental manipulation was undertaken.

### 2.2 Drug and test substance

Coartemether or COARTEM<sup>®</sup> (artemether-lumefantrine) was procured from the Pharmacy Department, Lagos State University Teaching Hospital, Lagos, Nigeria. Sunflower oil was procured from Feedwell Stores, Oyo Road, Ibadan, Oyo State, Nigeria.

## 2.3 Methods

### 2.3.1 Experimental design

Rats were randomly distributed into four groups of five animals each. The experimental groups were subjected to treatment twice daily for 3 days in line with the therapy regimen for uncomplicated malaria (Ehrhardt & Meyer, 2009).

Group I: control animals received a placebo solution (normal saline, 0.9% NaCl solution)

Group II: animals were administered with oral dose of 1.0 ml sunflower oil

Group III: animals were administered with oral dose of 28 mg coartemether

Group IV: animals were administered with oral dose of 28 mg coartemether and 1.0 ml of sunflower oil.

Coartemether preparation and normal saline were stored in a refrigerator at 4°C and were administered orally using an appropriate oral dosing needle. At the end of treatment period, animals were starved over night and subjected to cervical dislocation and immediately, blood samples were collected through cardiac puncture using a 5 ml hypodermal syringe into EDTA containers. The collected blood samples were centrifuged to obtain plasma. The liver, kidney and heart were encapsulated and perfuse in potassium chloride and homogenized in phosphate buffer solution. The homogenates were centrifuged at 3500 rpm for 10 min to obtain supernatant. Plasma and tissue samples were used to assess the levels enzymatic and non enzymatic antioxidant status and hepatic function enzymes.

### 2.3.2 Protein estimation

Protein estimation was determined by means of biuret method as described by Gornal *et al.* (1949) with some modification: Bovine serum albumin (BSA) was used as standard.

### 2.3.3 Determination of superoxide dismutase (SOD) activity

Plasma and tissue SOD activity were determined by the method of Misra and Fridovich (1972). 0.5 ml of sample was added to 2.5 ml of 0.05 M carbonate buffer, pH 10.2 to equilibrate in spectrophotometer curvette and the reaction started by addition of 0.3 ml freshly prepared 0.3 mM epinephrine to mixture. Increase in absorbance at 480 nm was monitored every 30 s for 1 min.

1 unit of SOD activity was given as amount of SOD required to cause 50% inhibition of the oxidation of adrenaline.

### 2.3.4 Determination of catalase activity

Plasma and tissue catalase activity were determined according to the method of Sinha (1972). 0.1 ml of sample was mixed with 4.9 ml distilled water to give 1:50 dilution. Assay mixture contained 2 ml of H<sub>2</sub>O<sub>2</sub> solution and 2.5 ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. 1 ml of properly diluted sample was rapidly mixed with the reaction withdraw and blown into 2 ml of dichromate/acetic acid reagent at 60 s interval for 3 min. Hydrogen

peroxide contents of the withdrawn sample were determined as above.

### 2.3.5 Determination of glutathione S-transferase activity

This was estimated by the method of Habig *et al.* (1974). Following the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate. The values were expressed as millimoles of CDNB-GSH conjugate formed/min/mg protein.

### 2.3.6 Determination of reduced glutathione (GSH)

This was measured by the method of Sedlak and Lindsay (1968) and Jollow *et al.* (1974). Two ml of 10% trichloroacetic acid was added to homogenized sample mixture of liver and kidney samples. The resulting mixture was centrifuged at 3000 g for 10 min. 0.5 ml of the supernatant was added to 4 ml of phosphate buffer and finally 0.5 ml of Ellman's reagent was added. The optical densities were read within 30 min of colour development at 412 nm using spectrophotometer.

### 2.3.7 Assessment of lipid peroxidation

This was estimated using the method of Stocks and Dormandy (1971). Malondialdehyde (a secondary product of lipid peroxidation), reacts with thiobarbituric acid (TBA) in acid medium to give a pink coloured pigment. The pink colour was extracted with butanol and the absorbance read at 535 nm. Values were expressed as nanomoles per deciliter.

### 2.3.8 Determination of plasma alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin levels

The levels of ALT, AST and total bilirubin were determined spectrophotometrically using commercial diagnostic kits (Randox, USA).

### 2.4 Statistical analysis

This was done with the aid of SPSS for windows, SPSS Inc., Chicago, Standard version 15.0 to determine difference between mean using Analysis of Variance (ANOVA). Data were reported as mean  $\pm$  standard deviation

**Table 1. Effect of coartemether, oil and combine coartemether-oil treatment on protein concentration and antioxidant activity**

Parameters		Control (normal saline)	Oil (1 ml)	Coartemether (28 mg/kg b.w.)	C-O
Protein content (mg/ml)	Plasma	104.38 $\pm$ 27.30	102.25 $\pm$ 18.40	121.0 $\pm$ 4.90	92.18 $\pm$ 5.23*
	Liver	175.57 $\pm$ 39.90	118.30 $\pm$ 12.31	100.27 $\pm$ 20.05	87.41 $\pm$ 14.70*
	kidney	29.00 $\pm$ 4.97	33.23 $\pm$ 4.83	61.35 $\pm$ 3.65	26.71 $\pm$ 5.25*
	Heart	23.44 $\pm$ 3.85	23.18 $\pm$ 1.57	21.76 $\pm$ 1.13	26.96 $\pm$ 6.39*
SOD activity (U/L)	Plasma	2.03 $\pm$ 0.01	2.02 $\pm$ 0.14	2.04 $\pm$ 0.24	2.00 $\pm$ 0.49
Catalase ( U/L)	Liver	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02	0.06 $\pm$ 0.03	0.10 $\pm$ 0.11*
	kidney	0.21 $\pm$ 0.08	0.15 $\pm$ 0.02	0.07 $\pm$ 0.03	0.17 $\pm$ 0.03*
	Heart	0.20 $\pm$ 0.06	0.23 $\pm$ 0.01	0.23 $\pm$ 0.02	0.26 $\pm$ 0.13
GSH activity (U/L)	Liver	14.04 $\pm$ 5.71	17.54 $\pm$ 2.34	14.34 $\pm$ 1.07	16.24 $\pm$ 1.21*
	kidney	19.81 $\pm$ 0.01	19.85 $\pm$ 0.17	19.73 $\pm$ 0.13	19.73 $\pm$ 0.16
	Heart	19.58 $\pm$ 0.29	20.01 $\pm$ 0.04	19.57 $\pm$ 0.28	19.81 $\pm$ 0.31
GST( $\mu$ mole/min/mg protein)	Liver	7.67 $\pm$ 0.89	0.11 $\pm$ 0.01	0.13 $\pm$ 0.02	0.25 $\pm$ 0.03*
	kidney	0.42 $\pm$ 0.07	0.38 $\pm$ 0.07	0.15 $\pm$ 0.01	0.71 $\pm$ 0.12*
	Heart	0.59 $\pm$ 0.13	0.53 $\pm$ 0.04	0.50 $\pm$ 0.06	0.85 $\pm$ 0.02
MDA ( $\mu$ mole/ml)	Plasma	42.89 $\pm$ 9.55	50.71 $\pm$ 20.80	72.69 $\pm$ 17.69	29.73 $\pm$ 10.73*
	Liver	33.46 $\pm$ 11.64	56.44 $\pm$ 0.60	36.78 $\pm$ 10.16	50.13 $\pm$ 16.14*
	kidney	43.41 $\pm$ 17.09	25.34 $\pm$ 3.82	36.92 $\pm$ 9.85	25.29 $\pm$ 16.87
	Heart	28.37 $\pm$ 3.40	10.46 $\pm$ 2.95	4.18 $\pm$ 1.67	7.42 $\pm$ 3.17*

**Table 2. Effect of oil, coartemether and combine coartemether-oil treatment on liver function enzymes and total bilirubin**

Parameters		Control	Oil (1 ml)	Coartemether (28 mg/kg b.w.)	C-O
Alkaline phosphatase activity (U/L)		24.42±4.43	23.93±4.95	25.58±4.15	25.58±5.64
Alanine aminotransferase activity (U/L)		0.005±0.02	0.007±0.06	0.006±0.01	0.009±0.02
Aspartate transferase	amino	14.04±2.81	15.14±7.32	17.60±3.35	16.28±3.62
Total Bilirubin		1.11±0.20	1.20±0.43	1.20±0.10	0.96±0.40

### 3.0 Results

Data from this study showed that there was no significant difference ( $P>0.05$ ) in plasma superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels in all studied tissues between C-O treated animals and control (Table 1). Catalase activity (CAT) in liver and heart was found to be significantly high ( $P<0.05$ ) in C-O groups compared to control while the CAT levels in the kidney of coartemether animals was low compared to control (Table 1). Glutathione *S*-transferase level was low in liver while it was elevated in the kidney and heart of C-O treated group compared to control. Plasma, kidney and heart malondialdehyde (MDA) content were significantly reduced ( $P<0.05$ ) in C-O animals compared to control. However, there was no significant difference ( $P>0.05$ ) in the liver of treated animals.

Hepatic function enzymes (ALP, ALT, AST) in plasma were also evaluated and result showed elevated levels of these enzymes in all coartemether and C-O treated animals compared to control while total bilirubin levels was high and low coartemether and C-O treated groups respectively.

### 4.0 Discussion

Studies have shown that the absorption of lipophilic drugs such as artemisinin combined therapy (coartemether) is dependent on its co-administration with fatty diet thus enhancing its bioavailability and efficacy. This notion led to the investigation of the potential of dietary lipid in enhancing the toxic effect of coartemether on the antioxidant system and hepatic function enzymes.

In this study, there was significant reduction of plasma, liver, kidney protein concentrations in C-O treated animals compared with control group except in the heart. Protein content alteration could have resulted from short duration (3 days) of administered compounds in the body (Olufemi *et al.*, 2009) as well as effect of the drug in modulating synthesis of drug metabolizing enzymes, antioxidant enzymes and associated proteins (Bibi, 2008).

There was no significant difference in the superoxide dismutase activity in all treated animals compared with control. This might have resulted from the short duration of drug administration. Superoxide dismutase has been known to catalyze the dismutation of superoxide anion ( $O_2^-$ ) into hydrogen peroxide and molecular oxygen (Usuh *et al.*, 2005).

The elevated liver and heart catalase activity in C-O and coartemether treated animals could have resulted in response of the hepatocytes to the presence of reactive intermediate dihydroxyartemisinin (DHA) inducing catalase enzyme synthesis (Chi-Tai & Gow Chin, 2006). Artemether undergoes biotransformation in the liver to produce DHA and other reactive intermediates with the potential for reacting with proteins (Ehrhart & Meyer, 2009) and possibly upsetting metabolic processes thus generating more reactive species such as peroxy radicals (Park *et al.*, 1998). Furthermore, oil may also contribute to the increased generation of reactive DHA by enhancing the absorption and bioavailability of coartemether drug from the gastrointestinal tract through the hepatic portal veins to the liver (Ezzet *et al.*, 2000). However, the reduction in kidney catalase activity in C-O and coartemether could be due to the effect of overall operation mechanism in the kidney (Kumar *et al.*, 1980).

The hepatic GST activity showed that all treated groups were significantly low ( $P<0.05$ ) compared with control animals. It has been reported that decreased or impaired GST activity would increase oxidative stress since GSTs are intracellular proteins that detoxify electrophilic compounds of endogenous and exogenous origin by conjugating them with GSH, predisposing them for export out of the cell by multi-drug resistant proteins (Reginald *et al.*, 2006). On the other hand, C-O group had a significantly high in kidney and heart GST activity ( $P<0.05$ ) compared with other treatment groups. This suggests protective role of GST in the tissues against the products of oxidative stress (Ketterer, 1988) and evidence shows glutathione *S*-transferase is

implicated in the disposal of xenobiotics as mercapturic acid (Pegg & Hook, 1977). There was no significant difference ( $P>0.05$ ) in GSH levels among the treatment groups in all tissues examined. This could have resulted from utilizing glutathione by the detoxifying enzymes against generated reactive species in the treated groups (Masella *et al.*, 2005). Glutathione is a cysteine-containing tripeptide with reducing and nucleophilic properties which plays an important role in cellular protection from oxidative stress. Reaction of reactive species with cellular antioxidants causes antioxidant depletion that may result in oxidative stress (Persie *et al.*, 2006).

Increase plasma malondialdehyde level in coartemether treated group reflects an increase in the peroxidation of membrane lipids. However, this trend was reversed in the C-O treated animals, suggesting a possible synergistic counteraction of the peroxidation of membrane lipids by the antioxidant system present in the oil. Lipid peroxidation has been reported to be one of the earliest major indicators of oxidative stress in cells of organism (Jones, 2008).

The effect of co-administered coartemether and oil on hepatic function biomarkers was also investigated. Both coartemether and C-O treated groups had slightly elevated ALP activity compared to the control animals. An elevated level of ALP serves as biomarker for hepatic damage to cells that undergo toxic liver injury or liver ischemia (Giannini *et al.*, 2005). In the oil, coartemether and C-O treated groups there was increased ALT activity compared to the control animals. Increased activities of ALT and ALP enzymes show that the integrity of hepatocytes was abnormal. This could result in the release of intracellular enzymes into systemic circulation (Adekunle *et al.*, 2009). More so, AST and ALT are enzymes that catalyze the transfer of alpha-amino groups from aspartate and alanine to the alpha-keto group of ketoglutarate acid to generate oxalacetic and pyruvic acids respectively, which are important contributors to citric acid cycle (Giannini *et al.*, 2005).

Furthermore, coartemether and C-O groups were high for AST activity compared to the other treatment groups. There is however, a possibility that coartemether drug increased the enzyme levels attributed to liver damage in the both coartemether and C-O groups. AST is diffusely represented in the heart, skeletal muscle, kidney, brain and red blood cells, and ALT has low concentrations in skeletal muscle and kidney; an increase in ALT serum levels is, therefore more specific for liver damage (Giannini *et al.*, 2005). Also, it is also known that reactive oxygen species are generated during the process of drug biotransformation and these ROS generated can

bind and react with cellular components in the liver to cause hepatic injury, thus impairing liver function (Adekunle *et al.*, 2009).

The total bilirubin content in the plasma was low in the C-O group when compared to other treatment groups. Though, the coartemether and oil treated groups showed elevated bilirubin levels than other treatment groups. It has been known that increased levels of total or conjugated bilirubin in systemic circulation is an indication of impairment of liver functions enzymes or an obstruction of the bile duct system that is supposed to eliminate it (Giannini *et al.*, 2005). This study has shown that the antimalarial artemether-lumefantrine drug combination in the presence of oil could modulate the activities of liver function enzymes and oxidative stress biomarkers.

#### Acknowledgement:

With sincere gratitude, we thank Babcock University Administration for the financial support. We also thank the Head of Department Chemical and Environmental Sciences, School of Science and Technology, Babcock University Prof. Edward B. Esan for providing material and technical support. Our appreciation goes to Anyasor Chiamaka .O. for the manuscript typesetting and editing. The authors declare no competing interest.

#### Correspondence to:

Anyasor God'swill N.  
Department of Chemical and Environmental Sciences  
School of Science and Technology  
Babcock University  
Ilisan-Remo  
Ogun, Nigeria.  
Mobile: +2347034618325  
Email: [anyasorgodswill@yahoo.co.uk](mailto:anyasorgodswill@yahoo.co.uk);  
[anyasorg@babcockuni.edu.ng](mailto:anyasorg@babcockuni.edu.ng)

#### References

- [1] Adekunle AS, Falade CO, Agbedana EO, Egbe A. Assessment of side effect of administration of artemether in humans. *Biology and Medicine*. 2009; 1(3):15-19.
- [2] Anyasor GN, Ajayi E IO, Saliu JA, Ajagbonna O, Olorunsogo OO. Artesunate opens mitochondrial membrane permeability transition pore opening. *Annals of Tropical Medicine and Public Health*. 2009; 2(2): 37-41.
- [3] Bibi Z. Role of cytochrome P450 in drug interactions. *Nutrition and Metabolism*. 2008; 5: 27.

- [4] Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. *Am. J. Trop. Med. Hyg.*, 2004; 71: 1-15.
- [5] Chi-Tai Y, Gow-Chin, Y. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance associated protein 3 mRNA expression. *The Journal of Nutrition*. 2006; 136: 11-15.
- [6] Cousin M, Kummerer S, Lefevre G, Marrast, AC, Stein, D, Weaver M. Coartem (artemether-lumefantrine) tablets for the treatment of malaria in patients with acute uncomplicated infections due to *Plasmodium falciparum* or mixed infections including *ip. Falciparum*. Novartis, Africa. 2008; 14-20.
- [7] Ehrhardt S, Meyer CG. Artemether-Lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria. *Therapeutics and Clinical Risk Management*. 2009; 5: 805-815.
- [8] Ezzet F, van Vugt M, Nosten F, Looreesuwan S, White N. Pharmacokinetics and Pharmacodynamics of lumefantrine (benflumetol) in acute *falciparum* malaria. *Antimicrobial agent and Chemotherapy*. 2000; 44: 697-704.
- [9] Giannini E, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *Canadian medical association Journal*. 2005; 172 (3): 367-379.
- [10] Gornall AG, Bardwill CS, David MM. *Journal of Biological Chemistry*. 1949; 177: 751 - 766.
- [11] Habig WH, Pabst MJ, Jakoby WB. *Journal of Biological Chemistry*. 1974; 249: 7130 - 7139.
- [12] Jollow DJ, Mitchell JR, Zampaglione N, Gillette J. *Pharmacology*. 1974; 11: 151 - 169.
- [13] Jones D. Radical free biology of oxidative stress. *American journal of Physiology Cell physiology*. 2008; 295: 849-868.
- [14] Ketterer B. Protective Role of Glutathione and Glutathione S-Transferases in Mutagenesis and Carcinogenesis. *Mutation Research*. 1988; 202: 343-361.
- [15] Kumar A, Husain M, Mukhtar H, Krishna M C. Hepatic and extra-hepatic glutathione S transferase activity in wild pigeons (*Columba livia*) *Journal of Biosciences*. 1980; 2(3): 181-189.
- [16] Masella R, DiBenedetto R, Vari R, Filese C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *Journal of Nutritional Biochemistry*. 2005; 16: 577-86.
- [17] Misra HP, Fridovich I. *Journal of Biological Chemistry*. 1972; 247: 3170 - 3175.
- [18] Mwesigwa J, Parikh S, McGee B, German P, Drysdale T, Kalyangp JN, Clark TD. Pharmacokinetics of artemether-lumefantrine and artesunate and amodiaquine in Children in kampala, Uganda. *Antimicrobial Agents and Chemotherapy*. 2010; 54: 52-59.
- [19] Olufemi MA, Gabriel O, Sheriff O, Oladele AA. Effects of Short term administration of artemether-lumefantrine on testicular functions and antioxidant defence in the rats. *Research Journal of Medicine and Medical Sciences*. 2009; 4(2): 165-170.
- [20] Park B, O'Neil P, Maggs J, Pirmohamed M. Safety assessment of peroxide antimalarials: clinical and chemical perspective. *British Journal of Clinical Pharmacology*. 1998; 46(6): 521-529.
- [21] Pegg DG, Hook JB. Glutathione S transferases: an evaluation of their role in renal organic anion transport. *Journal of Pharmacology and Experimental Therapeutics*. 1977; 200(1): 65-74.
- [22] Persie LA, Krotoski WH, Collins WT *et al.* Antioxidants and free radicals. *Antioxidant Free Radical Damage*. 2006; 19 (12), 1145-1150.
- [23] Pilar CM, Avery TD, Hanssen E, Fox E, Robinson TV, Valente P, Taylor DK. Artemisinin and a series of novel endoperoxide antimalarials exert early effects on digestive vacuole morphology. *Antimicrobial agents and Chemotherapy*. 2008; 52(1): 98-109.
- [24] Reginald A, Kavishe JB, Koenderink MB, McCall, HM *et al.* Short report: severe *Plasmodium falciparum* malaria in Cameroon: associated with the glutathione s-transferase M1 null genotype K. *American Journal of Tropical Medicine and Hygiene*. 2006; 75(5): 827-829.
- [25] Sedlak J, Lindsay RH. *Analytical Biochemistry*. 1968; 25 (1): 192-205.
- [26] Sinha AK. *Analytical Biochemistry*. 1972; 47: 389-394.
- [27] Stocks J, Dormandy TL. The auto-oxidation of human red cells lipid induced by

- hydrogen peroxide. Br. J. of Haematology. 1971; 20: 95-111.
- [28] Usuh IF, Akpan EJ, Etim EO, Farombi EO. Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa* L. on sodium arsenite-induced oxidative stress in rats. Pakistan Journal of Nutrition. 2005; 4(3): 135-141.
- [29] Van Agtmael M, Gupta V, van der Wosten T, Rutten J, van and Boxtel C. Grapefruit juice increases the bioavailability of artemether. European Journal of Clinical Pharmacology. 1999;55: 405-410.
- [30] Van Vugt *et al.*, Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug resistant *Plasmodium falciparum* malaria. American Journal of Tropical Medicine and Hygiene. 1999; 60:936-942.
- [31] White N, van Vugt, M, Ezzet F. Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clinical pharmacokinetics. 1999;37: 105-125.
- [32] Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. Lancet Infectious Diseases. 2002; 2: 209-218.

**Submission date : 13/6/2011**