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Comparative Erythrocyte Glutathione S-Transferase Activity Profile of Non-Malarious Guinea Pigs (*Cavia tschudii*) Administered with Pyrimethamine/Sulphadoxine and Artemether/Lumefantrine Combination Therapies

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ABSTRACT

Cellular level of enzyme activity can serve as reliable biomarker in the event of environmental/chemical insults. The present study sought to investigate time-dependent alterations of erythrocyte glutathione S-transferase (Ery-GST) activity of non-malarious guinea pigs (*Cavia tschudii*) administered with pyrimethamine/sulphadoxine and artemether/lumefantrine combination therapies. Eighteen guinea pigs were allotted into three groups of six (n = 6), composed of the control (C1) and two test groups T1; pyrimethamine/sulphadoxine treated group and T2; artemether/lumefantrine treated group. Accordingly, single dose intra-peritoneal injection of pyrimethamine = 3.7 mg/sulphadoxine 0.7 mg mixture and artemether = 0.3 mg/lumefantrine = 1.8 mg mixture per kg of body weight (b.wt.) were administered to the guinea pigs. The animals were fasted for 16 h prior-treatment and blood samples were drawn at time intervals of 3, 6, 9 and 24 h and measured for GST activity using spectrophotometric methods. Ery-GST activity of group C1 ($C1_{GST}$) within 0 h≤t≤24 h was fairly constant and did not exhibit significant alterations ($p>0.05$), whereas the Ery-GST activity profile of the test groups (T1 and T2) were biphasic. Ery-GST activity of group T1 ($T1_{GST}$) varied within the range of 5.04±0.98-5.60±0.59 IU/gHb with peak enzyme activity at t = 0 h. At t = 9 h, $T2_{GST}$ activity was not significantly different ($p>0.05$) from $T1_{GST}$ activity. The Ery-GST activity profile indicated perturbation of erythrocyte physiochemistry, which could be of relevance from toxicological and therapeutic standpoints.

Key words: Artemether, erythrocyte, glutathione S-transferase, lumefantrine, pyrimethamine

INTRODUCTION

Combination therapy of sulphadoxine (50 mg) and pyrimethamine (250 mg) exist under the trade mark name of Fansidar™, often administered as prophylaxis and for treatment of certain chloroquine resistant strains of *Plasmodium falciparum* malaria (WHO, 2001). This drug combination effectively inhibits two enzymes involved in the biosynthesis of folinic acid within the parasite (Milhous *et al*., 1985; Nzila, 2006). Sulphadoxine {N^1-(5, 6-dimethoxy-4-pyrimidinyl) sulphanilamide} is a structural analogue of para-aminobenzoic acid (PABA) that competitively inhibits protozoan dihydropteroate synthetase (DHPS), the enzyme required for incorporation of PABA into dihydropteroic acid and immediate precursor of folic acid. The resultant depletion of folic acid, an essential cofactor in the biosynthesis of nucleic acids, interferes with protozoan nucleic acid and protein biosynthesis (Nzila, 2006). Sensitive malarial parasites are those that must synthesize their own folic acid, whereas those that can utilize preformed folate are not affected.

Pyrimethamine {5-(4-chlorophenyl)-6-ethyl-pyrimidine-2, 4-diamine} is a competitive inhibitor of dihydrofolate reductase (DHFR), the enzyme that reduces dihydrofolate to tetrahydrofolate (FH_4), required for one carbon transfer reactions. Because malaria parasites do not incorporate exogenous thymine or thymidine for DNA synthesis, thymidylate must be synthesized *de novo* in a process that requires active FH_4. The inhibition of this pathway effectively interferes with DNA synthesis in the malaria parasite with attendant deleterious consequence.

The combination of artemether and lumefantrine is available from Novartis under the brand name Coartem™. It is the most effective available treatment for malaria in children in areas of Africa where resistance to conventional antimalarial drugs is high (WHO, 2001). Coartem™ is currently the only fixed-dose artemisinin-based combination therapy (ACT) pre-qualified by the World Health Organization (WHO) (Lefevre *et al*., 2013). This fixed-dose combination is beneficial to patients as it facilitates treatment compliance and supports optimal clinical effectiveness.

The two active principles of Coartem™ show synergistic anti-protozoan activity against *P. falciparum* and a 1:6 ratio of artemether and lumefantrine has been described as optimal *in vitro* (Bakshi *et al*., 2000; Nzila, 2006; Lefevre *et al*., 2013). Artemether therapeutic action depends on its endoperoxide bridge, which interacts with haem iron to cause free radical mediated damage to malaria parasites (Tracy and Webster, 2001; Crespo-Ortiz and Wei, 2012). Lumefantrine, most likely, interferes with haem polymerization, which is an obligatory and critical detoxifying pathway in malaria parasites (Bakshi *et al*., 2000). Both agents have secondary actions that probably include inhibition of parasite nucleic acid and protein synthesis (MacArthur, 2000). The concept of combination therapy is based on the synergistic or additive potentials of two or more drugs to improve therapeutic efficacy and delay the development of resistance to the individual components of the drug combination (WHO, 2001).

The glutathione S-transferases (GSTs) activity among other functions (Sherratt and Hayes, 2001; Ziglari and Allameh, 2013) catalyzes the transfer of reduced glutathione (GSH) to reactive electrophiles. The GST activity pathways serve to protect cellular macromolecules from the deleterious effects of reactive oxygen and nitrogen species (RONS), which in turn, ensure cellular functionality and structural integrity (Sherratt and Hayes, 2001; Gilliland *et al*., 2004; Primavera *et al*., 2008). Several GST isoforms exist in different tissue/organ types (Sherratt and Hayes, 2001; Noce *et al*., 2012) with remarkable high occurrence in the liver, kidney and intestine (Harvey and Beutler, 1982; Ziglari and Allameh, 2013). Studies have shown that human erythrocyte glutathione S-transferase (Ery-GST) isoforms are immunologically distinct from the human hepatocytes GST isoforms (Awasthi and Singh, 1984). As at present, the physiologic role of Ery-GST is not fully known but there are suggestions that the presence of GST in erythrocytes is ideal for the detoxification and elimination of circulating xenobiotics (Ziglari and Allameh, 2013; Noce *et al*., 2012). There are also indications that Ery-GST function physiologically as a haemin-binding and/or transport protein in developing erythroid cells (Harvey and Beutler, 1982). Protein binding studies have shown that Ery-GST appear to bind haemin with an affinity equal to, if not greater than that of hepatocyte GST, the so-called ligandins or GST-B. Ligandins that have been posited to be responsible for the transport of haem from mitochondria to cytoplasm (Harvey and Beutler, 1982; Hamza and Dailey, 2012). However, bilirubin appears to be a poor substrate for Ery-GST compared to hepatocyte GST isoforms (Takikawa and Kaplowitz, 1988). These reports affirmed GST binding and transport functions in concurrence with its catalytic actions.

Previous studies have reported the correlation between GST activity and level of exposure of human and animal models to xenobiotics and pathogens (Sherratt and Hayes, 2001; Ismert *et al*., 2002; Primavera *et al*., 2008; Ezeji *et al*., 2012; Ziglari and Allameh, 2013). The reproducibility and reliability of GST activity, to serve as a biomarker in the event of environmental/chemical insults, informed the use of Ery-GST activity, to serve as an index to ascertain the level of distortion in erythrocyte physiochemical status of guinea pigs administered with pyrimethamine/sulphadoxine and artemether/lumefantrine combination therapies. The present study will give an insight into time-dependent alterations of Ery-GST activity of animal models following the administration of the two antimalarial combination therapies as prophylaxis.

MATERIALS AND METHODS

Collection/preparation of anti-malarial drug suspensions: The two antimalarial drugs, Fansidar™ {Swiss (Sipha) Pharmaceuticals Nigeria Ltd} and Coartem™ (Beijing Norvatis Pharmaceutical Company Beijing China), were purchased from Cympok Pharmaceuticals, Owerri, Nigeria. Tablets of the two antimalarial drugs, each weighing 2 g, were pulverized separately into powder form using ceramic mortar and pestle. The two ground drug samples were suspended in separate 50 mL of phosphate buffer saline (PBS) solutions (pH = 7.4) and allowed to stand in a water bath at thermostatically controlled temperature of 25±5°C under continuous agitation for 30 min after which the mixtures were separated using Whatman No. 24 filter paper.

Experimental animals: Healthy non-malarious male guinea pigs (*Cavia tschudii*) (8-10 weeks old) weighing 550-600 g were generous gift from Professor A.A. Uwakwe of the Department of Biochemistry, University of Port Harcourt, Nigeria. The guinea pigs were maintained at room temperature (25±5°C), 30-55% of relative humidity on a 12 h light/12 h dark cycle, with access to distilled water (DW) and standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) ad libitum for 2 weeks acclimatization period.

Ethics: The institutional review board of the Department of Biochemistry, Imo State University, Owerri, granted approval to this study. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Study design: The animals were fasted for 16 h before commencement of treatment (control and test experiments) (Ibegbulem and Chikezie, 2013). A single dose equivalents of pyrimethamine = 3.7 mg, sulphadoxine 0.7 mg and artemether = 0.3 mg, lumefantrine = 1.8 mg kg^{-1} b.wt. were prepared from corresponding stock solutions of the drugs and administered by intra-peritoneal injection to the guinea pigs. A total of eighteen guinea pigs were allotted into three groups of six (n = 6) each as follows:

- **Group C1 (Control):** Guinea pigs received only PBS (Vehicle; 2.0 mL kg^{-1} b.wt., i.p.
- **Group T1:** Guinea pigs received pyrimethamine = 3.7 mg/sulphadoxine 0.7 mg mixture/kg b.wt.; i.p.
- **Group T2:** Guinea pigs received artemether = 0.3 mg/lumefantrine = 1.8 mg mixture/kg b.wt.; i.p.

Collection of blood and preparation of erythrocyte haemolysate: Blood samples were drawn from the anterior region of the guinea pigs, using hypodermic syringe, at time intervals of 3, 6, 9 and 24 h post-treatment for analyses. The blood volumes obtained were transferred into test tubes containing PBS solution (blood to PBS ratio 1:4; v/v). The blood suspension was subjected to bench centrifugation for 10 min. The pelleted erythrocytes were washed by methods of Tsakiris *et al.* (2005) as described by Chikezie *et al.* (2009). Within 2 h of collection of blood specimen, 0.5 mL of harvested erythrocytes were introduced into centrifuge test tubes containing 3.0 mL of buffer solution pH = 7.4: 250 mM tris (hydroxyl methyl) amino ethane-HCl (Tris-HCl)/140 mM NaCl/1.0 mM $MgCl_2$/10 mM glucose). The erythrocyte suspension was further centrifuged at 1200 g for 10 min and repeated for 3 times. According to Chikezie (2011), the pelleted erythrocytes were re-suspended in 3.0 mL of PBS solution and passed twice through newly packed columns (3.5 cm in a 30 mL syringe) of cellulose-microcrystalline cellulose (ratio 1:1; w/w) to obtain erythrocyte suspension sufficiently devoid of leucocytes and platelets (Kalra *et al.*, 1981). Finally, the erythrocyte suspension was stored at 4°C and lysed by freezing/thawing as described by Galbraith and Watts (1980) and Kamher *et al.* (1984). The erythrocyte haemolysate was used for the determination of Ery-GST activity.

Erythrocyte haemolysate haemoglobin concentration: The cyanomethaemoglobin reaction modified method of Baure (1980), as described by Chikezie *et al.* (2009), was used for measurement of haemolysate haemoglobin concentration. A 0.05 mL portion of erythrocyte haemolysate was added to 4.95 mL of Drabkins reagent (100 mg NaCN and 300 mg $K_4Fe(CN)_6$ per liter). The mixture was left to stand for 10 min at 25±5°C and absorbance measured with a spectrophotometer (Digital Blood Analyzer®; SPECTRONIC 20, Labtech) at λ_{max} = 540 nm against a blank. The absorbance was used to evaluate haemolysate haemoglobin concentration by comparing the values with the standards.

Erythrocyte glutathione S-transferase: Ery-GST activity was measured by the method of Habig *et al.* (1974) as described by Pasupathi *et al.* (2009) with minor modifications according to Chikezie *et al.* (2009). The reaction mixture contained 1.0 mL of 0.3 mM phosphate buffer (pH = 6.5), 0.1 mL of 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 1.7 mL of DW. After pre-incubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 mL of erythrocyte haemolysate and 0.1 mL of GSH substrate. The absorbance was measured at time intervals of 30 sec for 5 min at λ_{max} = 340 nm. Ery-GST activity was expressed in international unit per gram haemoglobin (IU/gHb) using an extinction coefficient (Σ) of 9.6 mM^{-1} cm^{-1} in a reaction mixture in which 1 mole of GSH was oxidized (Eq. 1).

Calculation of Ery-GST activity:

$$E_A = \frac{100}{Hb} \times \frac{OD/min}{\Sigma} \times \frac{V_c}{V_H} \quad (1)$$

Where:

E_A = Enzyme activity in IU/gHb
Hb = Haemolysate haemoglobin concentration (g dL^{-1})
OD/min = Change per min in absorbance at 340 nm
V_C = Cuvette volume (total assay volume) = 3.0 mL
V_H = Volume of haemolysate in the reaction system (0.05 mL)

Calculation of Area Under the Curve (AUC): Cumulative Ery-GST per h (IU/gHb.h) within the experimental time (0 h≤t≤24 h) was evaluated using the Simpson's Rule. Thus:

$$f(X_1)h_1+f(X_2)h_2+...+f(X_n)h_n$$

Area Under the Curve (AUC) of the plot of IU/gHb versus time (h) is given by:

$$AUC\left(\frac{IU}{gHb}\times h\right)=\frac{h}{2}\left(y_n+2y_{n-1}+2y_{n-2}+2y_{n-3}+...\right) \quad (2)$$

Where:
h = Time intervals (h)
y = Ery-GST activity (IU/gHb) at corresponding time interval

Statistical analyses: The data collected was analyzed by the analysis of variance (ANOVA) procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the Statistical Analysis System (SAS) package of 9.1 version.

RESULTS

Table 1 showed that within 0 h≤t≤24 h, Ery-GST activity of group C1 ($C1_{GST}$) was fairly constant and did not exhibit significant alterations ($p<0.05$). Conversely, Ery-GST activity of group T1 ($T1_{GST}$) varied within the range of 5.04±0.98-5.60±0.59 IU/gHb with peak Ery-GST activity at the commencement of the experiment (t = 0 h) (Table 1). In addition, $T1_{GST}$ activity showed lower enzyme activity relative to $C1_{GST}$ activity. Specifically, at t = 6 h, $T1_{GST}$ activity was significantly ($p<0.05$) lower than $C1_{GST}$ activity, whereas no significant difference ($p>0.05$) was exhibited at t = 3 h. The lowest $T1_{GST}$ activity was registered at t = 9 h, which represented 90% relative enzyme activity compared to $T1_{GST}$ activity at t = 0 h (Fig. 1).

A cursory look at Table 1 showed that at t = 0 h, $T1_{GST}$ and $T2_{GST}$ activities were not significantly different ($p>0.05$) from $C1_{GST}$ activity. Similarly, at t = 9 h, $T2_{GST}$ activity was not significantly different ($p>0.05$) from $T1_{GST}$ activity. The lowest $T2_{GST}$ activity was at t = 3 h, which corresponded

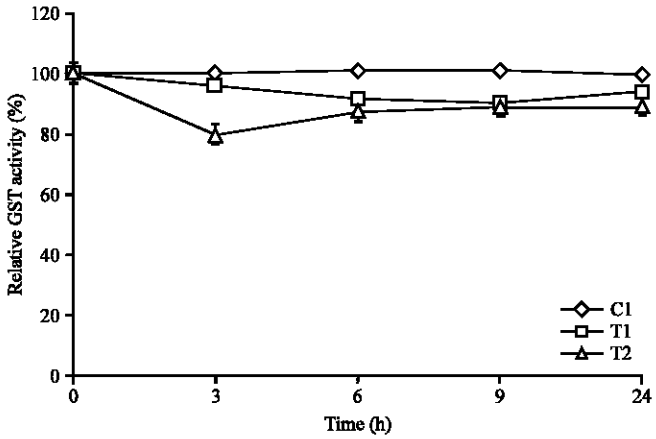

Fig. 1: Profile of guinea pigs erythrocyte glutathione S-transferase activity

Table 1: Guinea pigs erythrocyte glutathione S-transferase activity

	Experimental groups (n = 6)		
Time (h)	C1	T1	T2
0	5.63±0.31[a]	5.60±0.59[a,b,c]	5.73±0.37[a,b,c]
3	5.64±0.21[a]	5.38±0.11[a,b]	4.58±0.73[c]
6	5.67±0.16[a]	5.15±0.83[b]	4.99±0.27[c]
9	5.68±0.15[a]	5.04±0.98[b]	5.09±0.28[b,c]
24	5.61±0.11[a]	5.26±0.28[b]	5.10±0.28[c]

Results are mean (X)±SD of six (n = 6) determination, Means in the row with the same letter are not significantly different at p>0.05 according to LSD

Table 2: Area under the curve ($AUC_{0\,h \le t \le 24\,h}$) of guinea pigs erythrocyte glutathione S-transferase activity

	Experimental groups (n = 6)		
Parameter	C1	T1	T2
AUC (IU/gHb.h)	124.29±0.19[a]	113.99±0.56[b]	111.48±0.39[b,c]

Results are mean (X)±SD of six (n = 6) determination, Means in the row with the same letter are not significantly different at p>0.05 according to LSD

to 79.93% relative Ery-GST activity post-treatment (Fig. 1). Generally, within 0 h≤t≤24 h, Ery-GST activity of the three experimental groups was in the order; C1>T1>T2.

Figure 1 showed that the Ery-GST activity profile of the test groups (T1 and T2) were biphasic, $T1_{GST}$ and $T2_{GST}$ activities were lower compared to $C1_{GST}$ activity. For instance, within the experimental time of 24 h, t<9 h represented the inhibition phase of $T1_{GST}$ activity followed by the enzyme recovery phase, exemplified by relative increase in $T1_{GST}$ activity at t = 9 h by 3.97% compared to $T1_{GST}$ activity at t = 24 h ($p<0.05$). Likewise, t<3 h represented decreasing $T2_{GST}$ activity, whereas t>3 h showed progressive increase in $T2_{GST}$ activity relative to pre-treatment time at t = 0 h (Fig. 1).

The cumulative Ery-GST activity profile and capacity of the two drugs to alter erythrocyte enzyme activity in the various experimental groups are presented in Table 2. Thus, $AUC_{0\,h \le t \le 24\,h}$ showed that the level of alterations in $T1_{GST}$ activity was comparable to $T2_{GST}$ activity and was significantly different ($p<0.05$) from $C1_{GST}$ activity.

DISCUSSION

Cellular levels of GST activity are of immense application in providing insights into the etiology and diagnosis of diverse pathologic states and for monitoring toxicological and therapeutic events. There are reports on over-expression of GST in erythrocytes of individuals with chronic renal failure with attendant uremia (Romeu *et al*., 2010) and hyperbilirubinemia (Noce *et al*., 2012). Also, toxicological studies of human erythrocytes exposed to heavy metals (Goodrich and Basu, 2012) and rainbow trout erythrocytes incubated in aminoglycoside antibiotics *in vitro* (Comakli *et al*., 2011) as well as animal models exposed to particulate matters (Gilliland *et al*., 2004), gas substances, aromatic/organic chemicals *in situ* (Ismert *et al*., 2002) exhibited decreased levels of Ery-GST activity in a dose/concentration dependent manner. Furthermore, the activities of redox enzymes, Ery-GST activity inclusively serve as reliable biomarkers in environmental and toxicological studies. For instance, Ery-GST activity previously had been employed as a tool for

assessing levels of pesticide contaminations (Domingues *et al*., 2010) and exposure to pollutants involving two crustacean species; water flea *Daphnia magna*, terrestrial isopod *Porcellio scaber* (Jemec *et al*., 2010) as well as fish (*Cyprinus carpio* L.) (Valon *et al*., 2013).

The present study has equally shown that non-malarious guinea pigs administered with pyrimethamine/sulphadoxine and artemether/lumefantrine combination therapies exhibited reduced levels of Ery-GST activity, which was a spinoff effect of the biotransformational pathways of the drugs *in vivo*. The concerted role of cellular GST and cytochrome P450 superfamily in the metabolism of xenobiotics and pathogenic metabolites has been described (Ziglari and Allameh, 2013), which serves to explain the basis for the pattern of Ery-GST activity of present study. Also from the present study, the subsequent time-dependent increasing levels of Ery-GST activity (recovery phases) after initial decay/attenuation of enzyme activity (inhibition phase) was in a manner similar to those earlier reported by Ayalogu *et al*. (2001). They noted that rats injected with gasoline exhibited early short term low activity of GST, which also affirmed the outcome of Chiapotto *et al*. (1995) report on inactivation of GST activity by different concentrations of acetaldehyde. Their study further suggested that detoxification process and eventual systemic clearance of the causative metabolites accounted for the subsequent increasing levels in GST activity of rats administered with kerosene and crude oil (bonny light) as the experimental time progressed. Rathore *et al*. (1998) had reported similar characteristic pattern of GST activity in oxidative stressed rats induced by the administration of isoproterenol. They noted that inactivated GST activity partially recovered 12 h after the administration of isoproterenol in efforts by antioxidant system to counteract and ameliorate oxidative stress. The capability of artemether, lumefantrine and sulphadoxine to generate RONS (Bakshi *et al*., 2000; MacArthur, 2000; Nzila, 2006; Chikezie *et al*., 2009; Crespo-Ortiz and Wei, 2012) could be responsible for the time-dependent biphasic Ery-GST activity profile of guinea pigs administered with pyrimethamine/sulphadoxine and artemether/lumefantrine combination therapies.

The initial decreasing levels of Ery-GST activity was in connection with the consumption of GST cofactor-GSH, one of the first lines of antioxidant defense mechanism against xenobiotics, which might have accounted for depletion of intracellular GSH with resultant decreasing levels of relative Ery-GST activity (Sherratt and Hayes, 2001; Primavera *et al*., 2008; Ezeji *et al*., 2012). However, increasing levels of Ery-GST activity after the initial inactivation phase, occasioned by low levels of GSH among other factors, could have prompted the positive activation and up-regulation of the redox enzymes in effort to restore homeostasis and prevent cellular damage (Anosike *et al*., 1991) consequent upon the propensity of these chemical agents to overwhelm cellular antioxidant system. More recently, reports have shown that xenobiotics promote the expression and activation of transcriptional factors, particularly the nuclear factor erythroid-2 (Nrf2) that has been implicated in up-regulation of antioxidant enzymes (Lodovici and Bigagli, 2011). The processes leading to up-regulation of redox enzymes are to ensure that the cell is equipped with adequate and sustainable quantity of requisite antioxidant enzymes for neutralization of potentially noxious chemical agents.

CONCLUSION

The findings of the present study showed that Ery-GST activity of non-malarious guinea pigs administered with pyrimethamine/sulphadoxine and artemether/lumefantrine combination therapies gave a characteristic biphasic profile. The perturbation of erythrocyte physiochemistry of non-malarious guinea pigs administered with pyrimethamine/sulphadoxine and

artemether/lumefantrine combination therapies was typified by the disparities in cumulative Ery-GST activity (IU/gHb.h) among the experimental groups (C1, T1 and T3), which could be of relevance from toxicological and therapeutic standpoints.

REFERENCES

Anosike, E.O., A.A. Uwakwe, M.O. Monanu and G.I. Ekeke, 1991. Studies on human erythrocyte glutathione-*S*-transferase from HbAA, HbAS and HbSS subjects. Biochem. Biomed. Acta, 50: 1051-1056.

Awasthi, Y.C. and S.V. Singh, 1984. Purification and characterization of a new form of glutathione S-transferase from human erythrocytes. Biochem. Biophys. Res. Commun., 125: 1053-1060.

Ayalogu, E.O., N.H. Igboh and E.B. Dede, 2001. Biochemical changes in the serum and liver of albino rats exposed to petroleum samples (gasoline, kerosene and crude petroleum). J. Applied Sci. Environ. Manage., 5: 97-100.

Bakshi, R., I. Hermeling-Fritz, I. Gathmann and E. Alteri, 2000. An integrated assessment of the clinical safety of artemether-lumefantrine: A new oral fixed-dose combination antimalarial drug. Trans. R. Soc. Trop. Med. Hyg., 94: 419-424.

Baure, J.D., 1980. Laboratory Investigation of Hemoglobin. In: Gradwohl's Clinical Laboratory Methods and Diagnosis, Sonnenwirth, A.C. and L. Jarett (Eds.). Mosby, St. Louis, MO., pp: 809-902.

Chiapotto, E., F. Biasi, A. Scavazza, S. Camandola and M. Aragno *et al*., 1995. Acetaldehyde involvement in ethanol-induced potentiation of rat hepatocyte damage due to the carcinogen 1,2-dibromoethanol. Alcohol. Alcoholism, 30: 721-728.

Chikezie, P.C., 2011. Glutathione S-transferase activity of human erythrocytes incubated in aqueous solutions of five antimalarial drugs. Free Radicals Antioxid., 1: 26-30.

Chikezie, P.C., C.C. Monago and A. Uwakwe, 2009. Studies of methemoglobin concentrations of three human erythrocyte genotypes (Hb AA, Hb AS and Hb SS) in the presence of five anti-malarial drugs. IJBC, 1: 151-157.

Comakli, V., M. Ciftci and O.I. Kufrevioglu, 2011. Purification of glutathione S-transferase enzyme from rainbow trout erythrocytes and examination of the effects of certain antibiotics on enzyme activity. Hacettepe J. Biol. Chem., 39: 413-419.

Crespo-Ortiz, M.P. and M.Q. Wei, 2012. Antitumor activity of artemisinin and its derivatives: From a well-known antimalarial agent to a potential anticancer drug. J. Biomed. Biotechnol. 10.1155/2012/247597

Domingues, I.S., A.R. Agra, K. Monaghan, A.M.V.M. Soares and A.J.A. Nogueira, 2010. Cholinesterase and glutathione-S-transferase activities in freshwater invertebrates as biomarkers to assess pesticide contamination. Environ. Toxicol. Chem., 29: 5-18.

Ezeji, E.U., E.A. Anyalogbu, T.N. Ezejiofor and J.U. Udensi, 2012. Determination of reduced glutathione and glutathione S-transferase of poultry birds exposed to permethrin insecticide. Am. J. Biochem., 2: 21-24.

Galbraith, D.A. and D.C. Watts, 1980. Changes in some cytoplasmic enzymes from red cells fractionated into age groups by centrifugation in Ficoll™/Triosil™ gradients: Comparison of normal human and patients with Duchenne muscular dystrophy. Biochem. J., 191: 63-70.

Gilliland, F.D., Y.F. Li, A. Saxon and D. Diaz-Sanchez, 2004. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: Randomised, placebo-controlled crossover study. Lancet, 363: 119-125.

Goodrich, J.M. and N. Basu, 2012. Variants of glutathione S-transferase pi 1 exhibit differential enzymatic activity and inhibition by heavy metals. Toxicol. *In vitro*, 26: 630-635.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hamza, I. and H.A. Dailey, 2012. One ring to rule them all: Trafficking of heme and heme synthesis intermediates in the metazoans. Biochim. Biophys. Acta Mol. Cell Res., 1823: 1617-1632.

Harvey, J.W and E. Beutler, 1982. Binding of heme by glutathione S-transferase: The first step in mercapturic acid formation. J. Biol. Chem., 245: 7130-7139.

Ibegbulem, C.O. and P.C. Chikezie, 2013. Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi*, *Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattusnorvegicus*). Pharmacog. Commun., 3: 12-16.

Ismert, M., T. Oster and D. Bagrel, 2002. Effects of atmospheric exposure to naphthalene on xenobiotic-metabolising enzymes in the snail *Helix aspersa*. Chemosphere, 46: 273-280.

Jemec, A., D. Drobne, T. Tisler and K. Sepcic, 2010. Biochemical biomarkers in environmental studies: Lessons learnt from enzymes catalase, glutathione *S*-transferase and cholinesterase in two crustacean species. Environ. Sci. Pollut. Res., 17: 571-581.

Kalra, V.K., S.C. Sikka and G.S. Sethi, 1981. Transport of amino acids in gamma-glutamyl transpeptidase-implanted human erythrocytes. J. Biol. Chem., 256: 5567-5571.

Kamher, E., A. Poyiagi and G. Delikonstantinos, 1984. Modifications in the activities of membrane-bound enzymes during *in vivo* ageing of h uman and rabbit erythrocytes. Comp. Biochem. Physiol. B: Comp. Biochem., 77: 95-99.

Lefevre, G., P. Bhad, J.P. Jain, S. Kalluri, Y. Cheng, H. Dave and D.S. Stein, 2013. Evaluation of two novel tablet formulations of artemether-lumefantrine (Coartem®) for bioequivalence in a randomized, open-label, two-period study. Malaria J., Vol. 12. 10.1186/1475-2875-12-312

Lodovici, M and E. Bigagli, 2011. Oxidative stress and air pollution exposure. J. Toxicol. 10.1155/2011/487074

MacArthur, J.D., 2000. Cinchona, microsoft encartal online. Encyclopedia 1997-2000, Microsoft Corporation.

Milhous, W.K., N.F. Weatherly, J.H. Bowdre and R.E. Desjardins, 1985. *In vitro* activities of and mechanisms of resistance to antifol antimalarial drugs. Antimicrob. Agents Chemother., 27: 525-530.

Noce, A., M. Ferrannini, R. Fabrini, A. Bocedi and M. Dessi *et al*., 2012. Erythrocyte glutathione transferase: A new biomarker for hemodialysis adequacy, overcoming the Kt/V(urea) dogma? Cell Death Dis., Vol. 3. 10.1038/cddis.2012.112

Nzila, A., 2006. Inhibitors of *de nove* folate enzymes in *Plasmodium falciparum*. Drug Discovery Today, 11: 939-944.

Pasupathi, P., V. Chandrasekar and U.S. Kumar, 2009. Evaluation of oxidative stress, antioxidant and thyroid hormone status in patients with diabetes mellitus. J. Med., 10: 60-66.

Primavera, A., S. Fustinoni, A. Biroccio, S. Ballerini and A. Urbani *et al*., 2008. Glutathione transferases and glutathionylated hemoglobin in workers exposed to low doses of 1,3-butadiene. Cancer Epidemiol. Biomarkers Prev., 17: 30-34.

Rathore, N., S. John, M. Kale and D. Bhatnagar, 1998. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol. Res., 38: 297-303.

Romeu, M., R. Nogues, L. Marcas, V. Sanchez-Martos and M. Mulero *et al*., 2010. Evaluation of oxidative stress biomarkers in patients with chronic renal failure: A case control study. BMC Res. Notes, Vol. 3. 10.1186/1756-0500-3-20

Sherratt, P.J. and J.D. Hayes, 2001. Glutathione S-Transferases. In: Enzyme Systems That Metabolise Drugs and Other Xenobiotics, Ioanides, C. (Ed.). Wiley and Sons, New York, USA., ISBN-13: 978-0-471-89466-7, pp: 319-352.

Takikawa, H. and N. Kaplowitz, 1988. Comparison of the binding sites of GSH S-transferases of the Y_a- and Y_b-subunit classes: Effect of glutathione on the binding of bile acids. J. Lipid Res., 29: 279-286.

Tracy, J.W. and L.T. Webster, 2001. Drugs Used in the Chemotherapy of Protozoal Infections. In: Good-man and Gilman's the Pharmacological Basis of Therapeutics, Joel, G.H. and L.E. Limbird (Eds.). 10th Edn., McGraw Hill, New York, USA., pp: 1097-1120.

Tsakiris, S., A. Giannoulia-Karantana, I. Simintzi and K.H. Schulpis, 2005. The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. Pharmacol. Res., 53: 1-5.

Valon, M., A. Valbona, G. Fahri, M. Qenan, K. Dhurat and G. Fatmir, 2013. Evaluating environmental pollution by applying oxidative stress biomarkers as bioindicators of water pollution in fish. Pol. J. Environ. Stud., 22: 1519-1523.

WHO, 2001. Antimalarial drug combination therapy: Report of a WHO technical consultation. WHO/CDS/RBM/2001.35, World Health Organisation, Geneva, Swizerland. http://whqlibdoc.who.int/hq/2001/WHO_CDS_RBM_2001.35.pdf.

Ziglari, T. and A. Allameh, 2013. The Significance of Glutathione Conjugation in Aflatoxin Metabolism. In: Aflatoxins-Recent Advances and Future Prospects, Razzaghi-Abyaneh, M. (Ed.). InTech Publication, New York, USA., ISBN-13: 9789535109044.