

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

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Background: Cellular level of enzyme activity can serve as a reliable biomarker in the event of environmental/chemical insults.

Objectives: The present study investigated time-dependent alterations of erythrocyte glutathione S-transferase (Ery-GST) activity of non-malarious guinea pigs (*Cavia tschudii*) administered with pyrimethamine/sulfadoxine and artemether/lumefantrine combination therapies.

Materials and Methods: Eighteen guinea pigs were divided into three groups of six, composed of the control (C1) and two test groups: T1: pyrimethamine/sulfadoxine treated group and T2: artemether/lumefantrine treated group. Accordingly, single dose intra-peritoneal injections equivalents of mixture of pyrimethamine = 3.7 mg/kg bw and sulfadoxine = 0.7 mg/kg bw was administered to T1, whereas a mixture of artemether = 0.3 mg/kg bw and lumefantrine = 1.8 mg/kg bw was administered to T2. The animals were not fed for 16 hours prior to treatment and blood samples were drawn at time intervals of three, six, nine and 24 hours and measured for GST activity using spectrophotometric methods.

Results: The Ery-GST activity of group C1 ($C1_{GST}$) within $0 \leq t \leq 24$ hours 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 with a range of 5.04 ± 0.98 to 5.60 ± 0.59 IU/gHb with peak enzyme activity at $t = 0$. At $t = 9$ hours, $T2_{GST}$ activity was not significantly different ($P > 0.05$) from $T1_{GST}$ activity.

Conclusions: The Ery-GST activity profile indicated perturbation of erythrocyte physiochemistry, which could be of relevance from toxicological and therapeutic standpoints.

Keywords: Erythrocyte; Glutathione S-transferase; Guinea pigs; Pyrimethamine; Sulfadoxine

1. Background

Combination therapy of sulfadoxine (50 mg) and pyrimethamine (250 mg) exist under the trade mark name of FansidarTM, often administered as prophylaxis and for treatment of certain chloroquine resistant strains of *Plasmodium falciparum* malaria (1). This drug combination effectively inhibits two enzymes involved in the biosynthesis of folinic acid within the parasite (2, 3). Sulfadoxine (N^1 -(5, 6-dimethoxy-4-pyrimidinyl) sulfanilamide) 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 the protozoan nucleic acid and protein biosynthesis (3). Sensitive malarial parasites are those that must synthesize their own folic acid, whereas those that can utilize existing folate are not affected. Pyrimethamine (5-(4-chlorophenyl)-6-ethyl-pyrimidine-2, 4-diamine) is a competitive inhibitor of dihydrofolate re-

ductase (DHFR), the enzyme that reduces dihydrofolate to tetrahydrofolate (FH4), required for one of the carbon transfer reactions. Since malaria parasites do not incorporate exogenous thymine or thymidine for DNA synthesis, thymidylate must be synthesized de novo in a process that requires active FH4. The inhibition of this pathway effectively interferes with DNA synthesis in the malaria parasite with attendant deleterious consequences. The combination of artemether and lumefantrine is available from Novartis under the brand name CoartemTM. It is the most effective available treatment for malaria of children in African areas where resistance to conventional antimalarial drugs is high (1). CoartemTM is currently the only fixed-dose artemisinin-based combination therapy (ACT) pre-qualified by the World Health Organization (WHO) (4). This fixed-dose combination is beneficial for patients as it facilitates treatment compliance and supports optimal clinical effectiveness.

The two active principles of CoartemTM show synergistic anti-protozoan activity against *P. falciparum* and a 1:6

ratio of artemether and lumefantrine has been described as optimal *in vitro* (3-5). Artemether therapeutic action depends on its endoperoxide bridge, which interacts with haem irons to cause free radical mediated damage to malaria parasites (6, 7). Lumefantrine most likely interferes with haem polymerization, which is an obligatory and critical detoxifying pathway in malaria parasites (5). Both agents have secondary actions that probably include inhibition of parasite nucleic acid and protein synthesis (8). 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 (1). The glutathione S-transferases (GSTs) activity among other functions (9, 10) 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 (9, 11, 12). Several GST isoforms exist in different tissue/organ types (9, 13) with remarkable high occurrence in the liver, kidney and intestine (10, 14). Studies have shown that human erythrocyte glutathione S-transferase (Ery-GST) isoforms are immunologically distinct from human hepatocytes GST isoforms (15). At present, the physiological role of Ery-GST is not fully known yet there are suggestions that the presence of GST in erythrocytes is ideal for the detoxification and elimination of circulating xenobiotics (10, 13). There are also indications that Ery-GST functions physiologically as a haemin-binding and/or transport protein in developing erythroid cells (14). Protein binding studies have shown that Ery-GST appears to bind haemin with an affinity equal to, if not greater than that of hepatocyte GST; the so-called ligandins or GST-B. Ligandins have been posited to be responsible for the transport of haem from mitochondria to cytoplasm (14, 16). However, bilirubin appears to be a poor substrate for Ery-GST compared to hepatocyte GST isoforms (17). 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 (9, 10, 12, 18, 19). 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 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.

2. Objectives

The present study will give insight into time-dependent alterations of Ery-GST activity of animal models following the administration of two antimalarial combination therapies as prophylaxis.

3. Materials and Methods

3.1. Collection Preparation of Anti-Malarial Drug Suspensions

The two antimalarial drugs, FansidarTM (Swiss (Sipha) Pharmaceuticals Nigeria Ltd) and CoartemTM (Beijing Novartis 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 by using a ceramic mortar and pestle. The two ground drug samples were suspended separately in 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 minutes after which the mixtures were separated using Whatman No 24 filter paper.

3.2. Experimental Animals

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

3.3. Ethics

The institutional review board of the Department of Biochemistry, Imo State University, Owerri granted approval of 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).

3.4. Study Design

The animals were not fed for 16 hours before initiation of treatment (control and test experiments) as described elsewhere (20). A single dose Intra-peritoneal injections equivalents of mixture of pyrimethamine = 3.7 mg/kg bw and sulfadoxine = 0.7 mg/kg bw was administered to the first animal test group (T1), whereas a mixture of artemether = 0.3 mg/kg bw and lumefantrine = 1.8 mg/kg bw was administered to the second animal test group (T2). 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 bw; i.p.)
- Group T1; guinea pigs received pyrimethamine = 3.7 mg/sulfadoxine 0.7 mg mixture/kg bw; i.p.
- Group T2; guinea pigs received artemether = 0.3 mg/lumefantrine = 1.8 mg mixture/kg bw; i.p.

3.5. Collection of Blood and Preparation of Erythrocyte Hemolysate

Blood samples were drawn from the anterior region of the guinea pigs, using a hypodermic syringe, at time intervals of three, six, nine and 24 hours 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 minutes. The pelleted erythrocytes were washed by methods described by Tsakiris et al. (21) and Chikezie et al. (22). Within two hours of collection of blood specimens, 0.5 mL of harvested erythrocytes was 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 MgCl₂/10 mM glucose). The erythrocyte suspension was further centrifuged at 1200 g for 10 minutes and this was repeated 3 times. According to Chikezie (23), 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 (24). Finally, the erythrocyte suspension was stored at 4°C and lysed by freezing/thawing as described by Galbraith and Watts (25) and Kamber et al. (26). The erythrocyte hemolysate was used for the determination of Ery-GST activity.

3.6. Erythrocyte Hemolysate Hemoglobin Concentration

The cyanomethaemoglobin reaction modified method of Baure, (27) as described by Chikezie et al. (22) was used for measurement of hemolysate hemoglobin concentration. A 0.05 mL portion of erythrocyte hemolysate was added to 4.95 mL of Drabkin's reagent (100 mg NaCN and 300 mg K₄Fe(CN)₆ per liter). The mixture was left to stand for 10 minutes at 25 ± 5°C and absorbance was measured with a spectrophotometer (Digital Blood Analyzer®; SPECTRONIC 20, Labtech) at λ_{max} = 540 nm against a blank. The absorbance was used to evaluate hemolysate hemoglobin concentration by comparing the values with the standards.

3.7. Erythrocyte Glutathione S-Transferase

The Ery-GST activity was measured by the method of Habig (28) as described by Pasupathi et al. (29) with minor modifications according to Chikezie et al. (22). 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 minutes, the reaction was started by the addition of 0.1 mL of erythrocyte hemolysate and 0.1 mL of GSH substrate. Absorbance was measured at time intervals of 30 seconds, for 5 minutes at λ_{max} = 340 nm. The Ery-GST activity was expressed by

the international unit of per gram hemoglobin (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 (Equation 1).

3.8. Calculation of Ery-GST Activity

$$E_A = 100/(\text{Hb}) \times (\text{OD}/\text{min}) / \Sigma \times V_C/V_H \text{ Equation 1}$$

Where,

EA = Enzyme activity in IU/gHb

[Hb] = Hemolysate hemoglobin concentration (g/dL)

OD/min = Change per minute with absorbance at 340 nm.

VC = Cuvette volume (total assay volume) = 3.0 mL.

VH = Volume of hemolysate in the reaction system (0.05 mL).

3.9. Calculation of Area Under the Curve (AUC)

Cumulative Ery-GST per hour (IU/gHb h⁻¹) within the experimental time (0 h ≤ t ≤ 24 h) was evaluated using the Simpson's Rule. Thus:

$$f(X_1)h_1 + f(X_2)h_2 + \dots + f(X_n)h_n$$

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

$$\text{AUC (IU/gHb h}^{-1}\text{)} = h/2 (y_n + 2y_{n-1} + 2y_{n-3} + \dots) \text{ Equation 2}$$

Where:

h = Time intervals in hours.

y = Ery-GST activity (IU/gHb) at corresponding time interval.

3.10. Statistical Analyses

The results were expressed as mean ± SEM, and statistically analyzed by one way ANOVA followed by Dunnett's test, with level of significance set at P < 0.05.

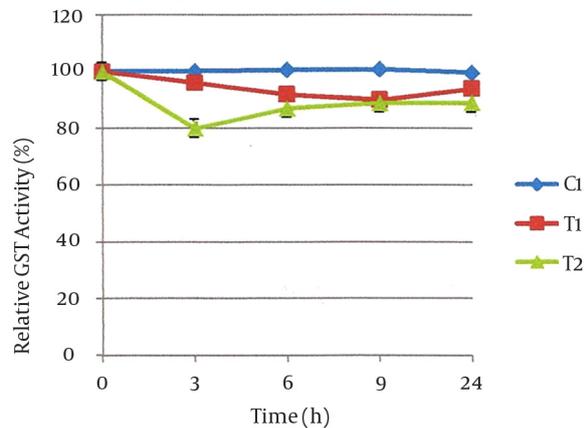
4. Results

An overview of Table 1 showed that within 0 ≤ t ≤ 24 hours, 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 hour) (Table 1). In addition, T1_{GST} activity showed lower enzyme activity relative to C1_{GST} activity. Specifically, at t ≥ 6 hours, 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 hours. The lowest T1_{GST} activity was registered at t = 9 hours, which represented 90% relative enzyme activity compared to T1_{GST} activity at t = 0. (Figure 1).

A look at Table 1 shows that at t = 0, T1_{GST} and T2_{GST} activities were not significantly different (P > 0.05) from C1_{GST} activity. Similarly, at t = 9 hours, T2_{GST} activity was not significantly different (P > 0.05) from T1_{GST} activity. The lowest T2_{GST} activity was at t = 3 hours, which corresponded to 79.93% relative Ery-GST activity post-treatment (Figure 1). Generally, within 0 ≤ t ≤ 24 hours, Ery-GST activity of the three experimental groups was in the following order: C1 > T1 > T2.

Table 1. Guinea Pigs Erythrocyte Glutathione S-Transferase Activity^a

Time, h	Experimental Groups (n = 6) ^b		
	Control	Test 1	Test 2
0	5.63 ± 0.31	5.60 ± 0.59	5.73 ± 0.37
3	5.64 ± 0.21	5.38 ± 0.11	4.58 ± 0.73
6	5.67 ± 0.16	5.15 ± 0.83	4.99 ± 0.27
9	5.68 ± 0.15	5.04 ± 0.98	5.09 ± 0.28
24	5.61 ± 0.11	5.26 ± 0.28	5.10 ± 0.28

^a The results are Mean ± SD.^b Means are not significantly different at P > 0.05.**Figure 1.** Profile of Guinea Pigs Erythrocyte Glutathione S-Transferase Activity**Table 2.** Area Under the Curve (AUC_{0 h ≤ t ≤ 24 h}) of Guinea Pigs Erythrocyte Glutathione S-Transferase Activity^{a,b}

Experimental Groups (n = 6)	Control	Test 1	Test 2
AUC (IU/gHb h ⁻¹)	124.29 ± 0.19a	113.99 ± 0.56b	111.48 ± 0.39b,c

^a The results are Mean ± SD.^b Means are not significantly different at P > 0.05.

Figure 1 shows that the Ery-GST activity profile of the test groups (T1 and T2) were biphasic, and T1_{GST} and T2_{GST} activities were lower compared to C1_{GST} activity. For instance, within the experimental time of 24 hours, t < 9 hours 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 hours by 3.97% compared to T1_{GST} activity at t = 24 hours (P < 0.05). Likewise, t < 3 hours represented decreasing T2_{GST} activity, whereas t > 3 hours showed progressive increase in T2_{GST} activity relative to pre-treatment time at t = 0 (Figure 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, area under the curve (AUC 0 ≤ t ≤ 24 hours)

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.

5. Discussion

Cellular levels of GST activity have immense application for 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 (30) and hyperbilirubinemia (13). Also, toxicological studies of human erythrocytes exposed to heavy metals (31) and rainbow trout erythrocytes incubated in aminoglycoside antibiotics in vitro (32) as well as animal models exposed to particulate matters (11), gas substances, aromatic/organic chemicals in situ (18) showed that there were decreased levels of Ery-GST activity in a dose/concentration dependent manner. Furthermore, the activities of redox enzymes, Ery-GST activity inclusive, serve as reliable biomarkers in environmental and toxicological studies. For instance, Ery-GST activity has previously been employed as a tool for assessing levels of pesticide contamination (33) and exposure to pollutants involving two crustacean species: water flea *Daphnia magna* and terrestrial isopod *Porcellio scaber* (34) as well as fish (*Cyprinus carpio*. L) (35). The present study has equally shown that non-malarious guinea pigs administered FansidarTM and CoartemTM 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 elsewhere (10), which serves to explain the basis for the pattern of Ery-GST activity of the 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. (36). They noted that rats injected with gasoline exhibited early short-term low activity of GST, which also affirmed the outcome of the study by Chiarotto et al. (37) 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. (38) had reported a similar characteristic pattern for GST activity in oxidative stressed rats induced by the administration of isoproterenol. They noted that inactivated GST activity partially recovered 12 hours after the administration of isoproterenol in efforts by the antioxidant system to counteract and ameliorate oxidative stress. The capability of artemether, lumefantrine and sulfadoxine to generate RONS as reported elsewhere (3, 5, 7, 8, 22) could be

responsible for the time-dependent biphasic Ery-GST activity profile of guinea pigs administered with pyrimethamine/sulfadoxine and artemether/lumefantrine combination therapies.

The initial decreasing levels of Ery-GST activity was related to 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 (9, 12, 19). 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 an effort to restore homeostasis and prevent cellular damage (39); 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 (40). 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. The findings of the present study showed that Ery-GST activity of non-malarious guinea pigs administered with pyrimethamine/sulfadoxine and artemether/lumefantrine combination therapies gave a characteristic biphasic profile. The perturbation of erythrocyte physiochemistry of non-malarious guinea pigs administered with pyrimethamine/sulfadoxine and artemether/lumefantrine combination therapies was typified by the disparities in cumulative Ery-GST activity (IU/gHb h⁻¹) among the experimental groups (C1, T1 and T3), which could be of relevance from toxicological and therapeutic standpoints.

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References

- World Health Organization.. *Antimalarial drug combination therapy. Report of a Technical Consultation*. Geneva: WHO; 2001.
- Milhouh WK, Weatherly NF, Bowdre JH, Desjardins RE. In vitro activities of and mechanisms of resistance to antifol antimalarial drugs. *Antimicrob Agents Chemother*. 1985;27(4):525-30.
- Nzila A. Inhibitors of de novo folate enzymes in Plasmodium falciparum. *Drug Discov Today*. 2006;11(19-20):939-44.
- Lefevre G, Bhad P, Jain JP, Kalluri S, Cheng Y, Dave H, et al. Evaluation of two novel tablet formulations of artemether-lumefantrine (Coartem) for bioequivalence in a randomized, open-label, two-period study. *Malar J*. 2013;12:312.
- Bakshi R, Hermeling-Fritz I, Gathmann I, Alteri E. An integrated assessment of the clinical safety of artemether-lumefantrine: a new oral fixed-dose combination antimalarial drug. *Trans R Soc Trop Med Hyg*. 2000;94(4):419-24.
- Tracy JW, Webster LT. *Drugs used in the chemotherapy of protozoan infections*. In: *Goodman and Gilman's Pharmacological Basis of Therapeutics*. 10th edUSA: McGraw-Hill; 2001.
- Crespo-Ortiz MP, Wei MQ. Antitumor activity of artemisinin and its derivatives: from a well-known antimalarial agent to a potential anticancer drug. *J Biomed Biotechnol*. 2012;2012:247597.
- MacArthur JD. Cinchona. Microsoft Encarta Online Encyclopedia editor. : Microsoft Corporation; 2000.
- Sherratt PJ, Hayes JD. Glutathione S - transferases. In: Loanides C editor. *Enzyme Systems That Metabolise Drugs and Other Xenobiotics*. New York: Wiley and Sons; 2001. pp. 319-52.
- Ziglar T, Allameh A. *The Significance of Glutathione Conjugation in Aflatoxin Metabolism. Aflatoxins-Recent. Advances and Future Prospects*. InTech Publication; 2013.
- Gilliland FD, Li YF, Saxon A, Diaz-Sanchez D. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. *Lancet*. 2004;363(9403):119-25.
- Primavera A, Fustinoni S, Biroccio A, Ballerini S, Urbani A, Bernardini S, et al. Glutathione transferases and glutathionylated hemoglobin in workers exposed to low doses of 1,3-butadiene. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3004-12.
- Noce A, Ferrannini M, Fabrini R, Bocedi A, Dessi M, Galli F, et al. Erythrocyte glutathione transferase: a new biomarker for hemodialysis adequacy, overcoming the Kt/V(urea) dogma? *Cell Death Dis*. 2012;3.
- Harvey JW, Beutler E. Binding of heme by glutathione S-transferase: a possible role of the erythrocyte enzyme. *Blood*. 1982;60(5):1227-30.
- Awasthi YC, Singh SV. Purification and characterization of a new form of glutathione S-transferase from human erythrocytes. *Biochem Biophys Res Commun*. 1984;125(3):1053-60.
- Hamza I, Dailey HA. One ring to rule them all: trafficking of heme and heme synthesis intermediates in the metazoans. *Biochim Biophys Acta*. 2012;1823(9):1617-32.
- Takikawa H, Kaplowitz N. Comparison of the binding sites of GSH S-transferases of the Ya- and Yb-subunit classes: effect of glutathione on the binding of bile acids. *J Lipid Res*. 1988;29(3):279-86.
- Ismert M, Oster T, Bagrel D. Effects of atmospheric exposure to naphthalene on xenobiotic-metabolising enzymes in the snail *Helix aspersa*. *Chemosphere*. 2002;46(2):273-80.
- Ezeji EU, Anyalogbu EA, Ezejiofor TN, Udensi JU. Determination of reduced glutathione and glutathione S-transferase of poultry birds exposed to permethrin insecticide. *Am J Biochem*. 2012;2(3):21-4.
- Ibegbulem CO, Chikezie PC. Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi*, *Viscum album* and *Allium sativum* administered to alloxan induced diabetic albino rats (*Rattus norvegicus*). *J Biol Chem Res*. 2012;29(1):16-25.
- Tsakiris S, Giannoulia-Karantana A, Simintzi I, Schulpis KH. The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. *Pharmacol Res*. 2006;53(1):1-5.
- Chikezie PC. Comparative methaemoglobin concentrations of three erythrocyte genotypes (HbAA, HbAS and HbSS) of male participants administered with five antimalarial drugs. *Afr J Biochem Res*. 2009;3(6):266-71.
- Chikezie PC. Glutathione S-transferase activity of human erythrocytes incubated in aqueous solution of five antimalarial drugs. *Free Rad Antiox*. 2011;1(2):25-9.
- Kalra VK, Sikka SC, Sethi GS. Transport of amino acids in gamma-glutamyl transpeptidase-implanted human erythrocytes. *J Biol Chem*. 1981;256(11):5567-71.
- Galbraith DA, Watts DC. Changes in some cytoplasmic enzymes from red cells fractionated into age groups by centrifugation in Ficoll/Triosil gradients. Comparison of normal humans and patients with Duchenne muscular dystrophy. *Biochem J*. 1980;191(1):63-70.
- Kamber E, Poyiagi A, Deliconstantinos G. Modifications in the activities of membrane-bound enzymes during in vivo ageing of human and rabbit erythrocytes. *Comp Biochem Physiol B*. 1984;77(1):95-9.
- Baure JD. Laboratory investigation of hemoglobin. In: Sonnenwirth AC, Jarett L editors. *Gradwohl's Clinical Laboratory Methods and Diagnosis*. St Louis: Mosby; 1980. pp. 809-902.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The

- first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974;**249**(22):7130-9.
29. Pasupathi P, Chandrasekar V, Kumar US. Evaluation of oxidative stress, antioxidant and thyroid hormone status in patients with diabetes mellitus. *J Med.* 2009;**10**:60-6.
 30. Romeu M, Nogues R, Marcas L, Sanchez-Martos V, Mulero M, Martinez-Vea A, et al. Evaluation of oxidative stress biomarkers in patients with chronic renal failure: a case control study. *BMC Res Notes.* 2010;**3**:20.
 31. Goodrich JM, Basu N. Variants of glutathione s-transferase pi 1 exhibit differential enzymatic activity and inhibition by heavy metals. *Toxicol In Vitro.* 2012;**26**(4):630-5.
 32. Comakli V, Ciftci M, Kufrevioglu O. Purification of glutathione S-transferase enzyme from rainbow trout erythrocytes and examination of the effects of certain antibiotics on enzyme activity. *Hacetatepe J Biol Chem.* 2011;**39**(4):413-419.
 33. Domingues I, Agra AR, Monaghan K, Soares AM, Nogueira AJ. Cholinesterase and glutathione-S-transferase activities in freshwater invertebrates as biomarkers to assess pesticide contamination. *Environ Toxicol Chem.* 2010;**29**(1):5-18.
 34. Jemec A, Drobne D, Tisler T, Sepcic K. Biochemical biomarkers in environmental studies—lessons learnt from enzymes catalase, glutathione S-transferase and cholinesterase in two crustacean species. *Environ Sci Pollut Res Int.* 2010;**17**(3):571-81.
 35. Valon M, Valbona A, Fahri G, Qenan M, Dhurat K, Fatmir C. Evaluating Environmental Pollution by Applying Oxidative Stress Biomarkers as Bioindicators of Water Pollution in Fish. *Pol J Environ Stud.* 2013;**22**(5):1519-23.
 36. Ayalogu OE, Igboh NM, Dede EB. Biochemical Changes in the Serum and Liver of albino rats exposed to Petroleum Samples (gasoline, kerosene, and crude Petroleum). *J Appl Sci Environ Manag.* 2001;**5**(1):97-100.
 37. Chiarpotto E, Biasi F, Scavazza A, Camandola S, Aragno M, Tamagno E, et al. Acetaldehyde involvement in ethanol-induced potentiation of rat hepatocyte damage due to the carcinogen 1,2-dibromoethane. *Alcohol Alcohol.* 1995;**30**(6):721-8.
 38. Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol Res.* 1998;**38**(4):297-303.
 39. Anosike EO, Uwakwe AA, Monanu MO, Ekeke GI. Studies on human erythrocyte glutathione-S-transferase from HbAA, HbAS and HbSS subjects. *Biomed Biochim Acta.* 1991;**50**(9):1051-6.
 40. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. *J Toxicol.* 2011;**2011**:487074.