JASEM ISSN 1119-8362
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J. Appl. Sci. Environ. Mgt. 2003
Vol. 7 (2) 45 - 48

# Activation of human erythrocyte glutathione – s – transferase (EC. 2.5.1.18) by caffeine (1, 3, 7 – trimethylxanthine)

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**ABSTRACT:** Caffeine (1,3,7 – trimethylxanthine) was extracted from Nescafe ® (product number CC5AA) a brand of "instant coffees" produced by Nestle foods, Lagos, Nigeria, using Harris method adapted from FDALS (1982) and confirmed by Wagenar test (Arnand, 1984). Various concentrations (5.0µM, 10.0µM, 15.0µM, 20.0µM, 25.0µM and 50.0µM) of the isolated caffeine were tested *in-vitro* on their possible effect on human erythrocyte (red cell) glutathione – S – transferase (EC. 2.5.1.18) activity. The result indicated significant ($P < 0.05$) activation of the erythrocyte enzyme (GST) by caffeine in a concentration – dependent manner i.e. higher concentrations produced greater activation of the enzyme. For instance at caffeine concentrations of 5.0µM, 25.0µM and 50.0µM, red cell GST activity was increased by 1.99 folds (299.43%), 4.99 folds (598.85%) and 6.98 folds (798.28%) respectively. These results may point to a possible binding – deterioration of caffeine by the human erythrocyte GST @ JASEM

Caffeine (1,3,7 – trimethylxanthine – figure 1) is an alkaloid of pharmacological importance with wide spread usage in drugs, drinks and beverages. This usage is based on its stimulatory properties and the slight degree of bitterness that it impacts (Harold, 1992).

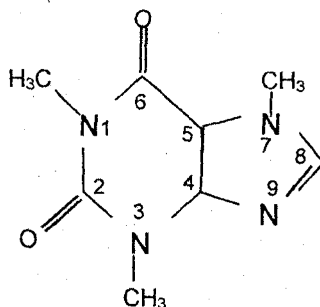


**Fig. 1**: Caffeine (1, 3, 7 – trimethylxanthine)

Caffeine (Mwt 194.2g/mol) was first isolated from coffee in 1920 (Arnand, 1984). It was subsequently identified in cocoa, mate, kola nuts and other plants. Total synthesis of caffeine was first achieved in 1895 starting with dimethylurea. Dimethyluric acid, chlorotheophyline and chlorocaffeine were intermediates (fischer and Ach, 1895). Anhydrous caffeine obtained by crystallization from non-aqueous solvents is a white crystalline substance that melts at 235 – 237°C. It begins to sublime without decomposition at 120°C at atmospheric pressure and at 80°C under high vacuum. When crystallized from water, the monohydrate forms long, silky, white needless which become anhydrous at 80°C. Caffeine is soluble to the extent of 0.6% in water at O°C, 2.13% at 25°C and 66.7% at 100°C. The pH of dilute solutions is 6.9. (Thakkar, 1970). The caffeine molecule is sufficiently hydrophobic to pass freely through biological membranes. It is completely absorbed from the gastrointestinal tract and rapidly attains peak plasma levels; Lethal plasma concentration is in the range of 0.5 – 1.0mM (Von-Borstel, 1983). Aside from its effect on the cardiovascular, neurological and renal systems (Von-Borstel and Wurtman, 1984), caffeine produces a mild diuresis in humans and increases the excretion of sodium, potassium, and chloride ions (Arnand, 1984). The effects of caffeine on the cardiovascular, neurological and renal systems and on lipolysis are primarily triggered by the blockage of adenosine receptors through competitive antagonism (Von-Borstel and Wurtman, 1984). Caffeine has also been reported to be an inhibitor of the enzyme, phosphodiesterase (Harold 1992). However, the effect of caffeine on some other key metabolic enzymes such as the red cell glutathione-S-transferase (GST), remained speculative. Glutathione–S–transferases (EC. 2.5.1.18) are a group of enzymes, which use reduced glutathione (γ-glutamyl, cysteinyl-glycine-GSH) and a wide variety of hydrophobic compounds as substrates (Jacoby, 1978). They are present in rats and human liver, pigeon, locust gut, housefly and other sources (Ketley *et al*, 1975). Functionally, the glutathione-S-transferases catalyse the conjugation of electrophilic groups of hydrophobic drugs and xenobiotics to form glutathione thiol esters. The thiol esters are in turn converted to mercapturic acid following a sequential action of gamma (γ) – glutamyl – transpeptidase, dipeptidase and acetylase (Boyland and Chasseaud, 1969). Glutathione-S-transferases help to detoxify certain extremely reactive substances by direct covalent binding of the electrophilic agent to protein (Jacoby, 1978). Thus GSTs protect cellular constituents from electrophiles and toxic xenobiotics.

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The location of GST in erythrocytes is ideal for the removal of circulating xenobiotics (Marcus *et al* 1978). The red cell GST also functions physiologically as a haemin-binding and/or transport protein in developing erythroid cells (Keilin, 1960). It has, however, been suggested that the occurrence of GST in the erythrocytes is primarily for the protection of erythrocytes against electrophilic compounds rather than serving a general protective function in the body (Harvey and Beutler, 1987). Recently, the rat erythrocyte enzyme (GST) was found to be inhibited *in-vitro*, by the antimalaria, alkaloid drugs, chloroquine and fansidar (sulfadoxine + pyrimethamine); an effect that was interpreted to mean a possibility of these drugs being capable of raising the oxidant stress of the erythrocytes (Ayalogu *et al* 2000). The present study is to examine and/or elucidate, *in-vitro*, the possible effect of caffeine (1,3,7 – trimethylxanthine) another alkaloid xenobiotic, on human erythrocyte glutathione-S-transferase activity. The study was necessitated by (1) the documented important role of this enzyme in the functional integrity of the human erythrocyte and (2) the frequency and wide spread consumption of caffeine without recourse to its possible negative import on the oxygen function of human erythrocyte.

## MATERIALS AND METHODS

*Caffeine:* This was extracted from Nescafe ® (product number CC5AA) a brand of "instant coffees" produced by Nestle foods, Lagos, Nigeria, using Harris method adapted from FDALS (1982) and confirmed by Wagenar test (Arnand, 1984).Other chemicals used were from BDH and M&B, London.

*Blood Sample:* Blood samples were collected from ten (10) healthy volunteers of ages 18 – 25 years and of both sexes (6 males and 4 females), into citrate anticoagulant tubes. Erythrocytes were isolated from the blood samples by centrifugation at 10,000g for fifteen minutes using bench centrifuge (MSE minor). Following careful siphoning of the plasma (with a pasteur pipette), the erythrocytes were washed thrice with a stabilizing solution (2.7mM EDTA, 0.7mM 2-mercaptoethanol, pH 7.0) as described by Beutler (1984). The samples were then frozen and thawed for immediate use. Portions (0.02ml) of the prepared samples (haemolysates) were made use of for the determination of haemoglobin concentration of haemolysate, using Drabkin's solution (Drabkin and Austin, 1935, Van-kampen and Zijlstra, 1961).

*Enzyme assay:* Glutathione-S-transferase was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with glutathione (GSH) at 340nm at 37°C (Habig *et al* 1974). The 3ml assay mixture contained 0.5mM CDNB, 1mM GSH and 100mM phosphate buffer, pH 6.5. The CDNB was dissolved in ethanol and added to the phosphate buffer before use. The ethanol concentration in the assay mixture was 2%. The phosphate buffer-CDNB mixture was preincubated for 10mins at 37°C and the reaction was started by adding GSH followed immediately by an aliquot (0.15ml) of the haemolysate). The rate of increase in absorbance at 340nm was measured for 10mins at 37°C against a blank containing the reaction mixture without haemolysate.

*Effect of Caffeine on red cell GST activity:* Human erythrocyte (red cell) GST activity was determined in the presence of varied concentrations (5.0μM, 10.0μM, 10.0μM, 20.0μM, 25.0μM and 50.0μM) of caffeine.

Caffeine (1, 3, 7 – trimethylxanthine) was dissolved in the phosphate buffer prior to the assay.

*Statistical Analysis:* Student's t-test of statistical significance (Brokes *et al* 1979) was used to analyse the resultant data for statistical significance. Data were also analyzed by one-way analysis of variance (ANOVA) using Duncan's (1955) multiple range test.

## RESULTS

The activity of human erythrocyte glutathione –S–transferase, as determined for 10 samples, increased significantly ($P<0.05$) in a concentration – dependent manner in the presence of caffeine (at micromolar concentration range) (Table 1). For instance, at concentrations of 5.0μM, 25.0μM and 50.0μM, caffeine activated human red cell GST by 1.99 folds (299.43%) 4.99 folds (598.85%) and 6.98 folds (798.28%) respectively.

**Table 1**: Effect of Caffeine (CF) on red cell glutathione-S-transferase activity (E) at 37°C, pH 6.5.

| [CF] μM | E(μ/L) x± SD; n=10 | % E<br>x, n=10 | Fold increase in E<br>x; n = 10 |
|---|---|---|---|
| *0.0 | 1.74 ± 0.00$^a$ | 100.00$^a$ | – 0.00$^a$ |
| 5.0 | 5.21± 0.02$^b$ | 299.43$^b$ | 1.99$^b$ |
| 10.0 | 6.94 ± 0.04$^c$ | 398.85$^c$ | 2.99$^c$ |
| 15.0 | 7.95 ± 0.03$^d$ | 456.90$^d$ | 3.57$^d$ |
| 20.0 | 9.02 ± 0.05$^e$ | 518.39$^e$ | 4.18$^e$ |
| 25.0 | 10.42 ± 0.03$^f$ | 598.85$^f$ | 4.99$^f$ |
| 50.0 | 13.89 ± 0.05$^g$ | 798.28$^g$ | 6.98$^g$ |

- *Control*

*Values on the same column having different superscripts are statistically significant at 95% confidence level (P = 0.05).*

## DISCUSSION

Quite some work has been done on the properties and functions of liver and kidney forms of glutathione-S-transferase (GST) in both experimental animals and man (Boyland and Chasseand, 1969; Ketley *et al*, 1975; Awasthi *et al*, 1981; Harvey and Beutler, 1982). However, the physiological role of this enzyme in the human erythrocyte is not yet fully and conclusively defined. It has been suggested that red cell glutathione-S-transferase functions intracellularly to prevent superoxide-induced haemolysis in addition to curbing the toxic effect of red cell electrophiles/oxidants (Anosike *et al*, 1991). The present study has revealed significant (P<0.05) *in-vitro* activation of the human erythrocyte GST by caffeine (1, 3, 7 – trimethylxanthine) in the micromolar concentration range (5.0 to 50.0μM) and in a concentration-dependent manner (i.e. higher concentrations of caffeine produced greater activation of the enzyme). The activation of this erythrocyte enzyme (GST) by caffeine, could suggest a possibility of this alkaloid (caffeine) to raise the oxidant stress of the red cells in the course of their targeted therapeutic actions. In other words, this alkaloid could either be acting as an electrophile/oxidant or be involved in the direct generation of red cell electrophiles with the resultant increase in erythrocyte GST activity. This possibility agrees with the suggestion of Anosike *et al* (1991) that red cell GST functions intracellularly to prevent superoxide-induced haemolysis as well as the toxic effects of electrophiles. It is also in agreement with the suggested role of red cell GST in the protection of cellular constitutents from xenobiotics (Boyland and Chasseand, 1969). Caffeine, incidentally, is a xenobiotic compound. In his work, Jacoby (1978) suggested that the glutathione-S-transferases help to detoxify certain extremely reactive substances by direct covalent binding of the electrophilic agent to protein. It could therefore be concluded that human erythrocyte GST is involved in the binding detoxification of the alkaloid caffeine as a way of shielding red cell components/metabolic system from any deleterious effect of the alkaloid (caffeine). On the other hand, caffeine could be acting as an allosteric activator of the enzyme (GST) and hence the observed increase in the enzyme's activity. These speculations are however subject to further experimental confirmations which were not possible during this study.

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