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In Vitro Kinetic of Glutathione-S-Transferase Enzyme in (Buffalo, Camel, Sheep, Goat, and Cattle) and Inhibition by Aspirin and Paracetamol

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Abstract

Enzyme kinetic showed competitive inhibition pathways for both aspirin and paracetamol. The activity of glutathione-s-transferase of RBCs (buffalo, camel, sheep, goat, and cattle) was measured by the photometric method in the presence and absence of two NSDI analgesic drugs (aspirin and paracetamol). Two different concentrations (10 and 20 mM) of each analgesic drug were used, which covered the reported therapeutic index and toxic concentration range of the drugs. At a level of significance ($p < 0.05$)

Aim of the study
In vitro kinetic of (buffalo, camel, sheep, goat, and cattle) RBCs glutathione S-transferase enzyme and effective competitive inhibition of analgesic drugs aspirin and paracetamol.

Keywords: Aspirin, Paracetamol, RBCs, Glutathione -S-transferase.

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Introduction

The superfamily of glutathione transferases (GSTs) is composed of multifunctional proteins widely distributed in nature in both eukaryotes and prokaryotes [1,2,3,4,5]. In eukaryotes, GSTs are divided according to their cellular localization into at least three major families of proteins, namely cytosolic GSTs, mitochondrial GSTs and microsomal GSTs [1,2,6]. Cytosolic GSTs are widely distributed and divided into several major classes based on their chemical, physical and structural properties. Mitochondrial GSTs are also known as kappa class GSTs and are soluble enzymes which bear structural similarities with cytosolic GSTs. On the contrary, microsomal GSTs, also known as MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism), are integral membrane proteins not evolutionaryly related to the other major classes. Aspirin, also known as acetylsalicylic acid (ASA), is a nonsteroidal anti-inflammatory drug (NSAID) used to reduce pain, fever, and/or inflammation and as an antithrombotic.[7] Aspirin treats specific inflammatory conditions, including Kawasaki disease, pericarditis, and rheumatic fever.[7]

The medium lethal dose of aspirin is between 0.2 to 0.5g/kg body weight. Aspirin has many other pharmacology-therapeutic uses. In therapeutic concentrations, aspirin inhibits the uncoupled oxidative phosphorylation in the mitochondria. More than in high doses, they could inhibit intracellular oxidative enzymes, inhibit the activation of kinin synthesis enzymes, and antagonize the pharmacological effects of kinins [8].

Paracetamol is acetaminophen with a molecular weight of 151.16 Daltons. It is an analgesic and antipyretic, similar to aspirin, but it has no anti-rheumatic activity [8]. In addition, because of its absence of gastrointestinal tract irritation side effects, paracetamol is more suitable than aspirin for patients suffering from peptic ulcers or having sensitive reactions to aspirin [9].
Paracetamol's toxic plasma concentration has been reported at about 10mg /ml [9]. It can damage the liver, kidney, heart, and central nervous system. Liver damage shows within hours because of the accumulation of toxic metabolic substances and the depletion of the detoxifying agent glutathione from the liver [9, 10].

The effect of paracetamol reported by the decrease of hepatic glutathione concentration [9, 10] This study aims to report the effects of these analgesics on RBC glutathione S-transferase, a notable detoxifying enzyme that has glutathione (GSH) as its substrate [11].

Materials and Methods

Sample collection and preparation

Blood samples were collected from healthy animals (buffalo, camel, sheep, goat, and cattle) in the Al-Najaf slaughterhouse and put into EDTA anticoagulant tubes. RBCs were isolated from the whole blood by washing with normal saline and diluted 1:20. The hemolysates were pooled to assay.

Enzyme assay

Glutathione-S-transferase was kinetic assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB (Sigma, U.S.A).) with glutathione (GSH) (Sigma, U.S.A). [12].
The 1 ml assay mixture contained 0.5 mM CDNB, 1 mM GSH, and 100 mM potassium phosphate buffer, pH 6.5.
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 pre-incubated for 10 min at 37 ˚C, and the reaction was initiated by the addition of GSH, followed immediately by an aliquot (0.05ml) of the haemolysate. The rate of increase in absorbance at 340nm by using a semi-automatic chemistry analyzer (CYAN, Belgium) was measured for 8 min at 37˚C against a blank containing the reaction mixture without haemolysate [12].

Enzyme inhibition by Aspirin and Paracetamol

Glutathione-S-transferase activity was determined in the presence of two concentrations of aspirin (10 and 20 mM). Similar determinations by using two concentrations of paracetamol (10, and 20 mM). The drugs were dissolved in the 100mM phosphate buffer, pH 6.5.

Statistical Analysis

All data parameters were calculated as average to the baseline values and were expressed as mean ± standard error. Values were statistically analyzed by One-way ANOVA Values of $P<0.05$ [13].

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Results

The activity normal of RBCs (buffalo, camel, sheep, goat, and cattle) glutathione-S-transferase (GST) enzyme, Tables 1, Chart 1

Table (1) Normal RBCs glutathione-S-transferase mM

animals	RBCs glutathione-S-transferase mM (Mean ± S.E.)	
Buffalo	0.88333	± 0.001
Camel	0.9667	± 0.01
Goat	0.96667	± 0.002
Sheep	2.5*	± 0.003
Cattle	2.74667*	± 0.002

*Significantly different from the respective between groups $p<0.05$

The decreased notably while increasing concentrations of aspirin on the activity of RBCs (buffalo, camel, sheep, goat, and cattle) glutathione-S-transferase (GST) enzyme compared with normal activity, Tables 2, Chart1.

Table (2) level of RBCs glutathione-S-transferase mM inhibited by aspirin

animals	RBCs glutathione-S-transferase nM inhibited by aspirin 10 mM (Mean ± S.E.)		RBCs glutathione-S-transferase nM inhibited by aspirin 20 mM (Mean ± S.E.)	
Buffalo	0.13	± 0.021	0.05667	± 0.02
Camel	0.88333	± 0.011	0.6667	± 0.023
Goat	0.75333	± 0.032	0.697	± 0.05
Sheep	0.13*	± 0.043	0.26667*	± 0.04
Cattle	0.75333*	± 0.012	0.70333*	± 0.08

*Significantly different from the respective with table (1) same group value $p<0.05$

The decreased notably while increasing concentrations of paracetamol on the activity of RBCs (buffalo, camel, sheep, goat, and cattle) glutathione-S-transferase (GST) enzyme compared with normal activity, Tables 3, Chart 1.

Table (3) level of RBCs glutathione-S-transferase mM inhibited by paracetamol

animals	RBCs glutathione-S-transferase nM inhibited by paracetamol 10 mM (Mean ± S.E.)		RBCs glutathione-S-transferase nM inhibited by paracetamol 20 mM (Mean ± S.E.)	
Buffalo	0.13	± 0.021	0.05667	± 0.02
Camel	0.88333	± 0.011	0.26667	± 0.023
Goat	0.75333	± 0.032	0.697	± 0.05
Sheep	0.13*	± 0.043	0.26667*	± 0.04
Cattle	0.75333*	± 0.012	0.70333*	± 0.08

*Significantly different from the respective with table (1) same group value $p<0.05$

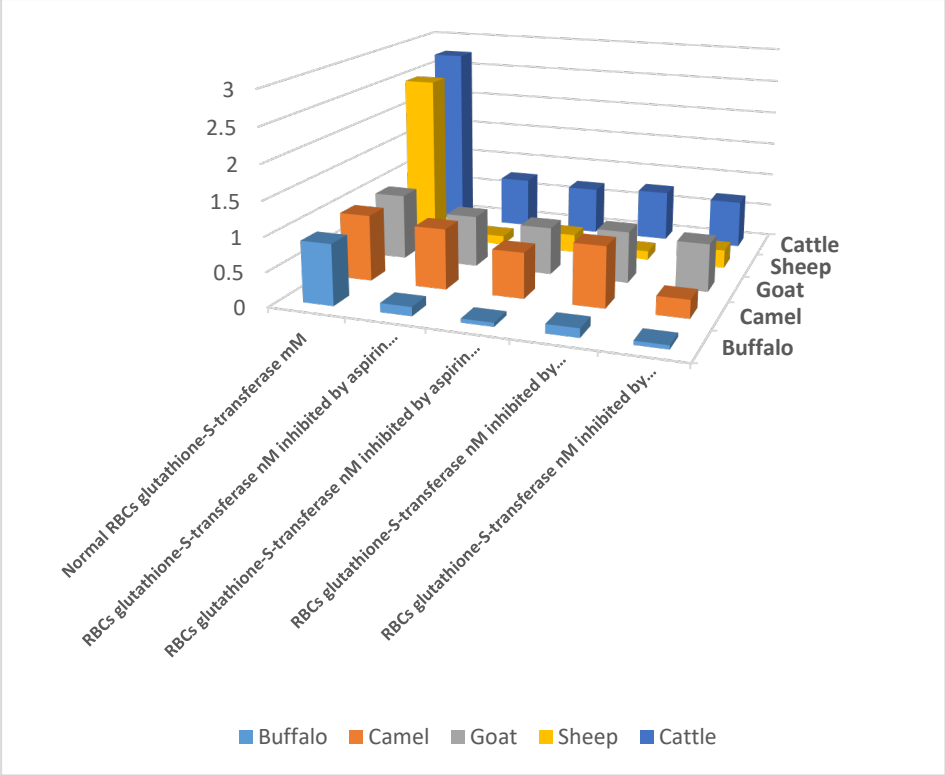

Chart (1) the *in vitro* kinetic of RBCs GST enzyme and inhibition with aspirin and paracetamol.

Discussion

Aspirin and paracetamol are the most common two analgesics used in the treatment of fever, inflammatory reactions and pains; these drugs are among the drugs most often abused in their usage without knowing it is their possible chemical and pharmacological side effects. The present in vitro study has induced an inhibitory effect of those drugs on (buffalo, camel, sheep, goat, and cattle) RBCs glutathione S-transferase (GST) [8].

Glutathione-S-transferases are a family of proteins having multiple detoxification functions [11]. They are catalyzing the reaction of glutathione (a tripeptide) with a large group of hydrophobic substances (mostly with xenobiotics) having an electrophilic centre [11,14].

RBCs GST were reported to have two major functions. The first function is binding or transporting protein [3], and the second protects cellular compounds from hydrophobic xenobiotic chemicals [15].

This study noted inhibition of this enzyme would induce a disturbance in RBCs by that increasing exposure to aspirin and paracetamol [10]

This could be referred to the hepatic damage caused by these drugs by reducing cellular GSH concentrations [10, 11], showing a competitive inhibition of the GSH enzyme by the analgesic drugs aspirin and paracetamol while increasing the inhibition rate by increasing the analgesic drugs aspirin and paracetamol concentration [17]

The administration of analgesic drugs aspirin and paracetamol has been done under precaution to avoid the degenerative effect on the RBCs [10].

Conclusion

The administration of analgesic drugs such as aspirin and paracetamol have an effective competitive inhibition on (buffalo, camel, sheep, goat, and cattle) RBCs glutathione S-transferase enzyme**.**

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