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The protective effect of caffeic acid and sesamol on human erythrocytes against hemin-induced hemolysis

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ABSTRACT

Hemin is a potential hemolytic agent which can induce hemolysis by accelerating the potassium leakage, dissociating erythrocyte membrane skeletal proteins and inhibiting a number of erythrocyte enzymes. The major aim of this work is to investigate the protective effect of caffeic acid and sesamol on hemin induced hemolysis of human erythrocytes. Hemin could induce hemolysis but did not elicit lipid peroxidation in human erythrocytes. Caffeic acid and sesamol offered significant protection to human erythrocytes against hemin induced hemolysis in in-vitro experimental system. Our results suggest that caffeic acid and sesamol inhibit hemin induced hemolysis by interference with heme incorporation into membrane and not by lipid peroxidation to form hemolytic holes.

Keywords: Caffeic acid, Flavone, Hemin, Hemolysis, Sesamol.

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INTRODUCTION

Heme (iron protoporphyrin ix) is the prosthetic group of several heme proteins like hemoglobin, myoglobin, cytochromes, NO synthase, catalase and peroxidases [1-4]. However, hemoglobin and myoglobin account for the major part of heme in the body. Heme is regularly released from degraded globins and in some pathological conditions such as hemoglobinopathies, oxidative stress and glucose-6-phosphate dehydrogenase deficiency [5-7]. Free heme is fairly hydrophobic and exerts toxic effects on cells by intercalating into lipid bilayer structure of membranes of erythrocytes [5, 8] and endothelial cells [9]. In erythrocytes, hemin induces potassium leakage [8] by colloid osmotic mechanism, causes dissociation of membrane skeletal proteins and inhibits several enzymes [10,11]. Heme oxygenase degrades heme and significantly reduces oxidative damage [12,13]. Additional protection against free hemin - mediated cell injury is made possible in vivo by albumin, haptoglobin, hemopexin, HDL and LDL which trap heme in plasma and ensure its complete clearance [14]. Vitamin E protects red cells against heme induced hemolysis by membrane stabilization [15]. Glutathione (GSH), a cellular antioxidant, inhibits hemin-induced hemolysis by promoting hemin degradation [16]. Desferrioxamine inhibits the hemolysis by blocking interaction of heme with red cell membrane [17].

Sesamol (3, 4-methylenedioxyphenol) is a constituent of roasted sesame having antioxidant properties due to phenolic and benzodioxole groups in its structure. It is slightly soluble in water but fairly soluble in oils. Caffeic acid (3, 4-dihydroxycinnamic acid) is a phenolic phytochemical present in many foods. It is a water soluble antioxidant which offers a number of health benefits. Since hemin monomers promote lipid peroxidation in biomembranes, it was of interest to find out whether sesamol and caffeic acid protect red cell membranes against oxidative damage and lysis.

MATERIAL AND METHODS

Chemicals

Hemin, Glutathione (GSH), caffeic acid, sesamol and flavone (2-phenylchromone) were obtained from M/S Sigma chemicals (Sigma, St. Louis, USA). Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Phosphate Buffered Saline (PBS) were purchased from Ashwini chemical, (Hyderabad, India). All other chemicals were of analytical grade.

Materials

Blood was drawn from apparently healthy individuals into heparin tubes after taking informed consent. Blood samples were centrifuged at 2000 x g for 10 minutes at 4^0C. The plasma was removed and packed erythrocytes were washed 3 times with PBS buffer (10mM Sodium phosphate, 135mM NaCl, pH 7.4). The buffy coat of white cells was removed.

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Erythrocyte membranes were prepared from the reaction mixtures according to the method reported by Hanahan and Ekholm.

Hemin was prepared fresh at the beginning of each experiment as 1mM stock in 5mM NaOH and stored in dark at 4^0C. Hemin concentration was determined using a millimolar extinction coefficient of 64.1 at 365nm.

Hemolysis assay

Suspensions of 1% erythrocytes were incubated with various reagents. One mL aliquots were removed and centrifuged for 3 minutes at 3000 g. The degree of hemolysis was determined from the absorbance of hemoglobin at 540nm in the supernatant. Absorbance corresponding to 100% hemolysis was determined by adding 10µL of Triton X-100 to 1 mL of erythrocyte suspension.

Measurement of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS assays were done as described by Stock and Dormandy [18]. The TBARS was determined as absorbance at 532nm with quantitation based upon a molar extinction coefficient of 1.56×10^5

RESULTS AND DISCUSSION

We have recently reported that several thiol cmpounds degrade heme in-vitro. Despite the existence of several heme detoxication systems in the body, it is desirable to explore novel therapeutic agents for protection against heme toxicity. Caffeic acid and sesamol are naturally occuring antioxidants available in food materials.They also differ in their hydrophobicity. Our results show that they offer protection against heme induced red cell hemolysis.

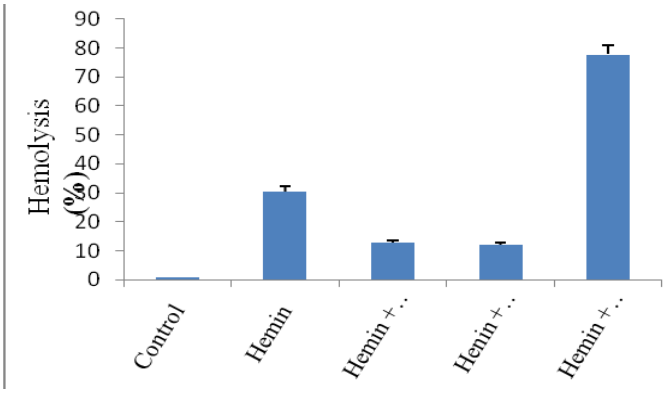

Fig. 1: Hemin induced hemolysis: Reaction mixture containing hemin (20µM), 250 µM caffeic acid, sesamol and flavone respectively, was incubated at 37^oC for 60 minutes. Hemolysis was determined by measuring the absorbance at 540 nm. Data represent mean ± SD of 3 independent experiments.

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The results in Fig. 1 show that caffeic acid and sesamol inhibit hemin-induced red cell hemolysis. In contrast, flavone, also a naturally occuring antioxidant, enhanced hemin mediated hemolysis substantially. Such observations underline the need for more comprehensive study of other similar compounds for their therapeutic suitability and efficiency.

Sesamol is reported to inhibit oxidative hemolysis brought about by free radicals generated by 2, 2′-Azo-bis (2-amidinopropane) dihydrochloride ex Vivo [19]. The protective effect of sesamol and caffeic acid appears to be unrelated to their antioxidant properties.The results in Fig. 2 show that the formation of TBARS is unaffected when RBC are incubated with hemin, hemin plus caffeic acid and hemin plus sesamol. Inhibition of heme induced hemolysis by sesamol and caffeic acid increased with increasing concentration upto 0.25mM. Fig. 3 shows the dose dependence of the compounds on hemin mediated hemolysis.

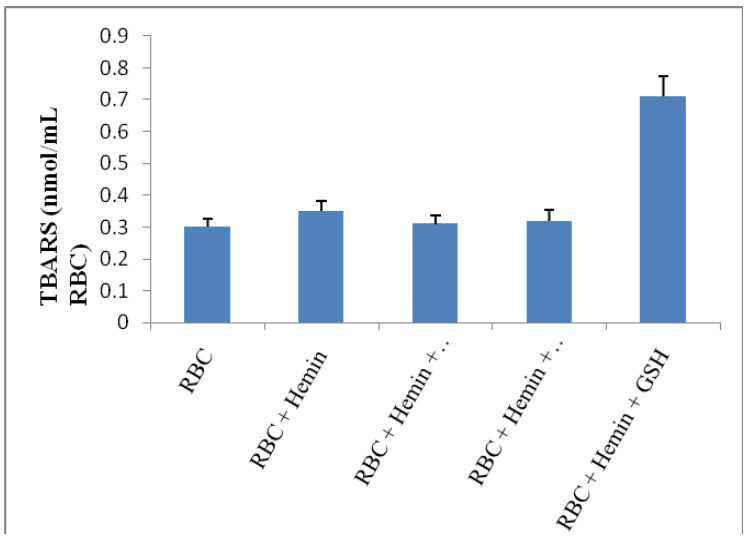

Fig. 2: Caffeic acid and sesamol have no effect on the formation of TBARS. Erythrocyte suspension (2.5%) were incubated with 40 µM hemin in the presence or absence of 250 µM caffeic acid or 250 µM sesamol or 2 mM GSH at 37 ^{0}C for 60 minutes. TBARS was determined as absorbance at 532 nm with quantification. Data represent mean ± SD of 3 independent experiments.

ISSN: 0975-8585

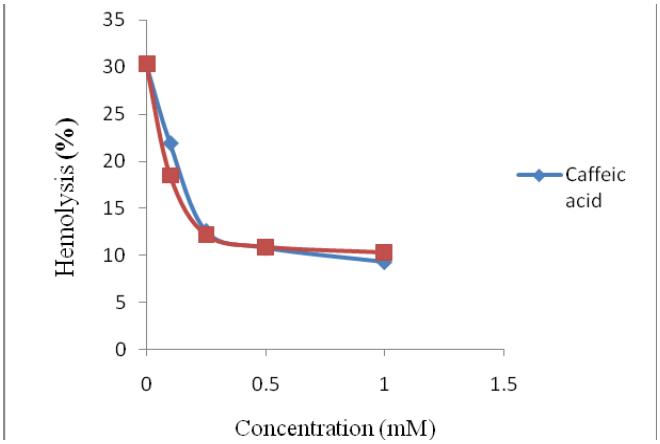

Fig. 3: Caffeic acid and sesamol inhibits hemolysis induced by hemin. Erythrocyte suspension (1%) was incubated with caffeic acid or sesamol at 37°C for 60 minutes, and then with 20 µM hemin for 60 minutes. Hemolysis was determined by measuring the absorbance at 540 nm. Data represent mean ± SD of 3 independent experiments.

Ingestion of diets containing sesame has been shown to increase the time for erythrocyte hemolysis [20]. This is due to quenching of free radicals which attack membrane components to increase fragility and breakdown.

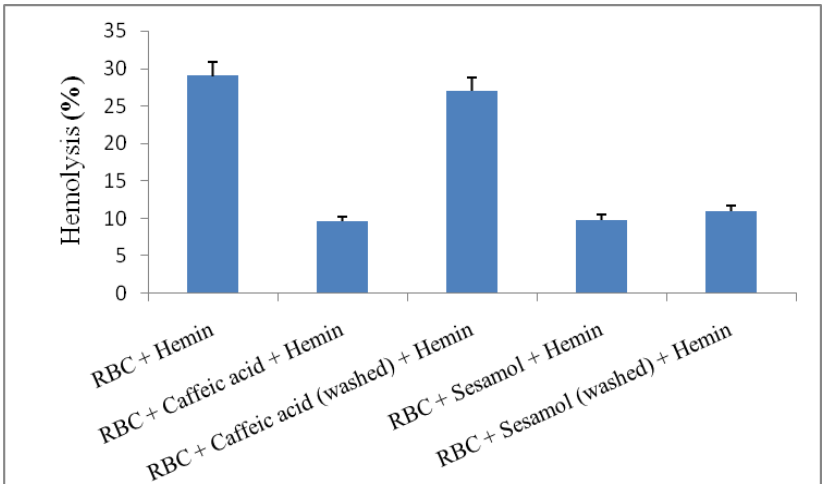

Fig. 4: Erythrocyte suspension (1%) was incubated with 250 µM Caffeic acid or Sesamol at 37°C for 60 minutes, and then washed with PBS buffer. Then incubated with 20 µM hemin for 60 minutes. Hemolysis was determined by measuring the absorbance at 540 nm. Data represent mean ± SD of 3 independent experiments.

Membrane stabilising and free radical scavenging property of caffeic acid are also reported by Kumaran K S and Manizen P.S [21]. Martin J.et al., showed that caffeic acid delays hemolysis of erythrocytes and partially prevents membrane loss of sulfhydryl goups in ghosts [22]. Antioxidant activity of caffeic acid, ferulic acid and related compounds on erythrocyte and microsomal membranes depends not only on the -OH groups or catechol groups but also on polarity and hydrophobicity of the compounds [23]. When erythrocytes were washed after preincubation with caffeic acid or sesamol, the protection against hemin induced hemolysis was

still maintained for sesamol and it exhibited nearly the same inhibitory effect as in unwashed erythrocytes whereas caffeic acid did not exhibit inhibitory effect in washed erythrocytes (Fig. 4). This indicates the relevance of the polarity of the compounds. Caffeic acid incorporated into membrane is removed by washing due to its hydrophilicity where as sesamol is not washed out due to its hydrophobicity. These compounds appear to prevent incorporation and intercalation of heme with erythrocyte membrane. Vitamin E is reported to protect red cells against heme induced hemolysis by membrane stabilization [15] and such possibility with caffeic acid and sesamolin needs to be explored.

CONCLUSION

There are several phytochemicals in vegetarian food materials which possess therapeutic potential. Sesamol and caffeic acid are common food constituents with well known antioxidant properties. Our results demonstrate the anti-hemolytic activity of these compounds in addition to their known antioxidant activity. The exact mechanism of their effect requires further studies.

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