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Oxidative damage of plasma proteins and lipids in epidemic dropsy patients: Alterations in antioxidant status☆

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Abstract

Epidemic dropsy is an acute food adulterant disease caused due to consumption of edible mustard oil contaminated with argemone oil. Our in vitro studies have shown that the toxicity of argemone oil is due to the production of reactive oxygen species. The present study was aimed to evaluate the development of oxidative stress in terms of oxidation of plasma proteins and lipids and its correlation to enzymatic and non-enzymatic antioxidants in epidemic dropsy patients. Total plasma protein and globulin contents were found to be significantly ($P<0.05$) enhanced with a concomitant decrease ($P<0.05$) in albumin/globulin ratio in dropsy patients when compared to controls. Total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol were found to be significantly ($P<0.05$) increased with a simultaneous decrease (51%) in high density lipoprotein cholesterol in dropsy patients. The oxidation of plasma proteins and lipids were substantially enhanced (162–175%) in dropsy patients when compared to controls. Further, significant ($P<0.05$) decrease in superoxide dismutase, catalase, glutathione reductase and glutathione-*s*-transferase with a concomitant increase (69%) in glutathione peroxidase activity was noticed in dropsy patients. A significant reduction in plasma total antioxidant capacity, α-tocopherol, glutathione, retinol and retinyl esters content was observed in dropsy patients when compared to healthy controls. The results suggest that there exists an unproportionate equilibrium between free radicals formation and enzymatic and non-enzymatic antioxidant scavengers, which may cause oxidative damage to proteins and lipids in dropsy patients.

Keywords: Epidemic dropsy; Argemone oil; Free radical; Oxidative stress; Lipid peroxidation; Protein carbonyl group; Total antioxidant capacity; Antioxidant enzyme and non-enzymatic antioxidant

1. Introduction

Epidemic dropsy is an acute food adulterant disease caused due to the consumption of edible mustard oil contaminated with argemone oil [1]. Due to the uncanny resemblance of mustard seeds and argemone seeds (*Argemone mexicana* sp) the contamination may occur accidentally or deliberately for economic gains [2]. Argemone oil poisoning was first reported from Calcutta in 1877. Since then, several outbreaks have occurred in different cities of India as well as in Mauritius, Fiji Island, South Africa and Madagascar [2,3]. The epidemic in 1998 at Delhi, India, is an eye opener and possibly the largest so far in which over 60 persons lost their lives and more than 3000 victims were hospitalized [3,4]. The symptoms of dropsy include nausea, vomiting, diarrhoea, anorexia, loss of taste, headache, fever, dyspnea, palpitation, hyperpigmentation of body parts, burning sensation of eyes, bilateral pitting oedema of lower limbs, erythema, breathlessness, tachycardia, hepatomegaly, crepitations in the lungs and gallop rhythm. In severe cases, glaucoma and even death due to cardiac and respiratory failure have been reported [2,3].

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The toxicity of argemone oil has been attributed to its physiologically active benzophenanthridine alkaloids, sanguinarine and dihydrosanguinarine [5]. Both are interconvertible by simple oxidation and reduction process. Histopathological lesions have been reported in the liver, lungs, kidney and heart by argemone oil intoxication in rats thus suggesting these to be the target sites [6]. In vitro studies have shown that the toxicity of argemone oil is due to the production of reactive oxygen species (ROS) [7–9] which in turn may cause enhancement in lipid peroxidation (LPO) in various hepatic subcellular fractions including microsomes and mitochondria of rats [10]. The damage in hepatic microsomal membrane causes loss of cytochrome P-450 and its dependent membrane bound enzymes responsible for xenobiotic metabolism thereby leading in a delay of sanguinarine excretion [11,12]. Benzacridine is one of the metabolite of sanguinarine identified in the urine of animals even after 96 h of exposure [12]. The toxicity of sanguinarine has also been shown to be dependent on the reactivity of iminium bond with nucleophilic sites like thiol groups, present at the active sites of the enzymes and thus suggesting the electrophilic nature of the alkaloid [11,13].

It has been suggested that levels of free radicals in the body are regulated by a variety of antioxidant defense system including enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) together with nonenzymatic antioxidants such as ascorbic acid, α-tocopherol, carotenoids, albumin, uric acid, bilirubin, etc. [14]. Since in vitro studies indicate the possibility of generation of ROS by argemone oil [7–9], the present study was aimed to evaluate the extent of oxidative stress and antioxidant defense mechanism against free radicals induced biomolecular damage in epidemic dropsy patients of Kannauj, Uttar Pradesh, India.

2. Materials and methods

2.1. Chemicals and reagent kits

Reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH), oxidized glutathione (GSSG), 5,5′-dithio-bis-(2-nitro benzoic acid) (DTNB), thiobarbaturic acid (TBA), epinephrine, l-chloro-2,4-dinitro benzene (CDNB), 2,4-dinitrophenyl hydrazine (DNPH), disodium ethylene diamine tetra acetic acid (Na_2 EDTA), guanidine hydrochloride, α-tocopherol, retinol and retinol acetate were purchased from Sigma Chemical, St. Louis, MO, USA. H_2O_2 (30%) solution and absolute alcohol were obtained from E. Merck, Mumbai, India, and Bengal Chemicals and Pharmaceuticals, India, respectively. Total protein, albumin, hemoglobin (Hb), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLc), and uric acid assay kits were procured from Accurex Biomedical Pvt Ltd. Mumbai, India. Water was purified by a Millipore system and had a resistivity >18 M Ωcm^{-1}. All the other chemicals used were of the highest purity available from commercial sources.

2.2. Patients

In June 2002, an outbreak of epidemic dropsy was reported in the Ismailpur village of Kannauj district, UP, India. Twenty one patients from three different families were affected and investigated at District Health Centre, Kannauj, Uttar Pradesh, India. Patients were of different age groups including children, adolescents and adults of both sexes. The samples of mustard oil consumed by these patients were found to be positive for argemone oil contamination by ITRC argemone oil detection kit [15].

2.3. Specimen collection and processing

After seeking history of the patients and the records at Kannauj District Health Centre, it was confirmed that the exposure of argemone oil adulterated mustard oil is acute (5–7 days). The blood of 21 dropsy patients and 7 healthy volunteers were collected in vacuum tubes (Vacutainer, Becton Dickinson Company, NJ, USA) containing EDTA as anticoagulant after 8–10 days of exposure. Blood samples were brought to the lab on the same day in an ice container. A portion of blood was used for the preparation of the lysate [16]. Briefly, blood was centrifuged at 2500×*g* for 15 min at 4 °C and the supernatant aspirated. The erythrocyte rich precipitate was washed three times with saline (3:1 v/v) and lysed by double distilled water. The particulate material was centrifuged at 15,000×*g* for 90 min at 4 °C, and the supernatant (erythrocyte lysate) was collected and stored at −80 °C.

Another portion of blood was transferred to glass tubes and kept in a refrigerator for 30 min to clot. Plasma was separated by centrifuging these tubes at 3000×*g* for 10 min and stored at −80 °C until further analysis. The remaining blood was used for hematological indices and glutathione content.

2.4. Hematological indices

Red blood cell (RBC), hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet count were carried out in whole blood samples on hematology analyser (ABACUS, 1.7, Diatron, Austria) [17].

2.5. Plasma protein fractions and lipid profile

Total protein and albumin (A) content in plasma was assayed by Accurex Biomedical kits (Mumbai, India). Globulin (G) and A/G ratio were calculated from these