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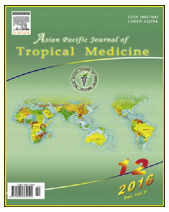

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Novel neuroprotective and hepatoprotective effects of citric acid in acute malathion intoxication

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ABSTRACT

Objective: To study the effect of citric acid given alone or combined with atropine on brain oxidative stress, neuronal injury, liver damage, and DNA damage of peripheral blood lymphocytes induced in the rat by acute malathion exposure.

Methods: Rats were received intraperitoneal (*i.p.*) injection of malathion 150 mg/kg along with citric acid (200 or 400 mg/kg, orally), atropine (1 mg/kg, *i.p.*) or citric acid 200 mg/kg + atropine 1 mg/kg and euthanized 4 h later.

Results: Malathion resulted in increased lipid peroxidation (malondialdehyde) and nitric oxide concentrations accompanied with a decrease in brain reduced glutathione, glutathione peroxidase (GPx) activity, total antioxidant capacity (TAC) and glucose concentrations. Paraoxonase-1, acetylcholinesterase (AChE) and butyrylcholinesterase activities decreased in brain as well. Liver aspartate aminotransferase and alanine aminotransferase activities were raised. The comet assay showed increased DNA damage of peripheral blood lymphocytes. Histological damage and increased expression of inducible nitric oxide synthase (iNOS) were observed in brain and liver. Citric acid resulted in decreased brain lipid peroxidation and nitric oxide. Meanwhile, glutathione, GPx activity, TAC capacity and brain glucose level increased. Brain AChE increased but PON1 and butyrylcholinesterase activities decreased by citric acid. Liver enzymes, the percentage of damaged blood lymphocytes, histopathological alterations and iNOS expression in brain and liver was decreased by citric acid. Meanwhile, rats treated with atropine showed decreased brain MDA, nitrite but increased GPx activity, TAC, AChE and glucose. The drug also decreased DNA damage of peripheral blood lymphocytes, histopathological alterations and iNOS expression in brain and liver.

Conclusions: The study demonstrates a beneficial effect for citric acid upon brain oxidative stress, neuronal injury, liver and DNA damage due to acute malathion exposure.

1. Introduction

Oxygen derived free radicals are produced in the cell from many sources. One important source is the mitochondrial electron transport chain where electrons that leaked from O_2 result in generation of superoxide anion radical. ($O_2^{\bullet-}$). The redox state of the cell is kept in balance due to a number of antioxidant mechanisms. These include both enzymatic (*eg.*, catalases, speroxide dismutases, and glutathione peroxidase) and non-enzymatic free radical scavengers (*eg.*, glutathione, α-tocopherol, ascorbic acid) [1,2]. Oxidative stress develops when there is an increase in oxidants and/or in adequate antioxidants [3]. Oxidative stress contributes to the development of several disease processes *eg.*, diabetes mellitus, cardiovascular disease, cancer, neurodegenerative and psychiatric disorders [4–7]. Owing to its high metabolic demand, the brain utilizes much O_2 with the consequent increased generation of reactive oxygen metabolites. Moreover, auto-oxidation of brain

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neurotransmitters generating O_2 and quinones and the presence of redox-active metals capable of catalyzing free radical reactions increase the brain' oxidant burden. The brain is also rich in polyunsaturated fatty acids, which is the preferred substrate for free radical attack. These factors coupled with modest antioxidant mechanisms make the brain tissue particularly susceptible to oxidative stress [1,2,8].

Reactive oxygen metabolites are likely to contribute to the neurotoxic effects of organophosphate insecticides. In this context, exposure to malathion caused increased lipid peroxidation [9,10] increased nitric oxide, and decreased reduced glutathione (GSH) [10] in the rat brain. Lipid peroxidation increased in blood, liver [11,12] and in the rat erythrocytes as well [13]. Studies also indicated decreased activities of the antioxidant enzymes glutathione reductase and glutathione peroxidase in rat cerebral cortex [14] and activities of superoxide dismutase, catalase and glutathione peroxidase in human erythrocytes [15] also decreased after exposure to malathion. Moreover, the chain breaking antioxidants α-tocopherol and ascorbate were able to reduce lipid peroxidation and ameliorate the changes in antioxidant enzymes caused by malathion in rat and human erythrocytes [13,15].

Citric acid (2-hydroxy-1,2,3-propane-tricarboxylic acid) is a weak organic acid found in all animal tissues [16]. Cellular citrate is synthesized inside the mitochondria [17] while rich dietary sources include lemon, orange, tangerine and grapefruit [18]. Intracellular citric acid is important in the intermediary energy metabolism of the cell. Citrate is produced in the mitochondria from acetyl-CoA and oxaloacetate and enters the citric acid cycle (tricarboxylic acid cycle or Krebs cycle). The resulting high-energy intermediates; the reduced coenzymes nicotinamide adenine dinucleotide and flavin adenine dinucleotide are then utilized in the respiratory chain in the inner mitochondrial membrane to make ATP (adenosine 5′-triphosphate) for the cell's energy needs. Citric acid released into the cytoplasm *via* specific mitochondrial carriers is converted to acetyl CoA for the biosynthesis of fatty acids, lipids, and cholesterol [19].

Besides its role in the generation of energy, citrates have other important actions including down regulation of inflammation and reduction of lipid peroxidation [20–23]. Citrate reduces polymorphonuclear cell degranulation and attenuate the release of inflammatory mediators *eg.*, myeloperoxidase, platelet factor 4, interleukin 1β [18–20] and tumor necrosis factor-alpha [23]. Citric acid displayed hepatoprotective effects where it reduced hepatocellular damage evoked by carbon tetrachloride in rats [24,25]. It also decreased brain lipid peroxidation and inflammation and liver damage in mice treated with bacterial lipopolysaccharide endotoxin [23].

A defect in mitochondrial bioenergetics might be involved in the neurotoxic effects of malathion. This is because organophosphates can cause mitochondrial impairment [9,26–29]. Moreover, the administration of methylene blue, an antioxidant [30] and an enhancer of the electron transport chain [31] protected against the malathion-induced neurotoxicity [10]. Thus, in view of the bioenergetic, antioxidant and anti-inflammatory effects reported above for citrate, it looked pertinent to investigate the effect of citric acid administration on oxidative stress and brain damage in rats intoxicated with the organophosphate malathion. We also examined the possible modulation by citric acid of the effect of atropine, the muscarinic receptor antagonist and the antidote employed in the management of acute organophosphate poisoning [32,33]. Since, malathion has been shown to cause hepatocellular damage [34,35], the study was extended to include the liver tissue.

2. Materials and methods

2.1. Animals

Male rats of the Sprague–Dawley strain with body weight of (130–140) g were used. Rats were obtained from Animal House Colony of the National Research Centre. Rats allowed free access to standard laboratory food and water. Animal procedures were done in accordance to the recommendations of the institutional Ethics Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and chemicals

Malathion (Commercial grade, 57%) was purchased from El-Naser Chemical Co., Cairo. Citric acid and atropine were obtained from Sigma–Aldrich (St Louis, MO, USA). Other chemicals and reagents were of analytical grade and purchased from Sigma–Aldrich.

2.3. Study design

Rats were randomly divided into different groups (6 rat/group). Group 1 was treated with *i.p.* saline (0.2 mL/rat) and served as negative control. Group 2–6 were *i.p.* treated with malathion at a dose of 150 mg/kg, along with saline (group 2), citric acid at 200 or 400 mg/kg (groups 3&4), atropine at 1 mg/kg (group 5) or citric acid at 200 mg/kg + atropine at 1 mg/kg (group 6). Rats were euthanized by decapitation 4 h after drug administration. Their brains were quickly removed on ice-plate and washed with ice-cold phosphate-buffered saline at pH 7.4. Brains were weighed and stored at −80 °C for later biochemical analyses. Homogenization of brain tissues were carried out using 0.1 M phosphate buffer saline (pH 7.4) to give a final concentration of 20% w/v for the biochemical assays.

2.4. Biochemical analyses

2.4.1. Lipid peroxidation

Malondialdehyde (MDA), a product of lipid peroxidation was determined in tissue homogenates by the method of Nair and Turne [36]. In this assay thiobarbituric acid reactive substances (TBA) react with thiobarbituric acid to form TBA-MDA adduct which can be measured colorimetrically at 532 nm.

2.4.2. Reduced glutathione

Reduced glutathione (GSH) was determined in tissue homogenates using the method of Ellman *et al.* [37]. The procedure is based on the reduction of Ellman's reagent [DTNB; 5, 5′-dithiobis (2-nitrobenzoic acid)] by the free sulfhydryl group on GSH to form yellow colored 5-thio-2-nitrobenzoic acid which can be determined using spectrophotometer at 412 nm.

2.4.3. Nitric oxide

Nitric oxide was determined using colorimetric assay where nitrate is converted to nitrite *via* nitrate reductase. Griess reagent

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