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Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats

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ABSTRACT

Aluminium is present in many manufactured foods and medicines and is also added to drinking water during purification purposes. Therefore, the present experiment was undertaken to determine the effectiveness of propolis in alleviating the toxicity of aluminium chloride ($AlCl_3$) on biochemical parameters, antioxidant enzymes and lipid peroxidation of male Wistar Albino rats. Animals were assigned to 1 of 4 groups: control; 34 mg $AlCl_3$/kg bw; 50 mg propolis/kg bw; $AlCl_3$ (34 mg/kg bw) plus propolis (50 mg/kg bw), respectively. Rats were orally administered their respective doses daily for 70 days. The levels of thiobarbituric acid reactive substances (TBARS) was increased, and the activities of glutathione S-transferase, superoxide dismutase, catalase and glutathione peroxidase were decreased in liver, kidney and brain of rats treated with $AlCl_3$. While, TBARS was decreased and the antioxidant enzymes were increased in rats treated with propolis alone. Plasma transaminases, lactate dehydrogenase, glucose, urea, creatinine, bilirubin, total lipid, cholesterol, triglyceride and LDL-c were increased, while total protein, albumin and high HDL-c were decreased due to $AlCl_3$ administration. The presence of propolis with $AlCl_3$ alleviated its toxic effects in rats treated with $AlCl_3$. It can be concluded that propolis has beneficial influences and could be able to antagonize $AlCl_3$ toxicity.

1. Introduction

Aluminum metal is abundantly present in the earth's crust. From the environment it gets access to the human body via the gastrointestinal and the respiratory tracts. Aluminium is a constituent of cooking utensils and medicines such as antacids, deodorants and food additives and this has allowed its easy access into the body (Yokel, 2000). The sources of aluminium are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, ware and containers. Also, it is present in medicines and is also added to drinking water for purification purposes (Ochmanski and Barabasz, 2000).

Aluminium has been proposed as an environmental factor that may contribute to some neurodegenerative diseases, and affects several enzymes and other biomolecules relevant to Alzheimer's disease (Domingo, 2006). Also, increased aluminium burdens, can cause neurological symptoms, biochemical responses leading to unhealthy bone metabolism and learning disabilities in children (Zafar et al., 2004). Salts of aluminium may bind to DNA, RNA, inhibit such enzymes as hexokinase, acid and alkaline phosphates, phosphodiesterase and phosphooxydase (Ochmanski and Barabasz, 2000). Strong et al. (1996) found that aluminium exposure caused impairments in glucose utilization, agonist-stimulated inositol phosphate accumulation, free radical-mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation. Yousef (2004) reported that aluminium-induced changes in hemato-biochemical parameters, increased lipid peroxidation and decreased the activities of the antioxidant enzymes in plasma and tissues of male rabbits. Also, Yousef et al. (2005, 2007) demonstrated that $AlCl_3$ caused deterioration in sperm quality, enhancement of free radicals and alterations in antioxidant enzymes in both *in vivo* and *in vitro*. The mechanism of aluminium-induced toxicity is that it potentiates the activity of Fe^{2+} and Fe^{3+} ions to cause oxidative damage (Xie and Yokel, 1996).

Abbreviations: Al, aluminium; $AlCl_3$, aluminium chloride; TBARS, thiobarbituric acid reactive substances; TBA, thiobarbituric acid; GST, glutathione S-transferase; SOD, superoxide dismutase; CAT, Catalase; GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; GSH-Px, glutathione peroxidase; GR, glutathione-reductase; NO, nitrogen oxide radical; LPO, lipid peroxidation. AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; HDL-c, high density lipoprotein; LDL-c, low density lipoprotein; GS, glutathione-synthase.

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Propolis or bee glue is a resinous hive product collected by honey bees from plant exudates and contains more than 160 constituents. Historically it has been used for various purposes, especially as a medicine (Ghisalberti, 1979). Flavonoids are thought to be responsible for many of its biological and pharmacological activities including anticancer (Padmavathi et al., 2006), anti-inflammatory (Paulino et al., 2008), and antioxidant effects (Nieva Moreno et al., 2000; Yousef et al., 2003a, 2003b, 2004a, 2004b). Flavonoids and various phenolic compounds are the most important pharmacologically active constituents in propolis that have been shown to be capable of scavenging free radicals and thereby protecting lipids from being oxidized or destroyed during oxidative damage (Nieva Moreno et al., 2000). Propolis has gained popularity and used extensively in healthy drinks and foods to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer (Padmavathi et al., 2006; Paulino et al., 2008). Therefore, oral supplementation with propolis may protect the animals from the harmful effect of aluminium. The role of propolis against aluminium-induced changes in biochemical parameters, lipid peroxidation, and antioxidant enzymes of rats have not so far been studied. Therefore, the present study was carried out to investigate: (1) the alterations in biochemical parameters, free radicals and antioxidant enzymes induced by aluminium chloride in liver, kidney and brain of male rats, (2) the role of propolis in alleviating the negative effects of aluminium chloride, and (3) the effect of propolis alone on the tested parameters.

2. Materials and methods

2.1. Chemicals

Aluminium chloride ($AlCl_3$) was purchased from Aldrich chemical Company, Milwaukee Wis, USA, while propolis was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other chemicals used in the experiment were of analytical grade. The doses of aluminium chloride ($AlCl_3$) and propolis were 34 mg/kg bw and 50 mg/kg bw, respectively.

2.2. Experimental design

Forty male Wistar Albino rats (average weight 180–200 g) were used in the present experiment. Animals were obtained from faculty of medicine, Alexandria University, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of 5 and given food and water *ad libitum*. After two weeks of acclimatization, animals were divided into four equal groups. The first group was used as control. While, groups 2, 3 and 4 were orally treated with aluminium chloride (34 mg/kg bw), propolis (50 mg/kg bw) and the combination of aluminium chloride (34 mg/kg bw) and propolis (50 mg/kg bw), respectively. Rats were orally administered their respective doses every day for 70 days. At the end of the experiment, animals were sacrificed by decapitation and brain, liver and kidney were immediately removed.

2.3. Biochemical parameters

Rats of each group were euthanized at the end of treatment period. Trunk blood samples were collected from the sacrificed animals and placed immediately on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 860 Xg for 20 min and stored at –60 °C till measurements. Brain, liver and kidney were immediately removed; washed using chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 Xg for 20 min at 4 °C, and the resultant supernatant was used for determination of biochemical parameters, assay of antioxidant enzyme and TBARS.

Plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine aspartate transaminase (ALT; EC 2.6.1.2) activities were determined with kits from SENTINEL CH. (via principle Eugenio 5–20155 MILAN, Italy). The activity of plasma lactate dehydrogenase (LDH; EC 1.1.1.27) was determined by the method of Martinek (1972).

Stored plasma samples were analyzed for total protein by the Biuret method according to Armstrong and Carr (1964). Albumin concentration was determined by the method of Doumas et al. (1977). The concentrations of glucose were determined with kits from Bio systems, S.A. Costa Brava, 30-Barcelona (Spain). Plasma urea and creatinine concentrations were determined by the method of Patton and Crouch (1977) and Henry et al. (1974), respectively. Plasma total bilirubin was measured using the method of Pearlman and Lee (1974). Plasma concentrations of total lipids were assayed by the method of Knight et al. (1972) and that of cholesterol and triglycerides were determined by the method Carr et al. (1993). High density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were determined according to the methods of Warnick et al. (1983) and Bergmenyer (1985), respectively.

2.4. Tissue thiobarbituric acid reactive substances and antioxidant enzymes

According to the method of Esterbauer and Cheeseman (1990), the extent of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) formation was measured. Plasma and tissue supernatants were mixed separately with 1 ml TCA (20%), 2 ml TBA (0.67%) and heated for 1 h at 100 °C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Glutathione S-transferase (GST; EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig et al. (1974). *P*-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 310 nm using UV-Double Beam spectrophotometer. The catalase enzyme (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity in plasma and tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Xu et al., 1997).

Super oxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction co-efficient was followed at 480 nm in a Spectrophotometer. Glutathione peroxidase (GSH-Px) activity assayed using the method of Chiu et al. (1976) in brain, liver and kidney.

2.5. Statistical analysis

Data are expressed as mean values ± SD of ten replicate determinations. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the post-Hoc Tukey's test was used for comparisons. The criterion for statistical significance was set at $p < 0.05$ or $p < 0.01$. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS® Inc., USA).

3. Results and discussion

3.1. Aluminium chloride

The present study was carried out to investigate the protective effects of propolis on aluminium-induced oxidative stress and biochemical alterations in rats. In the aluminium chloride treated rats, the levels of thiobarbituric acid reactive substances (TBARS) were found to be elevated but the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were decreased in liver, kidney and brain (Table 1). These observations are similar to the data reported by Yousef (2004), Yousef et al. (2005, 2007), Nehru and Anand (2005) who indicated that aluminium intake produces oxidative stress. Although aluminium is not a transition metal and therefore cannot initiate peroxidation, many investigations have searched for a correlation between aluminium accumulation and oxidative damage in the body tissues (Cherroret et al., 1995; Wilhelm et al., 1996; Nehru and Anand, 2005). An in vitro study has indicated that aluminium greatly accelerates iron-mediated lipid peroxidation (Xie and Yokel, 1996). In fact, aluminium, a non-redox-active metal, is a pro-oxidant both *in vivo* and *in vitro* (Exley, 2004).

The primary effects of aluminium on the brain, liver and kidney functions are thought to be mediated via damage to cell membranes. Lipid peroxidation of biological membranes results in the loss of membrane fluidity, changes in membrane potential, an increase in membrane permeability and alterations in receptor functions (Nehru and Anand, 2005). In the present experiment, there was a significant increase in lipid peroxidation after aluminium chloride exposure, measured in terms of TBARS levels in the brain,