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Propolis protection from reproductive toxicity caused by aluminium chloride in male rats

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ABSTRACT

Different forms of aluminium (Al) are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive toxicity. Propolis has been reported to be important antioxidant. Therefore, this study aimed at elucidating the protective effects of propolis against reproductive toxicity of aluminium chloride ($AlCl_3$) in male rats. The first group served as control. Group 2 received 34 mg $AlCl_3$/kg bw (1/25 LD_{50}). Group 3 was administered 50 mg propolis/kg bw/day. Group 4 was treated with $AlCl_3$ plus propolis. Treatment was continued for 70 days. $AlCl_3$ caused a decrease in testes, seminal vesicle and epididymis weights, sperm concentration, motility, testosterone level and the activities of 17-ketosteroid reductase, CAT and GST, and GSH content. While, dead and abnormal sperm and testes TBARS concentrations were increased. In the $AlCl_3$-treated group, histopathologic examinations revealed apparent alterations in the testes, where it induced marked lesions in seminiferous tubules. Propolis alone decreased dead and abnormal sperm and TBARS, and increased testosterone, GSH, 17-ketosteroid reductase, CAT and GST. Results showed that propolis antagonized the harmful effects of $AlCl_3$. This was proved histopathologically by the great improvement in testes. In conclusion propolis could be effective in the protection against the reproductive toxicity of $AlCl_3$.

1. Introduction

Aluminium compounds have many medical implications as, antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants and allergen injection (Exley, 1998). Aluminium absorption/accumulation in humans can occur via the diet, drinking water, ingestion with fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of aluminium in healthy subjects (Venturini-Soriano and Berthon, 2001). Aluminium chloride ($AlCl_3$) was found to be embryotoxic and teratogenic when given parenterally to animals (Cranmer et al., 1986). Results obtained from Yousef et al. (2005) revealed that rabbits orally administered $AlCl_3$ at 34 mg/kg BW every other day for 16 weeks showed significant decrease in ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, packed sperm volume, total functional sperm fraction, normal and live sperm, while dead and abnormal sperm were increased. Also, Yousef et al. (2007) reported that $AlCl_3$ showed reproductive toxicity on rabbit sperm *in vitro*.

Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals. High concentrations of aluminium in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability (Dawson et al., 1998). Testicular aluminium accumulation, necrosis of spermatocytes/spermatids and a significant decrease in fertility were found in both male mice and rats (Llobet et al., 1995; Sharma et al., 2003; Guo et al.,2005a,b). In addition, the suppressive effects of long-term oral $AlCl_3$ in drinking water on both sexual and aggressive behavior and fertility of male rats were also noted (Bataineh et al., 1998). There is evidence implicating androgenic hormones involved in mechanisms of aluminium toxicity on male reproduction (Sharpe, 1990). Also, Guo et al. (2005a) found that aluminium administration significantly increased nitric oxide (NO) production and decreased both testicular adenosine 3′,5′-cyclic monophosphate (cAMP) and testosterone levels. They demonstrated that excessive NO activated inducible NO synthase (NOS) which may be involved in reproductive toxicity of aluminium. In biological systems, NO is generated from L-arginine by the enzyme NOS, formed in a variety of tissues and involved in many physiological and pathological processes. Excessive NOS, in response to a variety of stressors that may induce the production of large amounts of NO metabolites, hence reducing rate and motility of sperm cells, and increasing

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their morphological abnormalities. In addition, NO related agent suppressed testosterone secretion in male rats (Guo et al., 2005a). Our studies have shown that aluminium enhanced lipid peroxidation in plasma, testes, brain, kidney, lung and liver of rabbits, and also in culture of rabbit sperm (Yousef,2004; Yousef et al., 2005, 2007).

To control the level of reactive oxygen species (ROS) and to protect cells under stress conditions, mammalian tissues contain several enzymes scavenging ROS such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione S-transferase (GST), and reduced glutathione (GSH). Some compounds also contribute to the detoxification process from ROS such as propolis (Mani et al., 2006; Castaldo and Capasso, 2002; Jasprica et al., 2007; Kanbura et al., 2009).

Propolis, a resinous sticky substance that honeybees produce by mixing their own waxes with resins collected from plants, is used as a sealant and sterilant in honeybee nests, and has been used as a folk medicine from ancient times. In modern times, it has been found to have a wide range of biological activities, such as antibacterial (Sforcin et al., 2000), anti-inflammatory (Khayyal et al., 1993), anticarcinogenic (Bazo et al., 2002), antioxidative (Matsushige et al., 1995; Khalil, 2006; Russo et al., 2006; Sobocanec et al., 2006; Jasprica et al., 2007; Kanbur et al., 2009), hepatoprotective effects (Gonzalez et al., 1994), and immunomodulatory (Sforcin et al. 2002). Propolis contains more than 300 components, including phenolic aldehydes, polyphenols, sequiterpene quinines, coumarins, steroids, amino acids, and inorganic compounds (Khalil, 2006). Phenolic compounds such as flavonoids are mainly responsible for the biological activity of propolis. Flavonoids are also responsible for antioxidant activity and this is principally based on their radical scavenging effect (Yousef et al., 2003a,b, 2004a,b; Mani et al., 2006). Propolis is also reported to inhibit the generation of superoxide anion. Furthermore, propolis has been determined to reverse the consumption of glutathione, which is synthesized in the liver and has radical scavenging activity (Castaldo and Capasso, 2002).

Although the knowledge of aluminium toxicity has markedly improved in recent years, information concerning the reproductive toxicity and testicular dysfunction of this element is still needs more research. Also, the role of propolis against aluminium induced deteriorations in reproductive performance of rats have not so far been studied. Therefore, the present study aimed to determine the reproductive toxicity of aluminium chloride in adult male rats. Also, to evaluate the protective effect of propolis against the possible testicular dysfunction caused by aluminium chloride.

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 dose of aluminium chloride ($AlCl_3$) was 34 mg $AlCl_3$/kg BW (1/25 LD_{50}). The LD_{50} of Al when administered orally to rats was reported to be 380–400 mg/kg bw (Krasovskii et al., 1979). The dose of propolis was 50 mg/kg BW. This dose was used according to the previous studies of Park and Kahng (1999).

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 feed and water *ad libitum*.

After two weeks of acclimation, 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, 1/25 LD_{50}), 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. The duration of the experiments lasts for 70-day for completion of the spermatogenic cycle and maturation of sperms in epididymis (Sarkar et al., 2003).

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*g* for 20 min and stored at −60 °C till measurements. The testes and accessory sex organs (seminal vesicles, prostates and epididymis) were dissected out, trimmed off the attached tissues and weighed. The relative weight of organs was calculated. The relative weight of organs (%) was calculated as g/100 g body weight. Specimens of the testes were fixed immediately in 10% buffered formalin for histological study.

2.3. Semen characteristics

The left epididymis was excised and placed in a warmed petri dish containing 0.2 ml of calcium and magnesium free Hank's solution at 37 °C. The tissue was minced with scalpels for approximately 1 min and placed in a 37 °C incubator for 15 min prior to determining sperm motility. The suspension was stirred, one drop was placed on a warmed microscope slide, and a 22 × 22 mm coverslip was placed over the droplet. At least 10 microscopic fields were observed at 400× magnification using a phase-contrast microscope, and the percentage of motile sperm was recorded (Linder et al., 1995; Llobet et al., 1995). The coverslip was removed and the spermatozoa suspension was allowed to dry in air. The sample was stained with 1% eosin Y/5% nigrosin and examined at 400× for morphological abnormalities. Three hundred spermatozoa from different fields were examined for each sample as described previously (Linder et al., 1995; Llobet et al., 1995).

The right epididymis and specimens of right testis were frozen immediately after weighing. After thawing at room temperature, the whole epididymis and specimens of testis were homogenized in 0.5 ml of a solution of 0.9% NaCl containing 2% of Triton X-100. Ten strokes of a manual glass homogenizer were used for each sample. The testis and epididymis homogenates were diluted with 1.5 ml of the homogenization solution and spermatozoa were counted at 400× magnification using a Neubauer hemocytometer. Three counts per sample were averaged (Llobet et al., 1995). Assessment of live, dead and abnormal spermatozoa was performed using an eosin–nigrosine blue staining mixture as described (Blom, 1950).

2.4. Assay of steroidogenic enzyme 17- ketosteroid reductase

The testicular tissues were homogenized with a Tekmar model TR-10, West Germany homogenizer in 10-volume 0.25 M-sucrose containing 0.05 mM-EDTA and 5 mM mercaptoethanol, buffered with 0.05 M potassium phosphate (pH 7.4), the homogenate was centrifuged at 401 °C in a cooling centrifuge (Heraeus Christ, West Germany). Aliquots of the resulting supernatant used as a crude preparation of 17-ketosteroid reductase according to the method of Katryna and Anita (1980).

2.5. Estimation of plasma testosterone

Plasma testosterone was assayed using electrochemiluminescence Immunoassay "ECLIA" Kit obtained from Roche Diagnostics GmbH. D-68298 Mannheim, USA, following the testosterone assay was 0.02 ng/ml.

2.6. Determination of testicular antioxidant enzymes, thiobarbituric acid reactive substances and reduced glutathione

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 enzyme catalase (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity 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). According to the method of Esterbauer and Cheeseman (1990), the extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured. Plasma and tissue supernatant was mixed 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 content (GSH) was determined using commercial glutathione reduced kits (Biodiagnostic for diagnostic reagents: Dokki, Giza, Egypt) according to the method of Beutler et al. (1963).

2.7. Protein estimation

The protein content of testicular tissues was determined by the method described by Lowry et al. (1951) using bovine serum albumin as a standard.