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Protective effect of propolis against aluminum chloride-induced reproductive toxicity in male rats

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Abstract

The aim of the present study was to investigate the toxic effects of aluminum chloride (AlCl₃) on the reproductive organs, as well as, the protective effect of propolis against AlCl₃-induced reproductive toxicity in male rats. Eighty adult male fertile Sprague Dawley albino rats were randomly divided into four groups of 20 each. Group 1: served as control group and received only distilled water. Group 2: received a daily ingestion of 80 mg/kg of AlCl₃. Group 3: received a daily ingestion of 200 mg/kg of an ethanol extract of propolis. Group 4: received a daily ingestion of 80 mg/kg of AlCl₃ in addition to 200 mg/kg of an ethanol extract of propolis. The duration of experiment was six weeks. At the end of the experiment, the testes, seminal vesicles, prostate glands and epididymides were dissected out, and weighed. Sperm characteristics were evaluated and plasma testosterone level was estimated. There were no significant changes between the control and the propolis-treated group. AlCl₃-treated group showed a highly significant decrease in the index weights of testes and prostate glands, a highly significant lower sperm count, motility and viability, a highly significant increase in the number of abnormal sperms, as well as, a highly significant decrease in serum testosterone level ($p < 0.001$), compared to control. Rats of AlCl₃+propolis-treated group showed a highly significant improvement in all previous alterations. In conclusion, propolis appeared to ameliorate AlCl₃-induced reproductive toxicity in male rats.

Keywords: Male rats; Aluminum chloride; Propolis; Reproductive organs; Sperm characteristics

1. Introduction

Aluminum is the most widely distributed metal in the environment [1]. Food consumption is the major source for aluminum intake under physiological conditions [2]. Aluminum-containing food products are mainly corn, yellow cheese, salts, herbs, spices, and tea, [3]. Aluminum, also, enters in the production of cosmetics such as antiperspirants and deodorant preparations [4]. It is incorporated in some medications such as antacids, buffered aspirin, antidiarrheal products, vaccines, and allergen injections, as well as, in some veterinary medicine products, glues, and disinfectants [5]. Aluminum sulfate is added as a coagulant agent during the purification process of drinking water [6].

In biological systems, aluminum is present only in trace amounts and have a definite biological function. Aluminum has been described mainly in bone, liver, testis, kidney, and brain; its accumulation in tissues and organs results in their dysfunction and toxicity [2]. It has been shown to alter the enzymatic activities, and subsequently, the metabolism in testis, epididymis and vas deferens, resulting into poor sperm motility and reduction in the fertility rate in rabbits [7], rats [8] and mice [9]. High concentrations of aluminum in human spermatozoa and seminal plasma were correlated with decreased sperm motility and viability [10]. In addition, long-term use of drinking water with high concentration of aluminum chloride (AlCl₃) leads to alteration of the sexual behavior and fertility of male rats [8].

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Propolis is a resinous substance that honeybees collect from the buds of certain trees, sap flows or other botanical sources, to be used as a sealant for unwanted open spaces in their hives [11]. Propolis is a hard and brittle substance, lipophilic in nature, that becomes soft, and gummy when heated [12]. It is widely used in traditional herbal medicine as antimicrobial, antiviral and antioxidant agent [13]. Its ingredients, phenols, flavonoids and cinnamic acid derivatives, are responsible for its immunomodulatory and its anti-inflammatory properties [14]. Propolis enters in manufacturing of cosmetics and medications for common cold, wounds, burns, and acne. It is commercially available in the form of capsules, creams, mouthwash solutions, toothpaste, and throat lozenges. [15].

The aim of the present study was to investigate the toxic effects of AlCl₃ on the reproductive organs, as well as, the protective effect of propolis, if any, against AlCl₃-induced reproductive toxicity in male rats.

2. Material and methods

2.1. Chemicals

AlCl₃ is obtained from Guangdong Guanghua Sci-Tech Co. Ltd., Shantou, Guangdong, China, and manufactured by Yueyang Jiazhiyuan Biological Co. Ltd., China. An ethanol extract of propolis is obtained from Boiron et Cie, Lyon, France.

2.2. Animals

Eighty adult male fertile Sprague Dawley albino rats, weighing 200 mg, were used in this study. Principles of laboratory animal care were followed throughout the experiment.

2.3. Experimental design

Animals were randomly divided into four groups of 20 each. Group 1 (Control group): rats of this group received a daily ingestion of 2.0 ml. of distilled water for six weeks. Group 2 (AlCl₃-treated group): rats of this group received a daily ingestion of 80 mg/kg of AlCl₃ (equal to 6 mg dissolved in 1 ml of distilled water) for six weeks [16]. Group 3 (propolis-treated group): rats of this group received a daily ingestion of 200 mg/kg of an ethanol extract of propolis (equal to 40 mg of propolis in 1 ml of ethanol) for six weeks [17]. Group 4 (AlCl₃+propolis-treated group): rats of this group received a daily ingestion of 80 mg/kg of AlCl₃ in addition to a daily ingestion of 200 mg/kg of an ethanol extract of propolis for six weeks. At the end of the experiment, animals were anesthetized with ether and then sacrificed. Trunk blood samples were collected from each animal and placed immediately on ice. Heparin was used as an anticoagulant, and plasma samples were obtained by centrifugation at 860 g for 20 minutes and stored at - 60° C till measurements. The testes, seminal vesicles, prostate glands and epididymides were dissected out, and weighed. The relative weight of organs was calculated as g/100 g body weight.

2.4. Sperm characteristics

Seminal content of epididymis was obtained by dissecting the cauda epididymis using surgical blades and squeezing its content into a sterile clean watch glass. This content was diluted 10 times with 2.9 % sodium citrate dehydrate solution and thoroughly mixed to estimate the progressive motility and sperm concentration. One drop of the suspension was smeared on a glass slide and stained by Eosin-nigrosine stain to determine the percentage of sperm cell viability and morphological abnormalities [18].

2.5. Estimation of plasma testosterone level

Plasma testosterone is assayed using electrochemiluminescence Immunoassay “ECLIA” Kit obtained from Roche Diagnostics GmbH. D-68298 Mannheim, USA.

2.6. Statistical analysis

Data are expressed as mean values ± SE of 10 replicate determinations. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. Differences were considered to be significant at $p < 0.05$ and highly significant at $p < 0.001$. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS, Inc., USA).

3. Results

There was a highly significant decrease in the index weights of testes and prostate glands ($p < 0.001$) and a non-significant decrease in the index weights of seminal vesicles in AlCl₃-treated group, compared to the other groups. There was a significant decrease in the index weights of testes and prostate glands in AlCl₃+propolis-treated group ($p < 0.05$) compared to control and propolis-treated groups. There were no significant changes between the control and the propolis-treated group. (Table 1).

Table 1 Index weights of testes, seminal vesicles and prostate glands

Experimental groups	Sex organs (g / 100 g body weight)		
	Testis	Seminal vesicle	Prostate
Control	0.922 ± 0.041	0.415 ± 0.023	0.130 ± 0.006
AlCl ₃	0.488 ± 0.018 ‡	0.384 ± 0.013	0.113 ± 0.004 ‡
Propolis	0.921 ± 0.040	0.412 ± 0.025	0.132 ± 0.005
AlCl ₃ +Propolis	0.780 ± 0.023 †*	0.404 ± 0.011	0.125 ± 0.002 †*

Each value represents the mean of 5 rats ± SE ; † Significant difference compared to control group and propolis-treated group ($p < 0.05$) ; ‡ Highly significant difference compared to control group and propolis-treated group ($p < 0.001$); * Highly significant difference compared to AlCl₃-treated group ($p < 0.001$)

AlCl₃-treated group showed a highly significant lower sperm count, motility and viability, as well as, a highly significant increase in the number of abnormal sperms ($p < 0.001$) compared to the other groups. AlCl₃+propolis-treated group showed a significant lower sperm count, motility, viability and a higher number of abnormal forms ($p < 0.05$) compared to control and propolis-treated groups. There was no significant change in any of the sperm characteristics between control and propolis-treated groups (Table 2).

Table 2 Sperm characteristics in experimental groups

Experimental groups	Sperm characteristics			
	Count (10 ⁶ / ml)	Motility (%)	Viability (%)	Sperm abnormalities (%)
Control	62.0 ± 3.63	82.4 ± 4.53	89.6 ± 4.31	6.6 ± 0.51
AlCl ₃	28.6 ± 1.29 ‡	42.0 ± 3.73 ‡	52.0 ± 3.65 ‡	18.4 ± 0.93 ‡
Propolis	64.0 ± 3.63	85.4 ± 4.53	90.6 ± 4.40	7.2 ± 0.54
AlCl ₃ +propolis	53.2 ± 3.22 †*	70.4 ± 2.16 †*	78.8 ± 2.85 †*	10.0 ± 0.71 †*

Each value represents the mean of 5 rats ± SE ; † Significant difference compared to control group and propolis-treated group ($p < 0.05$) ; ‡ Highly significant difference compared to control group and propolis-treated group ($p < 0.001$); * Highly significant difference compared to AlCl₃-treated group ($p < 0.001$)

Table 3 Serum testosterone level in experimental groups

Experimental group	Serum testosterone (ng/ ml)
Control	1.86 ± 0.049
AlCl ₃	0.936 ± 0.046 ‡
Propolis	1.88 ± 0.048
AlCl ₃ + propolis	1.556 ± 0.05 †*

Each value represents the mean of 5 rats ± SE ; † Significant difference compared to control group and propolis-treated group ($p < 0.05$) ; ‡ Highly significant difference compared to control group and propolis-treated group ($p < 0.001$); * Highly significant difference compared to AlCl₃-treated group ($p < 0.001$)

Table 3 showed a highly significant decrease in serum testosterone level in AlCl₃-treated group compared to the other groups. AlCl₃+propolis-treated group showed a significant decrease in serum testosterone level compared to control and propolis-treated groups. There was no significant difference in serum testosterone level between control and propolis-treated groups.

4. Discussion

The present study investigated the toxic effects of AlCl₃ on the reproductive organs of the male rats. Results showed a highly significant decrease in the index weights of testes and prostate glands in AlCl₃-treated rats compared to control rats. Similar results were reported by Bataineh et al. (1998) [8]. Also, a highly significant decrease in sperm counts, motility and viability, together with a highly significant increase in the percentage of abnormal forms of sperms were shown in AlCl₃-treated group compared to control group. The toxic changes of AlCl₃ shown were associated with a highly significant decrease in serum testosterone level in AlCl₃-treated group. The reduction in weights of testes and prostate glands might be attributed to the reduced availability of androgens. Defective sperm maturation and impairment of epididymal functions caused by androgen insufficiency might be responsible for such effects. Previous studies reported a reduction in testosterone level, in mice, following exposure to AlCl₃ [19, 20]. According to them, AlCl₃ would interfere with normal enzymatic activities resulting in a significant accumulation of cholesterol which, in turn, would reduce the level of testosterone and affect the metabolism of androgen-dependent reproductive organs. In addition, oxidative stress induced by AlCl₃ might be another possible cause. In accordance with the present suggestion, previous studies showed that AlCl₃ significantly induce oxidative stress in rabbits [21] and rats [22], resulting in a generation of reactive oxygen species (ROS) which include free radicals and peroxides. Oxidation of proteins might affect sperm maturation and might cause disturbances in the functions of the sperms. Furthermore, levels of ROS in semen were shown to be negatively correlated with the percentage of normal sperms [23]. In contrast to the present results, Llobet et al. (1995) [24] reported that, although the sperm counts were reduced, however sperm motility was not affected and the percentages of morphological normal spermatozoa in all mice exposed to aluminum nitrate were comparable to the values in control mice. The difference in the results between both studies might be due to species difference or due to the difference in the aluminum products and/or dosage used in each study.

The present study investigated the protective effect of propolis against AlCl₃-induced reproductive toxicity in male rats. Our results showed a highly significant increase in the index weights of testes and prostate glands in AlCl₃+propolis-treated group compared to AlCl₃-treated group. A highly significant increase in sperm counts, motility and viability, associated with a highly significant decrease in abnormal forms of sperms were demonstrated in AlCl₃+propolis-treated group compared to AlCl₃-treated group. The protective effect of propolis was confirmed by estimating the serum testosterone level which showed a highly significant increase in case of AlCl₃+propolis-treated group. Consistent with the present results, previous reports confirmed the protective effects of propolis against AlCl₃-induced hepatorenal toxicity in male rats [25]. Moreover, propolis has been shown to ameliorate sperm quality, reproductive organs and antioxidant status of male rats treated with cyclosporin-A [26]. Flavonoids and other phenolic compounds found in propolis seem to be capable of scavenging free radicals avoiding lipids and other substances such as vitamin C to be destroyed during oxidative damage [27].

5. Conclusion

The present study clearly demonstrated the reproductive toxicity produced by AlCl₃ on male rats, as evidenced by changes in organ weights, sperm characteristics and serum testosterone level. It also pointed out the protective role of propolis against AlCl₃-induced reproductive toxicity, possibly through inhibiting testicular lipid peroxidation. Consequently, it can be recommended that the exposure to aluminum in our daily life should be reduced, and the intake of diets rich with propolis might be a beneficial method to avoid the aluminum toxicity.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors disclose that there are no conflicts of interest.

Statement of ethical approval

Experimental protocols and procedures in this study were approved by the Deanship of Scientific Research - King Saud University through the research group no. (RG-1441- 517). All the experimental procedures were performed according to international guidelines for the care and use of laboratory animals.

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