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Protective role of propolis against reproductive toxicity of triphenyltin in male rabbits

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ABSTRACT

Triphenyltin (TPT) is known to cause endocrine disruption, reproductive toxicity and a decrease in testosterone production. It is involved in the production of reactive oxygen species. Propolis has been reported to be an important antioxidant. Therefore, the present study aimed to elucidate the possible protective effects of propolis in alleviating the toxicity of triphenyltin chloride (TPTCl) on reproductive performance, testosterone levels, lipid peroxidation and enzyme activities in seminal plasma of male New Zealand white rabbits. Animals were orally administered the doses of propolis, TPTCl and propolis plus TPTCl every day for 12 weeks. Results showed that semen quality was deteriorated following treatment with TPTCl. Also, testosterone levels, body weight (BW), relative weights of testes (RWT) and epididymis (RWE) were decreased. Thiobarbituric acid-reactive substances and lactate dehydrogenase were increased, while glutathione S-transferase, transaminases and phosphatases were decreased in seminal plasma of rabbits treated with TPTCl compared to control. Propolis alone significantly increased testosterone levels, BW, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of free radicals and lactate dehydrogenase. Furthermore, the presence of propolis with TPTCl alleviates its toxic effects. From the present study, it can be concluded propolis can be effective in the protection of TPTCl-induced reproductive toxicity.

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1. Introduction

The estimated annual amount of globally produced organotin compounds is 50,000 tons (Fent, 1996). Organotin compounds (OTC) are being, used extensively in a variety of industrial products like sportswear and plastic gloves, as heat stabilizers in the production of polyvinyl chloride (PVC), as stabilizers for improving resistance to ultraviolet radiation (Ohno et al., 2005), and used in agriculture as fungicides to protect crops (Golub and Doherty, 2004). Organotins are also used as components of ship-bottom paints as well as fishing-net anti-fouling to prevent growth of barnacles and other fouling organisms on aluminum hulled boats and ships (Kannan et al., 1995). Organotin compounds have been detected in the biota, water, and sediments from both freshwater and marine areas and their toxic effects have been observed on a variety of non-target organisms, such as fish (Fent and Meier, 1994), plankton (Fargasova and Kizlink, 1996) and gastropods (Horiguchi et al., 1997). Triphenyltin (TPT) belongs to the group of organotin compounds. Triphenyltin has been found to accumulate in the organs, muscles and head of sperms, caused distortion in reproductive hormonal regulators and altered reproduction in different animal classes (Horiguchi et al., 2002). Triphenyltin caused masculinization of female mollusks or increased the occurrence of imposex in certain water snail species (Whaley et al., 2001). It also caused a decrease in testosterone production (Ohno et al., 2005). Furthermore, triphenyltin was found to inhibit the activities of the antioxidant enzymes such as glutathione S-transferases (Yu and Huang, 2000) and to enhance the degree of phosphatidylcholine liposome membrane oxidation by the free radical forms of

Abbreviations: TPT, triphenyltin; TBT, tributyltin; TPTCl, triphenyltin chloride; OTC, organotin compounds; PVC, polyvinyl chloride; BW, body weight; FI, feed intake; RTW, relative testes weight; REW, relative epididymis weight; TBARS, thiobarbituric acid-reactive substances; GST, glutathione S-transferase; CAT, catalase; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; GSH, reduced glutathione; NO, nitrogen oxide; AST, aspartate transaminase; ALT, alanine transaminase; AcP, acid phosphatase; AlP, alkaline phosphatase; LDH, lactate dehydrogenase; ROS, reactive oxygen species; ROS, reactive oxygen species; EV, ejaculate volume; TSO, total sperm output; TMS, total motile sperm; PSV, packed sperm volume; TFSF, total functional sperm fraction, $AlCl_3$, aluminium chloride; $AlCl_3$, aluminium chloride; MDA, malondialdehyde; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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phenyltins (Gabrielske et al., 2006). The influence of reactive oxygen species (ROS) on fertility has become of increasing interest. In patients with asthenozoospermia, an elevated production of ROS in seminal plasma and increased ROS-mediated damage of sperm membranes has been detected. By altering membrane integrity, ROS may impair sperm motility as well as sperm viability. Therefore, protective agents against ROS may be useful therapeutic agents in the treatment of male infertility (Aitken, 1995).

Mammalian tissues contain several enzymes scavenging reactive oxygen species (ROS) such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione S-transferase (GST), and reduced glutathione (GSH) as controlling system of ROS and protecting cells under stress conditions. Also, there are some natural compounds contribute to the detoxification process from ROS such as propolis (Jasprica et al., 2007; Kanbura et al., 2009; Yousef and Salama, 2009).

Propolis is a resinous natural product collected from cracks in the bark of trees and leaf buds which are enriched with the salivary enzymes of honeybees. It has gained popularity and was used extensively in healthy drinks and foods to improve well-being and prevent diseases such as inflammation, heart disease, diabetes and even cancer. Propolis possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibiotic and antifungal activities (Banskota et al., 2000). Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids. In addition, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Tikhonov and Mamontova, 1987). Propolis, also contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids (Khalil, 2006). The antioxidant activity of propolis is mainly attributed to its flavonoid content, that is capable of scavenging free radicals and thereby protection against lipid peroxidation (Yousef and Salama, 2009). Propolis also induces the activation of antioxidant enzymes such as superoxide dismutase (Jasprica et al., 2007) and catalase (CAT) (Sobocanec et al., 2006) against free radicals.

It has been demonstrated that propolis provides protection against infertility by improving sperm production, motility, count and quality, and increased the process of steroidogenesis and hence testosterone production (Yousef and Salama, 2009). Furthermore, propolis protects sperm DNA from the oxidative damage caused by thiobarbituric acid-reactive substances (TBARS) (Russo et al., 2006).

There is no enough data concerning the reproductive toxicology and testicular dysfunction of triphenyltin. Also, the role of propolis against triphenyltin-induced deteriorations in reproductive performance of rabbits has not been studied so far. Therefore, the present study aimed to determine the reproductive toxicity of triphenyltin chloride in adult male rabbits, and also to assess the possible protective role of propolis in alleviating the expected reproductive toxicity and testicular dysfunction caused by the triphenyltin chloride.

2. Materials and methods

In this study triphenyltin chloride (TPTCl) and propolis were used. Triphenyltin chloride (purity 99.0%) was purchased from Sigma–Aldrich (USA) and 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 triphenyltin chloride (TPTCl) and propolis were 0.5 mg/kg bw and 50 mg/kg bw, respectively. The dose of TPTCl was selected based on a previous study of Grote et al. (2004), while the dose of propolis was used according to Newairy et al. (2009) and Yousef and Salama (2009).

Twenty-four male New Zealand white rabbits (age of 7 months and initial weight of 2.918 ± 0.029 kg) were used. 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 individually housed in stainless steel cages. Feed and water were provided *ad libitum*.

Rabbits were randomly divided into four equal groups of six rabbits each. Group 1 served as control. Groups 2, 3 and 4 were given propolis (50 mg/kg bw), triphenyltin chloride (TPTCl; 0.5 mg mg/kg bw) or their combination every day for 12 week, respectively. The doses of propolis and triphenyltin chloride were calculated according to the animal's body weight on the week before dosing.

Daily feed intake and body weight were recorded weekly. Semen collection occurred weekly over the 12 weeks of the study, so 72 ejaculates obtained per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. A weak eosin solution was used for the evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg 11, Germany) (Smith and Mayer, 1955). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in semen was carried out immediately after collection according to Mann (1948). Assessment of live and normal spermatozoa was performed using an eosin–nigrosine blue staining mixture (Blom, 1950). The percentage of motile sperm was estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. The total number of motile sperm was calculated by multiplying percentage of motile sperm and total sperm outputs. Reaction time (libido) for the buck is calculated as the time needs for mounting a doe until complete ejaculation; it was measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output (motility (%) multiplied by normal morphology (%) (Correa and Zavos, 1996). All rabbits were slaughtered at the end of the treatment period, and weights of testes and epididymis were recorded.

Blood samples were collected from the ear vein of each buck every other week and placed immediately on ice in heparinized tubes. Plasma was collected from blood by centrifugation at 860*g* for 20 min and stored at −60 °C. Testosterone concentration in plasma was measured by simple solid phase enzyme immunoassay utilizing horseradish peroxidase as a tracer (Equipar, via G. Ferrari, Saronno, Italy). Intra and inter-assay coefficient of variations were 3.9% and 6.2%, respectively.

Seminal plasma was obtained by centrifugation of semen samples at 860*g* for 20 min at 4 °C, and was stored at −60 °C until analysis. The activities of aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) activities were determined with kits from SENTINEL CH. (via principle Eugenio 5–20155 MILAN, Italy). For assaying acid phosphatase (AcP; EC 3.1.3.2) activity, the method of Moss (1984) was used. *p*-nitrophenyl phosphate is hydrolyzed in acid pH medium by the action of acid phosphatase. Liberated *p*-nitrophenyl is spectrophtometrically quantified. Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of (Principato et al., 1985). Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was determined by the method of Cabaud and Wroblewski (1958). Seminal plasma glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974), using *p*-nitrobenzylchloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in seminal at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M^{-1} Cm^{-1} was used for calculation (Tappel and Zalkin, 1959).

Data were analyzed as a randomized design (Steel and Torrie, 1981) using the General Linear Model procedure of SAS (1986). Dunnett Post hoc analysis was used to compare means of treatment groups against the control. *P* values <0.05 were accepted as significant.

3. Results

Body weight (BW), feed intake (FI), and relative weights of testes and epididymis and the levels of plasma testosterone were significantly ($P < 0.05$) decreased in rabbits treated with triphenyltin chloride (TPTCl) compared to control animals (Table 1). Propolis alone caused an increase ($P < 0.05$) in BW, FI, and relative weight of testes and epididymis and the levels of plasma testosterone. In addition, the presence of propolis with triphenyltin chloride (TPTCl) alleviated the reduction of these parameters (Table 1).

Treatment of rabbits with TPTCl caused a decrease ($P < 0.05$) in the overall means of semen ejaculate volume (EV), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal sperm, semen initial fructose and libido (by decreasing the reaction time) compared to control group (Table 2 and Figs. 1–4), while, dead sperm and initial hydrogen ion concentration (pH) were increased in animals treated with

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