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The ameliorative effect of propolis against methoxychlor induced ovarian toxicity in rat

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ABSTRACT

A study was designed to evaluate ameliorative effect of propolis against methoxychlor (MXC) induced ovarian toxicity in rat. The organochlorine pesticide (MXC) is a known endocrine disruptor with estrogenic, anti-estrogenic, and anti-androgenic properties. To investigate whether chronic exposure to MXC could cause ovarian dysfunction, two groups of Sprague–Dawley adult female rats were exposed to MXC alone in a dose of 200 mg/kg, twice/weekly, orally or MXC dose as previous plus propolis in a dose of 200 mg/l/day, in drinking water for 10 months. Another two groups of rat were given corn oil (control) or propolis. Multiple reproductive parameters, ovarian weight, serum hormone levels, ovarian oxidative status and ovarian morphology were examined. In MXC-exposed group, there is a significant decrease in body and ovarian weight vs. control. MXC decreases serum estradiol and progesterone levels. A significant increase in the levels of lipid peroxidation was obtained while a significant decrease of the total antioxidant was recorded. Ovarian histopathology showed primary, secondary and vesicular follicles displaying an atretic morphology. Increase in the ovarian surface epithelium height accompanied with vacuolated, pyknotic oocytes were obtained. The previous toxic effects were neutralized by the administration of propolis in MXC + propolis group. The present results suggest that propolis may be effective in decreasing of MXC-induced ovarian toxicity in rat.

1. Introduction

Endocrine disrupting chemicals (EDCs) such as pesticides, plasticizers, cosmetics, solvents, paints and pollutants are present in the environment. EDCs exert their toxicity by affecting the synthesis, secretion, transport, binding, action, and elimination of variety of hormones present in the body (Crisp et al., 1998). Methoxychlor (MXC) is an organochlorine pesticide that is widely used in many parts of the world, primarily to prevent or destroy pests that feed on crops and domestic animals (ATSDR, 2002). MXC gained popularity in the 1970s and replaced the potent chlorinated pesticide dichlorodiphenyltrichloroethane) DDT) after its usage was restricted in the US and other parts of the world. The use of MXC was banned in 2004 because of failure of registration with the Environmental Protection Agency (EPA) (Stuchal et al., 2006). Globally, humans and domestic animals are exposed to MXC through extensive usage of this chemical and in the US, through imported agricultural products. MXC has been shown to induce persistent vaginal estrus, decrease ovarian weights, and increase atresia (follicle death) of large follicles in adult female mice (Martinez and Swartz, 1991). Exposure to MXC in utero increases atretic follicles in F1a litters and the residual effect of MXC induces premature vaginal opening in F1b litters. In addition, mothers exposed to MXC have an increased gestation period and increased number of dead fetuses compared to vehicle controls during their first pregnancy (Swartz and Corkern, 1992). Neonatal exposure to MXC also induces ovarian atrophy, decreases relative ovarian weight, and decreases corpora lutea numbers (Eroschenko et al., 1995). Collectively, these previous studies indicate that MXC targets the ovary and affects fertility in laboratory rodents. More recent studies indicate that MXC

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damages the ovary by inhibiting antral follicle growth and increasing atresia (Borgeest et al., 2002; Miller et al., 2005; Gupta et al., 2006). While it is known that MXC inhibits growth and increases atresia of antral follicles, the mechanism by which it does so is unclear. To date, little is known about the mechanisms by which MXC induces atresia of antral follicles. Because atresia often results from oxidative stress (Hirshfield, 1991; Tilly and Tilly, 1995), and previous work suggests that MXC induces oxidative stress in male reproductive tissues (Gangadharan et al., 2001; Latchoumycandane et al., 2002; Latchoumycandane and Mathur, 2002a,b), this work was designed to determine whether MXC induces oxidative stress in the ovary.

Oxidative stress occurs when formation of reactive oxygen species (ROS) exceeds the ability of the cells to defend themselves from increased ROS. When oxidized molecules overwhelm the cell system, a cascade of detrimental effects ensues affecting the whole organ at biochemical and molecular levels (lipids, proteins, DNA, and/or RNA) (Pelicano et al., 2003). Often, there is increased cellular ROS when cells do not have enough defensive levels/activity of total antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Previous work has demonstrated that enzymes that protect against oxidative stress are important for ovarian function (Tilly and Tilly, 1995; Matzuk et al., 1998) and that MXC decreases the levels of SOD, GPX, and CAT in male reproductive tissues (Latchoumycandane and Mathur, 2002a).

There are some natural compounds contribute to the detoxification process from ROS such as propolis (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; Kanbura 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 (Mani et al., 2006).

Although the knowledge of MXC toxicity has markedly improved in recent years, little studies have examined whether MXC induces oxidative damage in the ovary. Also, the role of propolis against MXC induced deteriorations in reproductive performance of rats has not so far been studied. Thus, the purpose of this work was to test the hypothesis that MXC exerts its toxicity in the ovary by causing oxidative damage. Also was to evaluate the amelerative effect of propolis against the possible ovarian damage caused by MXC.

2. Materials and methods

2.1. Chemicals

Methoxychlor (1,1,1-trichloro-2,2-bis [methoxyphenyl] ethane, approximately 95%, was purchased from Sigma (St. Louis, MO, USA). MXC was dissolved in corn oil (1:100). The propolis samples were collected during (January–December 2012) from an apiary hive bee's located in Assiut Governorate by scraping the walls and frames of the hives. Aqueous propolis extraction (APE) was prepared according to Crane (1990): ten grams of crude propolis were added to 90 ml distilled water. The mixture was gradually heated, and allowed to boil for 3 min with shaking for 1/2 h. Then it was left at room temperature for 24 h. This procedure was repeated daily for 5 successive days. The extraction was filtered and stored at −4 °C until used. Total antioxidant capacity and lipid peroxide malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) were measured using commercial test kits supplied Bio-diagnostics (Bio-diagnostics, Cairo, Egypt). All other chemicals used in the experiment were of analytical grade.

2.2. Animals

One hundred adult female Sprague–Dawley rats, 4–6 weeks old, weighing about 100–120 g at the beginning of the experiment were used in all experiments. They were obtained from the Laboratory Animal House, Assiut University, Egypt. The animals were housed in plastic cages on wood chips for bedding and allowed to acclimatize two weeks before starting the experiment. Rats fed standard food pellets and tap water ad libitum. The rats were housed at 24–25 °C and humidity (65%) and in daily dark/light cycle. The studies were conducted in accordance with the principles and procedures outlined in the National Institute of Health of USA (NIH) guide for the Care and Use of the Laboratory Animals (National Research Council, 1996).

2.3. Experimental design

Rats were randomly divided into four groups of twenty-five animals each as follows: MXC-treated group received an oral dose of MXC 200 mg/kg b.w., twice/week, by gavage for 10 months. This dose was selected because it has been used in previous studies without demonstrating toxic effects in the exposed animals (Anway et al., 2005; Murono et al., 2006). MXC + propolis group was concomitantly treated with both MXC as previously described and propolis daily in a dose of APE 200 mg/l orally, in drinking water for 10 months. Propolis-treated group was received propolis daily as previous. This dose was used according to the previous studies of Bhadauria et al. (2007a,b). Control group received an oral dose of 2 ml corn oil, twice/week.

2.4. Sample collections

After 10 months of MXC exposure, all female rats were visually inspected and their body weight was recorded immediately before termination. They were anesthetized with CO_2 and decapitated. Trunk blood was collected from all animals after decapitation and allowed to clot at 4 °C. Sera were collected and stored at −80 °C until determination of steroid hormones by radioimmunoassay. Ovaries (left and right) were carefully removed from the body cavity, cleaned of adhering tissue and weighed for all animals. Left ovaries from the control group and from the treated groups were prepared for total antioxidant capacity and lipid peroxidation analyses while the right ones were used for histopathology for the same previous groups.

2.5. Serum steroid

Serum concentrations of progesterone (P_4) and estradiol (E_2) were measured as previously described by Terranova and Garza (1983).