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Antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos in rats

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ABSTRACT

Pesticides induce oxidative stress leading to generate free radicals and alternate the antioxidant or oxygen free radical scavenging enzyme system. This study was conducted to investigate the oral toxicity of chlorpyrifos toward male rat and the oxidative stress of the sub-lethal dose (9 mg/kg; 1/25 LD_{50}) on the lipid peroxidation level (LPO), reduced glutathione content (GSH) and antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities of testicular tissue. Also, the protective effects of propolis extract (50 mg/kg b.w.) alone or in combination with chlorpyrifos were investigated. The oral administration of chlorpyrifos significantly caused elevation in LPO level by 1.79-fold as compared to control. The activities of antioxidant enzymes including CAT, SOD, GPx and GST were decreased significantly (23.66%, 27.75%, 29.13% and 11.52%) as well as the level of GSH decreased by 21.97% in testicular tissue as compared to control animals. Co-administration of propolis extract with chlorpyrifos or alone in male rats decreased LPO level, normalized CAT, SOD GPx and GST activities, while GSH content was increased in testicular tissue. We conclude that propolis extract significantly reduces chlorpyrifos-induced oxidative stress in rat testis and the protective effect of the pre-treatment with propolis extract as attenuating agent could be due to its antioxidant properties.

1. Introduction

Occupational exposures to pesticides could diminish or destroy the fertility of workers sparked a concern about the effects of hazardous substances on male reproductive health. The issue of testicular toxicity is of growing concern as a large number of organophosphates viz., diazinon [1], and methyl parathion [2] adversely affect the testicular functions in experimental animals. Owing to the extensive use of organophosphate pesticides in agriculture there is a high risk of human exposure to these chemicals [3]. Pesticides may cause generation of reactive oxygen species (ROS), which may lead to oxidative stress, indicating the role of ROS in pesticide toxicity [4]. Pesticide-induced oxidative stress has been also a focus of toxicological research for the last decade as a possible mechanism of toxicity [5,6]. Antioxidant defenses such as catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) are involved to counteract the toxicity of ROS. Under normal conditions these antioxidants they protect the cells and tissues from oxidative damage.

Chlorpyrifos (CPF) is one of a conventional organophosphorous insecticide. It was used widely to control a variety of pests in agriculture and animal farm [7]. The active metabolite of CPF inhibited acetylcholinesterase [8]. The biotransformation of CPF is catalyzed by cytochrome P450 and associated enzymes, which are present in microsomal membranes of liver [9]. In fact, one of the molecular mechanisms of the toxicity of some pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the red blood cells, liver and kidney [10–12]. CPF treatment in these studies resulted in increased oxidative stress in the body, as evidenced by enhanced levels of thiobarbituric acid reactive substances (TBARS), accompanied by concomitant decrease in the levels of superoxide scavenging enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in serum, liver, kidney, retina and spleen [9–15].

Joshi et al. [16] assessed the effects of chlorpyrifos on testes, at the dose levels of 7.5, 12.5 and 17.5 mg/kg b.w./day administered

Abbreviations: b.w., body weight; CPF, chlorpyrifos; LPO, lipid peroxidation; GSH, reduced glutathione; GSSG, oxidized glutathione; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase; MDA, malondialdehyde; GST, glutathione S-transferase; ROS, reactive oxygen species.

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orally to male rats of Wistar strain for 30 days to evaluate the toxic alterations in testicular histology, hormone, sperm dynamics and testosterone levels. They found that CPF brought about marked reduction in epididymal and testicular sperm counts in exposed males and a decrease in serum testosterone concentration. Histopathological examination of testes also showed mild to severe degenerative changes in seminiferous tubules at various dose levels. Fertility test showed negative results whereas the protein was raised at significant levels. All these toxic effects are moderate at low doses and become severe at higher dose levels. El-Kashoury and Tag El-Din [7] investigated the toxic effects of three trade names of formulated CPF from different local manufactures (chlorozan, pestpan, and pyriban) on testicular oxidative stress and biochemical parameters administrated orally to male albino rats at dose of 23.43, 21.40 and 17.43 mg/kg b.w. with 5 doses per week for 28 days. Their findings demonstrated that CPF treatments alter markedly the testicular lipid peroxidation (LPO) levels, total protein (TP) level also exhibited an elevation in testicular tissue while, the decline in the total glutathione (GSH) was occurred only in two groups that treated with the high doses.

Reproductive behavior is also considered a promising tool in ecotoxicology and provides integrative measures of reproductive toxicity, reflecting biochemical and physiological reactions to the toxicant and broadly measures reproductive toxicity in human [18]. These studies are becoming prominent in toxicity assessments in rats [16–18], and mice [19]. The previous data showed that CPF (9 mg/kg b.w. for consecutive 70-days) was significantly decreased the sperm counts, spermatozoon survival and testosterone level as well as increase of sperm aberrations. CPF also increased significantly the lipid profile and the levels of serum liver marker enzymes [18].

Propolis has been mainly used as home remedies and a personal product [20]. EEP has antimicrobial and antibacterial properties. Propolis helps keep the hives free of germs. It is ideal for shampoos, conditioners and sprays [21]. Raw propolis typically concerts of waxes, resins, water, inorganics, phenolics and essential oils, the exact composition of which is dependent upon the source plant(s). After evaluating the IC_{50} found for propolis extract, it was concluded that the best antioxidant activity of the extracts of propolis studied was found for the superoxide radical generated, followed by lipid peroxidation inhibition and scavenging $^{\cdot}OH$ radicals in the deoxyribose assay [22]. The antioxidant activity of propolis extract is mainly attributed to its flavonoid content, that is capable of scavenging free radicals and thereby protection against lipid peroxidation [23]. Propolis also induces the activation of antioxidant enzymes such as superoxide dismutase and catalase (CAT) against free radicals [24]. *Baccharis dracunculifolia* (the main botanical source used by honeybees to produce Brazilian green propolis) exhibit potent antioxidant activity protecting liver mitochondria against oxidative damage and such action probably contribute to the antioxidant and hepatoprotective effects of green propolis [25]. This study is parallel with our another study [18] in which we found the exposure to CPF caused deterioration in semen quality, decrease of fertility indexes as well as alternation in lipid profile and decreased the enzyme activities of serum. Moreover, the testosterone decreased and there were many histological changes in the tests.

Testes are the main organ of male reproduction. So, the principle objective of the present study was to assess the oxidative damage sustained by testes following the exposure to CPF. This study was also planned to evaluate the role of propolis extract as a protective agent against chlorpyrifos-induced testes toxicity by measuring some oxidative and antioxidant parameters such as levels of LPO, TP, GSH and also activities of CAT, SOD, GPx and GST in testicular tissue.

2. Materials and methods

2.1. Chemicals

CPF technical grade (98%) was obtained from El-Helb Company for pesticides and chemicals, Egypt. Brazilian Ethanolic extract of propolis (EEP) was obtained from Sigma, St. Louis, MO, USA (P8904, pH 7.3). EEP contains flavonoid and caffeic acid phenethyl ester "CAPE" as active components of propolis as indicated by sigma. The biological activities of Brazilian propolis are mostly due to the high levels of phenolic acids [26].

Thiobarbituric acid, H_2O_2, S-2, 4-dinitrophenyl glutathione, 5,5′-dithiobis-(2-nitrobenzoic acid), phosphoric acid, butanol, sodium phosphate, sodium carbonate, sodium azide, EDTA, Tris–HCl, epinephrine ware brought from Sigma, St. Louis, MO, USA. Kit of GSH was obtained from Biodiagnostic for diagnostic reagents; Dokki, Giza, Egypt.

2.2. Animals

Healthy adult male albino rats of the Wistar strain *(Rattus norvegicus)* with proven fertility, (4–5) months of age and weighing 150–160 g, were supplied from the Animal Breeding House of the Medical Research Institute (MRI), Alexandria University, Alexandria, Egypt. Animals were maintained at the animal care facility in the Zoology Department, Faculty of Science, in plastic cages under controlled temperature (23 ± 2 °C), 12-h light/dark cycle and 50 ± 5% relative humidity. Water and food were available *ad libitum*. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments. The European Community Directive (86/609/EEC) and National rules on animal care have been followed.

2.3. Experimental design

After the period of acclimation, animals were divided into four groups with 25 animals in each. The first group was used as control. The animals of control group were orally given corn oil (4 ml/kg). The second male group was orally treated with CPF (9 mg/kg b.w.); about 1/25 LD_{50}. The third male group was orally treated with dissolved propolis extract (50 mg/kg b.w.) and fourth group was treated with combination of CPF (9 mg/kg b.w.) and propolis extracts (50 mg/kg b.w.). The selected dose of the CPF was based on studies of McCollister et al. [27] and the dose of propolis extract was according to the previous study of Park and Kahng [28]. The duration of the oral administration during the experiments lasts for 70-day for completion of the spermatogenic cycle and maturation of sperms in epididymis [3]. Ten animals only have been used for the investigation of the following parameters.

2.4. Preparation of homogenate tissue

The excised testicular tissue was washed with distal water for the removal of blood, and later the fatty parts were removed. Tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). The supernatant was separated by means of centrifugation at 1000*g* for 20 min at 4 °C. The supernatant were used for the analyzes of all antioxidant enzymes.

2.5. Determination of oxidative and antioxidant parameters

2.5.1. Lipid peroxidation (LPO) level

Lipid peroxidation process is determined in supernatant of homogenate testicular tissue by the thiobarbituric acid (TBA)