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RESEARCH ARTICLE

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EVALUATION OF PROTECTIVE EFFECT OF PROPOLIS AGAINST TESTICULAR OXIDATIVE DAMAGE, LIPID PEROXIDATION AND INFERTILITY INDUCED BY CHLORPYRIFOS IN ALBINO RATS

ABSTRACT:

Pesticides may induce oxidative stress leading to generate free radicals and alternate antioxidant or oxygen free radical scavenging enzyme system. This study was conducted to investigate the effect of the oral toxicity of chlorpyrifos toward male rat and the oxidative stress of the sub-lethal dose (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. Also, the protective effects of propolis (50 mg/kg body weight, BW) alone or in combination with chlorpyrifos were investigated**.** The oral administration of 9 mg/kg chlorpyrifos significantly caused elevation in LPO level. The activities of antioxidant enzymes including CAT, SOD, GPx and GST were decreased significantly as well as the level of GSH in testicular tissue. Co-administration of propolis with chlorpyrifos or alone in male rats decreased LPO level, normalized CAT, SOD GPx and GST activities, while GSH content increased in testicular tissue. In conclusion, propolis significantly reduces chlorpyrifos-induced oxidative stress in rats testes and the protective effect of the pre-treatment with propolis could be due to its antioxidant properties.

KEY WORDS:

Chlorpyrifos; Propolis; Fertility; lipid peroxidation; antioxidant enzymes

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INTRODUCTION

Owing to the extensive use of organophosphate pesticides in agriculture there is a high risk of human exposure to these chemicals (Sarkar *et al.*, 2000). The issue of testicular toxicity is of growing concern as a large number of organophosphates viz., diazinon (U.S. Department of Health and Human Services, 1994) and methyl parathion (Joshi *et al.*, 2003) adversely affect the testicular functions in experimental animals.

Chlorpyrifos (O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is a conventional organophosphorous insecticide that widely used to control a variety of pests in agriculture and animal farm (USEPA, 1986). Chlorpyrifos interferes with the activity of acetyl cholinesterase enzyme, which is necessary for normal nerve transmission (NRA, 2000). Oxidative stress is defined as a disruption of the pro-oxidant - antioxidant balance in favor of the former, leading to potential damage (Sies, 1991). It is a result of one of three factors: an increase in reactive oxygen species (ROS), an impairment of antioxidant defense system or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipid, DNA, and/or protein. The damage may alter cell function through changes in intracellular calcium or intracellular pH, and eventually can lead to cell death (Kehrer, 1993).

Under normal condition, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense system. This preventive defense system can be accomplished by enzymatic and non-enzymatic mechanisms including vitamins and glutathione. The antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) may have an important role in mitigating the toxic effects of ROS (Adali *et al*., 1999).

Recent studies indicated that the toxic manifestations induced by pesticides may be associated with the enhanced production of ROS, which may provide an explanation for the multiple types of toxic responses (Bagchi *et al.*, 1995; Verma *et al.*, 2007). Also, the production of ROS has been proposed as a mechanism by which xenobiotics and pathological conditions may produce oxidative stress and induce various tissue damages (Oncu *et al.*, 2002, Yu *et al.*, 2008). Several studies report that ROS have been implicated in the toxicology of organochlorine (Bagchi *et al.*, 1995) and organophosphates (Goel *et al.*, 2005; Verma *et al.*, 2007; Yu *et al.*, 2008).

Propolis (CAS No. 9009-62-5) (sometimes also referred to as 'bee glue') is the generic name for the resinous substance collected by honeybees from various plant sources. Propolis has been mainly used as home remedies and a personal product since 300 BC (Fu *et al.*, 2004), as well as Chinese traditional medicine. Extracts of propolis are receiving renewed attention worldwide because of their beneficial effects, among them, the effective antioxidant activity and a general "back to nature trend". Propolis typically conserts of waxes, resins, water, inorganics, phenolics and essential oils depending on the plant sources (Dobrowolski *et al.*, 1991). It was concluded that the best antioxidant activity of the extracts of propolis was found for the superoxide radical generated, followed by lipid peroxidation inhibition and scavenging •OH radicals in the deoxyribose assay (Marquele *et al.*, 2005). 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 and catalase (CAT) against free radicals (Jasprica *et al.*, 2007).

Testes are the main organ of male reproduction. The present study is planned to evaluate the role of propolis as a protective agent against chlorpyrifos-induced testes toxicity by measuring some antioxidant parameters such as lipid peroxidation, reduced glutathione and activities of CAT, SOD, GPx, and GST.

MATERIAL AND METHODS:

Chemicals:

Chlorpyrifos (CPF) technical grade (98%) was obtained from El-Helb Company for pesticides and chemicals, Egypt. Ethanolic extract of propolis was obtained from Sigma, St. Louis, MO, USA (P8904, EEP, pH 7.3).

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.

Experimental Design:

After the period of acclimation, animals were divided into four groups with twenty five animals per 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 BW); 1/25 LD_{50} (McCollister *et al.*, 1974). The third group was orally given propolis (50 mg/kg BW) and fourth group was treated with combination of CPF (9 mg/kg BW) and propolis (50 mg/kg BW).

Mating and fertility indexes:

After the end of the treatment course, males of both control and experimental groups of treated rats (n=25/ group), were mated 1:1 with untreated proven fertile, with regular estrus cycle, females for 5 days (complete one estrous cycle) (Fox and Laird, 1970). Mating was confirmed by the presence of vaginal plugs or visualization of spermatozoan in vaginal smear and day 0 of gestation was determined.

Pregnant females were anaesthetized by diethyl ether on day 20 of gestation. The uterine horns were macroscopically examined and number of implantation sites (Ambali *et al.* 2010). The % pre-implantation loss was calculated as described by US EPA (US EPA, 1996) and Bindali and Kaliwal (2002). Fertility mating indexes, number of implantation sites, resorptions, and dead and live fetuses were recorded.

Sperm Quality:

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 (Sarkar *et al.*, 2003). The left cauda epididymis was used for sperm motility and right cauda epididymis was used for sperm counts and morphology. The counting of both motile and immotile sperms was done at 40x magnification. Total number of the sperm head counted was counted using the hemocytometer and the results were finally expressed as percent motility (Freund and Carol, 1993).

Preparation of Homogenate Tissue:

The excised testicular tissue was washed with deionized water for the removal

of blood. Homogenization of known weight of testicular tissue was performed in a phosphate buffer solution with a pH 7.4, and the supernatant was separated by centrifugation at 1000 g for 30 min. The obtained supernatant and hemolysate were used for the analyses of the assayed antioxidant enzymes.

Biochemical analysis:

a- Lipid peroxidation (LPO) level:

Lipid peroxidation process is determined by the thiobarbituric acid (TBA) method via determining the malondialdehyde formation (MDA) according to Esterbauer and Cheeseman (1990). The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56×10^5 $M^{-1}cm^{-1}$). Lipid peroxidation is expressed as nano moles MDA/g tissue.

b- Antioxidant enzymes:

The specific activity of testicular superoxide dismutase (SOD) was determined according to the method described by Misra and Fridovich (1972). The enzyme catalase (CAT) converts H_2O_2 into water. Activity of SOD is expressed as units/mg protein. The testicular 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). Activity of CAT is expressed as units/mg protein. GPx catalyzes the reduction of hydroperoxides by utilizing GSH as a reluctant. Determination of testicular GPx activity was carried out according to the method of Chiu *et al.* (1976). The activity of this enzyme was estimated by measurement of the residual reduced glutathione remaining after the action of the enzyme with the Ellman's reagent (DTNB) in the presence of cumene hydroperoxide as a secondary substrate. Specific activity of this enzyme is expressed as OD/mg protein/min. Glutathione-S-transferase (GST) activity of testicular was measured spectrophotometrically by the method of Habig *et al.* (1974) using S-2,4-dinitrophenyl glutathione (CDNB) as a substrate. The activity of GST was expressed in terms of µmol/min/mg protein.

c- Reduced glutathione content:

Reduced Glutathione content (GSH) of supernatant estimation was performed by the method of Beutler *et al.* (1963) using commercial glutathione reduced kits (Biodiagnostic for diagnostic reagents: Dokki, Giza, Egypt). Determination of GSH is based on the reaction of DTNB (50,5- dithiobis-(2-nitrobenzoic acid)) with GSH and yield a yellow colored chromophore; 5-thio-nitrobenzoic acid with a maximum absorbance at 412 nm. The amount of GSH present in the testicular tissue was calculated as nmole/g tissue.

d- Serum protein assay:

The total protein level of supernatant was determined according to Henry (1964).

Statistical analysis:

Data are expressed as mean values ± SD (n=10). Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the post hoc Tukey's test was used for comparisons. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS® Inc., USA).

RESULTS:

Sperm quality:

Data of sperm quality of rat in control and propolis groups were 91.2 ± 9.85 and 90.4 ± 8.68 (million/ml), respectively (Table 1). Sperm count significantly ($P < 0.01$) decreased in treated-rat with CPF to 56.1 ± 4.65. Motility percentage of sperm in control rat was 59.4 ± 4.68. This percentage decreased to more than half in the treated-rat with CPF as compared to control. Treatment with propolis alone showed no significant effects on sperm concentration, motility (%) and sperm head abnormalities (%) as compared to control group. On the other hand, treatment with propolis in combination with CPF caused significantly decline in sperm concentration and motility, and significantly increased the percent of viability.

Table 1. The effect of chlorpyrifos and/or propolis on the sperm quality in male rats after oral administration for 70 days

Sperm Parameters	Control	Chlorpyrifos	Propolis	Chlorpyrifos and propolis	Value of P
Count (million/ml) %	91.2 ± 9.85	56.1 ± 4.65 [a]	90.4 ± 8.68	78.3 ± 6.98 [b]	0.012
Motility %	59.4 ± 4.68	25.1 ± 2.06 [a]	55.6 ± 4.65	42.8 ± 3.88 [b]	0.021
Head abnormalities %	13.5 ± 2.33	37.6 ± 2.96 [a]	14.7 ± 1.07	21.2 ± 2.41 [b]	0.013

The data are presented as mean ± S.D, n = 25.

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

Reproductive outcomes (Reproductivity):

No fetal death was recorded throughout the experimental groups. There were no significant differences between the controls and the propolis groups for any of the reproductive parameters assessed. However, there were statistically significant reduction in the number of viable fetuses/dam and significant increase in post-implantation loss and resorption/dam (Table 2).

Table 2. Reproductive outcomes of untreated females after cohabitation with treated male rats with chlorpyrifos (9 mg/kg BW) and/or propolis (50 mg/kg BW)

Parameters	Control	Chlorpyrifos	Propolis	Chlorpyrifos and propolis	Value of P
Number of pregnant females	25	7	24	14	
No of implantations/litter	10.47 ± 1.06	10.34 ± 1.02	10.42 ± 1.10	10.48 ± 0.96	> 0.05
Live (%)	10.42 ± 0.98 (99.52)	9.44 ± 0.98 (91.30)	10.33 ± 0.96 (99.14)	10.14 ± 1.07 (96.76)	> 0.05
Dead (%)	0.00	0.00	0.00	0.00	
Early resorption / litter (%)	0.05 ±0.0032 (0.48)	0.90 ± 0.036[a] (8.70)	0.09± 0.013[a] (0.86)	0.34 ± 0.021[b] (3.24)	0.013*
Late resorption / litter (%)	0.00	0.00	0.00	0.00	
Post implantation loss %	0.48 ± 0.016	8.70 ± 1.25[a]	0.86 ± 0.106[a]	3.24 ± 0.65[b]	0.031*

Data are presented as mean ± SD (n = 25).

Post implantation loss % = [(No. of implants–No. live fetuses)/No. of implants] X 100.

[a] Significant different as compared to control at P ≤ 0.05.

[b] Significant different as compared to chlorpyrifos group at P ≤ 0.05.

Testicular level of lipid peroxidation (TBARS):

Treating rats with CPF resulted in a significant increase in the levels of MDA (93.81 ± 9.55) as compared to control animals (52.43 ± 4.06). Treatment the rats with propolis only or with CPF decreased the MDA significantly as compared to control or CPF animals, respectively (Fig. 1).

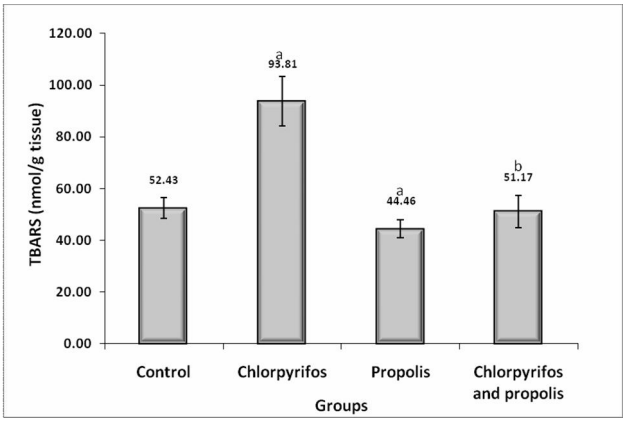

Fig. 1. Changes in the testicular level of lipid peroxidation in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± SD for groups of n = 10 rats.

[a] Significantly different from control group p < 0.001.

[b]Significantly different from chlorpyrifos group p < 0. 01.

Testicular activities of SOD, CAT, GPx and GST:

The present results revealed that chlorpyrifos produced a statistically significant decrease (p < 0.01) in SOD activity in male rats (Fig. 2) compared to the control value. Administration of propolis to chlorpyrifos-treated group of male rats improved the levels of SOD towards the control values although this treatment could not normalize it. Treatment with propolis alone did not result in significant alteration in SOD activity compared to control treatment.

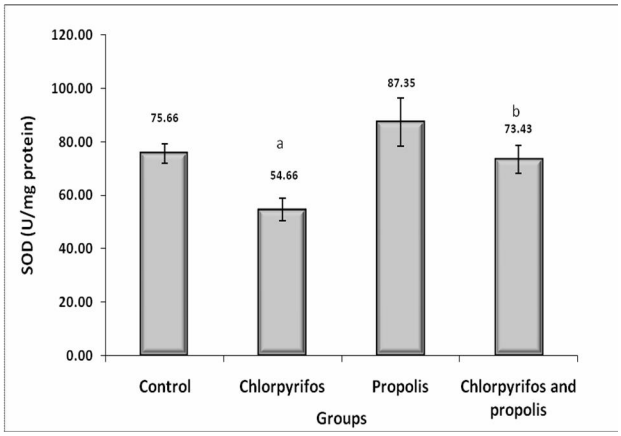

Fig. 2. Changes in the testicular activity of SOD (units) in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± SD (n = 10).

[a]Significantly different from control group p < 0.001.

[b]Significantly different from chlorpyrifos group p < 0. 001.

The result clearly indicated that treatment with CPF resulted in a significant decrease in the activities of testes CAT, GPx and GST as compared to control animals (Figs. 3-5). However, male rats treated with propolis showed significant increase (P < 0.01) in GPx, GST and CAT. When propolis administrated with CPF, there was amelioration of the activities of CAT and GST to almost normal values (Figs 3&5).

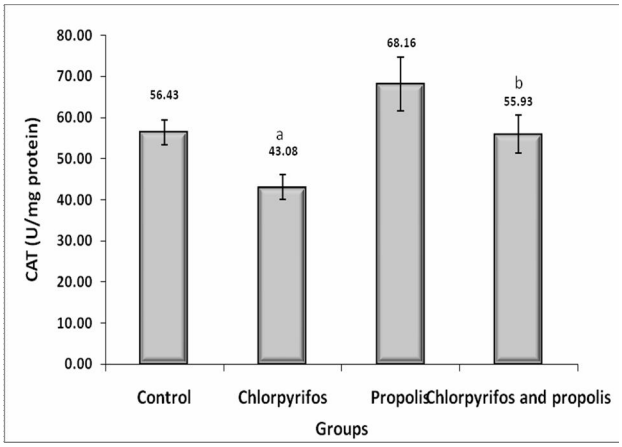

Fig. 3. Changes in the testicular activity of CAT (units) in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± S.D. (n = 10).

[a]Significantly different from control group (p < 0.001).

[b]Significantly different from chlorpyrifos group (p < 0. 01).

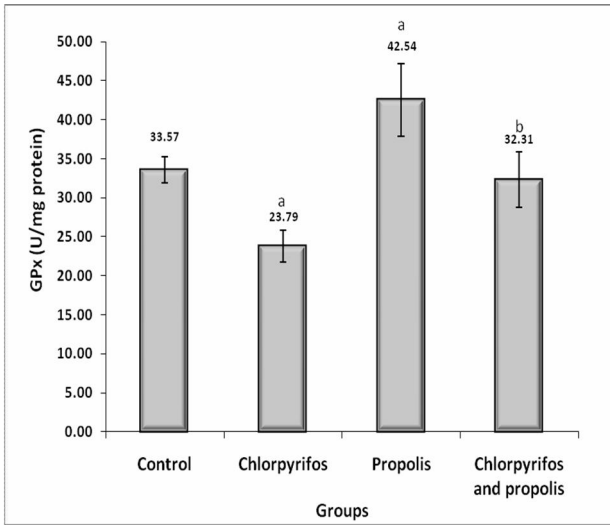

Fig. 4. Changes in the testicular activity of GPx (units) in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± S.D. (n = 10).

a Significantly different from control group ($p < 0.001$).

b Significantly different from chlorpyrifos group ($p < 0.01$).

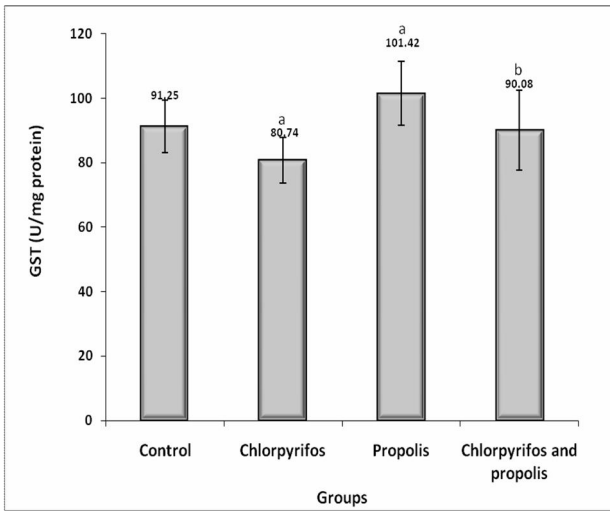

Fig. 5. Changes in the testicular activity of GST U/mg protein in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± S.D. (n = 10).

aSignificantly different from control group ($p < 0.001$).

bSignificantly different from chlorpyrifos group ($p < 0.01$).

Testicular level of reduced glutathione:

The result of testicular reduced glutathione (GSH) level is presented in figure 6. These results clearly indicated that treatment with CPF resulted in a significant decrease in the level of testes GSH comparing to control. However, applied propolis treatment showed significant increase in testes GSH content ($P < 0.05$). Combined treatment (GPF + propolis) revealed amelioration of the activity GSH.

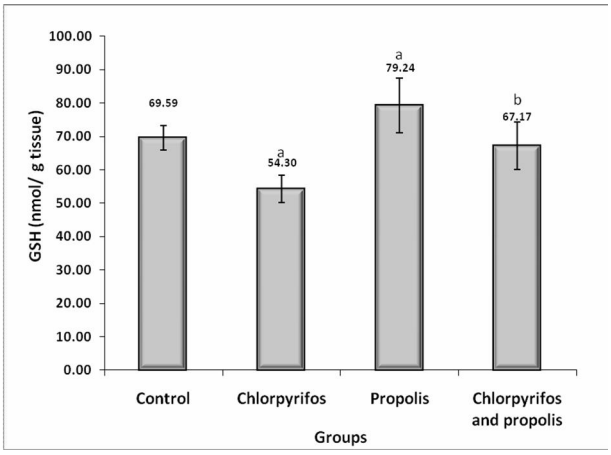

Fig. 6. Changes in the testicular activity of GSH in rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± S.D. (n = 10).

a Significantly different from control group ($p < 0.001$).

b Significantly different from chlorpyrifos group ($p < 0.01$).

Serum total proteins:

A significant decrease in the serum total protein was detected in chlorpyrifos treated group (Fig. 7). While, propolis administration significantly increased total protein and alleviated the negative effects for CPF treated group.

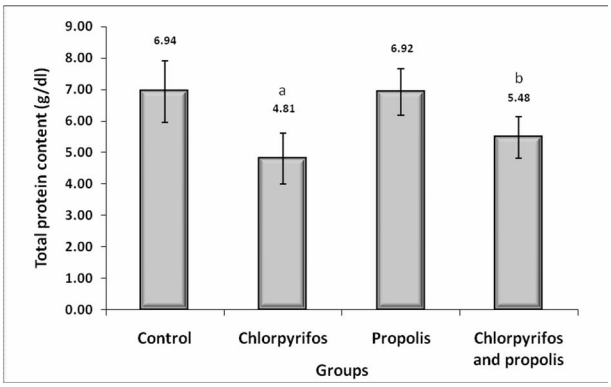

Fig. 7. Changes in serum total protein level of rats treated with chlorpyrifos, and/ or propolis.

Data are presented as mean ± S.D. (n = 10).

a Significantly different from control group ($p < 0.001$).

b Significantly different from chlorpyrifos group ($p < 0.01$).

DISCUSSION:

Because the use of organophosphate pesticides has been and remains pervasive in both developed and developing nations, concerns are increasing regarding the relative safety of these chemicals to the environment and human health (Saulsbury *et al.*, 2009). Organophosphates are chemicals which inhibit cholinesterase, however; several reports proved that these insecticides induce oxidative stress and apoptosis (Qiao *et al.*, 2005; Abdou and ElMazoudy, 2010). Oxidative stress caused by ROS has been reported in

membrane lipid peroxidation and DNA damage (Verma *et al.*, 2007).

The present work evaluates the protective role of propolis against the oxidative stress changes in testicular tissue resulting from the administration of CPF in rats. The biochemical mechanisms involved in the testis toxicity of CPF were studied by measuring the levels of LPO, GSH and by screening the activities of primary antioxidant enzymes such as SOD, CAT, GPx and GST.

Oxidative stress refers to disrupted redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these species. Defense against oxidative stress are maintained by using several mechanisms which include antioxidant machinery (Voellmy, 1999).

The main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in cell membrane), proteins (denaturation), carbohydrates and nucleic acids; this in turn can impair cellular structure and function (Bergamini *et al.*, 2004). It has been indicated that the LPO is one of the molecular mechanisms involved in pesticide-induced cytotoxicity (Abdollahi *et al.*, 2004). These findings support the occurrence of oxidative stress in the present study induced by CPF. The results showed that the LPO level was significantly increased in rat testicular tissue treated with CPF. The toxic manifestations induced by the tested pesticide may be associated with the enhanced production of ROS or the increase in MDA levels which is induced by the pesticide itself (degradation of phospholipids and ultimately result in cellular deterioration) or by a possible increase in free radicals caused by CPF (Gultekin *et al.*, 2001).

Among the antioxidant enzymes, SOD, CAT, GPx and GST are the first line of defence against oxidative injury. SOD is the primary step of the defence mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (Halliwell, 1994). H_2O_2 is neutralized by the combined action of CAT and GPx in all vertebrates (Wetscher et al, 1995). These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the levels of enzymes. The current data displayed CPF-induced reduction in the activities of the antioxidant enzymes (SOD, CAT, GPx and GST) and the content of non-enzymatic antioxidant (GSH). This effect might be due to increased H_2O_2 production and ROS generation which in turn induces oxidative stress.

In the present study, a significant decrease in the specific activity of SOD in testicular tissue was observed in CPF-treated testicular tissue, suggests an increased superoxide radical production and other ROS thereby induce oxidative damage (El-Shenawy and Al-Eisa, 2010). CAT activity of testicular tissue decreased in CPF-treated rat. These data are parallel with Bindhumol *et al.* (2003) and Banudevi *et al.* (2006) who found that bisphenol A and PCB's decreased the activity of both CAT and SOD. Also, Joshi *et al.* (2007) reported that sub-acute exposures to CPF induced oxidative stress in testes of rats. Since insecticides produced excessive ROS either directly or indirectly, the counter balancing effect of the antioxidant enzymes is lost (Banerjee *et al.*, 1999; Arthur, 2000; Seth *et al.*, 2001). The present results are coincident with Abdollahi *et al.* (2004), El-Shenawy and Al-Eisa (2010) and El-Kashoury and Tag El-Din (2010) who indicated that increasing CPF concentration caused a significant reduction in the activities of SOD and CAT and a significant increase in the level of LPO.

GPx is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biological function of GPx is to reduce lipid hydroperoxides conversion to their corresponding alcohols and to reduce free H_2O_2 reaction (Goel *et al.*, 2005; Ran *et al.*, 2007). In our experiment, GPx activity was decreased in testicular tissue of rats treated with CPF. This result is in contrast with many authors who found that GPx activity was not altered in rats exposed to CPF (Jett and Navoa, 2000; Gultekin *et al.*, 2000& 2001; El-Shenawy and Al-Eisa, 2010). Another studies observed that GPx activity was increased (Gultekin *et al.*, 2001; Goel *et al.*, 2005). However, it has been also reported that organophosphate pesticides caused a decrease in GPx activity both *in vivo* and *in vitro* (Altuntas *et al.*, 2003; Verma and Srivastava, 2003). Meanwhile, it has been reported that OPIs, such as phosphomidone, trichlorfom and dichlorvos caused a decrease in GPx activity (Naqvi and Hasan, 1992). Also, administration of mixture of pesticide including CPF reduced the activities of GPx in rat testes (Mattson *et al.*, 1996). In this study, a significant fall in GSH level and GSH-Px activity was observed in CPF treated animals, may be due to enhanced free radical production (as evidence by increase LPO) and apart from CAT, GSH-Px also involved in the removal of H_2O_2. H_2O_2 generated due to CPF toxicity, engage more GSH, which thereby get converted to oxidized glutathione (GSSG) in presence of GPx. Hence, the GSH, and GPx level decreases on CPF administration.

GST is a family of phase II detoxifying enzymes with broad substrate specificities that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (Hayes *et al.*,

2005; Lee-Hilz *et al.*, 2006). The present study found a decrease of GST activity in animals intoxicated with CPF. This decrease may be due to the GSH and glutathione dependent enzyme systems that provide major protection against the toxic agents.

A pronounced decrease of GSH level was found in testis of the rat intoxicated with CPF; this may be responsible for enhancement of LPO. Several studies observed depletion of GSH in CPF-intoxicated animals (Goel *et al.*, 2005; Verma *et al.*, 2007). GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST (Mascio *et al.*, 1991; Hayes *et al.*, 2005). Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increase of LPO (Cathcart, 1985). GSH in the testis acts either by directly scavenging the free radicals or by acting as a substrate to GPx and GST during the detoxification of hydrogen peroxides, lipid peroxides and electrophiles as well as by preventing oxidation of –SH groups of proteins (Hayes *et al.*, 2005). These results are in accordance with the study of El-Shenawy and Al-Eisa (2010); they reported that CPF decreased GSH content after 30 min of incubation with hepatocytes of rat.

The results of the present investigation clearly indicate that providing the rat with propolis found to be effective as improving the antioxidant level and decreasing the oxidative stress. Enhanced reduction in LPO levels observed with oral administration of propolis with CPF suggested that propolis consumption may be reducing or suppressing the release of free radicals. Propolis also increased the activities of enzymatic antioxidants of testicular tissue (SOD, CAT, GPx and GST).

Yousef and Salama (2009) studied the protective effect of propolis against the reproductive toxicity of aluminium chloride ($AlCl_3$). They found that the presence of propolis with $AlCl_3$ had protective effects against its reproductive toxicity and this may be due to the activity of propolis as antioxidant. Jasprica *et al.* (2007) reported that propolis can inhibit membrane LPO and free radical formation due to its free radical scavenging ability. The biological and antioxidant effects exhibited by propolis could be related to an overall effect of the phenolic compounds present in propolis (flavonol galangin; hydroxycinnamic acids, caffeic acid, p-cumaric acid, ferulic acid and caffeic acid phenethyl ester) (Russo *et al.*, 2006). The antioxidant capability of hydroxycinnamic acids (caffeic acid, p-cumaric acid and ferulic acid) as well as that of flavonol galangin is reported (Lee *et al.*, 2003). Kanbur *et al.* (2009) found a decrease in the plasma and tissue (liver, kidney and brain) malondialdehyde (MDA) levels, and an increase in the antioxidant enzyme parameters (SOD, CAT, and GPx) of animals that were administered propolis in association with propetamphos, in comparison to the group that was administered propetamphos alone. The primary mechanism of this effect of propolis may involve the scavenging of free radicals that cause LPO. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated, by propolis. Studies exist, which report xanthine oxidase to be inhibited by propolis (Harris *et al.*, 2000). Among other studies that demonstrate the mechanisms responsible for the antiradical and antioxidant activities of propolis, in a trial conducted by Matsushige *et al.* (1995), propolis has been determined to exhibit antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the superoxide radical by means of xanthine/xanthine oxidase and NADH/ phenazine reactions.

Sperm motility is an important functional measurement to predict sperm fertilizing capacity. Any negative impact on motility would seriously affect fertilizing ability (Joshi *et al.*, 2007). CPF has been shown to induce reproductive abnormalities in male rats causing reduced fertility by inducing oxidative stress in the epididymal sperm. These data could be confirmed by decreasing enzymatic and non-enzymatic antioxidants and increased levels of hydrogen peroxide and LPO after 70 days of treatment.

Regarding to the protective role of propolis, against CPF-induced testis toxicity, the present study reported that propolis afforded a significant improvements in rat sperm characteristics. Such improvements agree with recent study that reported benefits of propolis supplementation on sperm quality and male fertility of rabbit (Yousef and Salama, 2009).

CONCLUSION:

In conclusion, the present results showed that CPF increased LPO level and decreased the activities of antioxidant enzymes and GSH content by increasing the oxidative stress. The depletion of antioxidant enzyme activity was may be due to inactivation of the enzyme proteins by CPF-induced ROS generation, depletion of the enzyme substrates, and/or down-regulation of transcription and translation processes. Additionally, the treatment with propolis pre-chlorpyrifos administration can prevent or slow down the oxidative damage induced by CPF in testicular tissue of rats. The administration of propolis with known

antioxidant property caused alterations in oxidative stress parameters and alleviated the severity of oxidative stress. In addition, the administration of propolis was concluded to exhibit antiradical and antioxidant effect, and therefore to result in the alleviation of testicular oxidative stress and lipid peroxidation.

REFERENCES:

Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. 2004. Pesticides and oxidative stress: a review. Med. Sci. Monit., 10(6): RA141–147.

Abdou HM, ElMazoudy RH. 2010. Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats. J. Hazard. Mater., 182(1-3): 273–278.

Adali M, Inal-Erden M, Akalin A, Efe B. 1999. Effects of propothiourcil, propranolol and vitamin E on lipid peroxidation and antioxidant status in hyperthyroid patients. Clin. Biochem., 21: 363–367.

Altuntas I, Delibas N, Douc DK, Ozmen S, Gultekin F. 2003. Role of reactive oxygen species in organophosphate insecticide phosalone toxicity in erythtocytes *in vitro*. Toxicol. In Vitro, 17(2): 153–157.

Ambali FS, Henrieta OI, Shittu M, Kawu MU, Salami OS, Ayo OJ. 2010. Anti-implantation effect of chlorpyrifos in Swiss albino mice. Agr. Biol. J. N. Am., 1(2): 152-155.

Arthur JR. 2000. The glutathione peroxidises. Cell Mol. Life Sci**.,** 57(13-14): 1825–1835.

Bagchi D, Bagchi M, Hassoum EA, Stohs SJ. 1995. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104(1-3):129–140.

Banerjee BD, Seth V, Bhattacharya A, Pasha ST Chakraborty AK. 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. Toxicol. Lett., 107(1-3): 33–47.

Banudevi S, Krishnamoorthy G, Venkataraman P, Vignesh C, Aruldhas MM, Arunakaran J. 2006. Role of oe-tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats. Food Chem. Toxicol., 44(12): 2040–2046.

Bergamini CM, Gambetti S, Dondi A, Cervellati C. 2004. Oxygen, reactive oxygen species and tissue damage. Curr. Pharm. Design, 10(14): 1611–1626.

Beutler E, Duron O, Kelly BM. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882–888.

Bindali B, Kaliwal BB. 2002. Anti-implantation activity of carbamate fungicide mancozeb in albino mice. Ind. Health, 40(2): 191-197.

Bindhumol V, Chitra KC, Mathur PP. 2003. Bisphenol A induces reactive oxygen species generation in the liver of male rats. Toxicology, 188(2-3): 117–124.

Cathcart RF. 1985. Vitamin C: the nontoxic, nonrate-limited, antioxidant free radical scavenger. Med. Hypotheses, 18(1): 61–77.

Chiu DTY, Stults FH, Tappal AL. 1976. Purification and properties of rat lung soluble glutathione peroxidase, Biochim. Biophys. Acta, 445(3): 558–566.

Dobrowolski JW, Vohoraq SB, Sharma K, Shah SA, Naqvi SAH, Dandiya PC. 1991. Antibacterial, antifungal, antiamoebic, antiinflammatory, and antipyretic studies on propolis bee products. J. Ethnopharmacol. 35(1): 77–82.

El-Kashoury AA, Tag El-Din HA. 2010. Chlorpyrifos (from different sources): effect on testicular biochemistry of male albino rats. J. Am. Sci., 6(7): 252-261.

El-Shenawy NS, Al-Eisa RA. 2010. Mechanism of organophosphorus insecticide chlorpyrifos toxicity in isolated rat hepatocytes. J. Egypt. Soc. Toxicol., 43: 87-112.

Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Method. Enzymol., 186: 407 - 421.

Fox RP, Laird CW. 1970. Sexual cycles. In: "Reproduction and breeding techniques for laboratory animals, (Hafez ES. Ed.)". Philadelphia, PA: Lea and Febiger Press.

Freund M, Carol B. 1993. Factors affecting hemocytometer counts in sperm concentration in human semen. Toxicol. Environ. Health, 38: 393-398.

Goel A, Dani V, Dhawan DK. 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and histochitecture in chlorpyrifos-induced toxicity. Chem-Biol. Interact., 156(2-3): 131–140.

Gultekin F, Ozturk M, Akdogan M. 2000. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). Arch. Toxicol., 74(9): 533–538.

Gultekin F, Delibas N, Yasar S, Kilinc I. 2001: *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch. Toxicol., 75(2): 88–96.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249(22): 7130–7139.

Halliwell B. 1994. Free radical, antioxidants and human disease: curiosity, causes or consequence. Lancet, 344(8924): 721–724.

Harris SR, Panaro NJ, Thorgeirsson UP. 2000. Oxidative stress contributes to the anti-proliferative effects of flavone acetic acid on endothelial cells. Anticancer Res., 20(4): 2249–2254.

Hayes JD, Flanagan JU, Jowsey IR. 2005. Glutathione transferases, Annu. Rev. Pharmcol. Toxicol., 45: 51–88.

Henry RJ. 1964. Clinical Chemistry. Harper and Row publishers, New York. p. 181.

Jasprica D, Mornar A., Debeljak Z, Smolcic-Bubalo A, Medic-Saric M, Mayer L, Romic Z, Bucan K, Balog T, Sobocanec S, Sverko V. 2007. *In vivo*

study of propolis supplementation effects on antioxidative status and red blood cells. J. Ethnopharmacol., 110(3): 548–554.

Jett DA, Navoa RV. 2000. *In vitro* and *in vivo* effects of chlorpyrifos on glutathione peroxidase and catalase in developing rat brain. Neurotoxicology, 21(1-2)**:** 141–145.

Joshi SC, Mathur R, Gajraj A, Sharma T. 2003. Influence of Methyl Parathion on reproductive parameters in male rats. Environ. Toxicol. Pharmcol., 14(3): 91–98.

Joshi SC, Mathur R, Gulati N. 2007. Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat. Toxicol. Ind. Health, 23(7): 439–444.

Kanbur M, Eraslan G, Silici S. 2009. Antioxidant effect of propolis against exposure to propetamphos in rats. Ecotox.l EnvironSafe., 72(3): 900–915.

Kehrer JP. 1993. Free radicals as mediator of tissue injury and disease. Crit. Rev. Toxicol., 23(1): 21–48.

Lee KG, Shibamoto T, Takeoka GR, Lee SE, Kim JH, Park BS. 2003. Inhibitory effects of plant-derived flavonoids and phenolic acids on malonaldehyde formation from ethyl arachidonate. J. Agr. Food Chem., 51(24): 7203–7207.

Lee-Hilz YY, Boerboom AM, Westphal AH, Berkel WJ, Aarts JM, Rietjens IM. 2006. Pro-oxidant activity of flavonoids induces EpRE-mediated gene expression. Chem. Res. Toxicol., 19(11): 1499–1505.

Marquele FD, Di Mambro VM, Georgetti SR, Casagrande RY, Valim ML, Fonseca MJV. 2005. Assessment of the antioxidant activities of Brazilian extracts of propolis alone and in topical pharmaceutical formulations. J. Pharmaceut. Biomed., 39(3-4): 455–462.

Mascio PD, Murphy ME, Sies H. 1991. Antioxidant defense system: the role of carotenoids, tocopherols, and thiols. Am. J. Clin. Nutr., 53(1): 194–200.

Matsushige KI, Kusumoto, T., Yamamoto, Y., Kadota, S., Namba, T., 1995. Quality evaluation of propolis. 1. A comparative study on radical scavenging effects of propolis and vespae nidus. J. Trad.l Med., 12: 45–53.

Mattson JP, Wilmer JW, Shankar MR, Berdasco NM, Crissman JW, Maurissen JP, Bond DM. 1996. Single-dose and 13- week repeated-dose neurotoxicity screening studies of chlorpyrifos. Food Chem. Toxicol., 34(4): 393-405.

McCollister SB, Kociba RJ, Humiston CG, McCollister DD, Gehring PJ. 1974. Studies of the acute and long-term oral toxicity of chlorpyrifos (o,o-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate). Food Cosmet. Toxicol., 12: 45–61.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247(10): 3170–3175.

Naqvi SM, Hasan M. 1992. Acetylhomocystein thiolactone protection against phosphamidon-induced alteration of regional superoxide dismutase activity in the central nervous system and its correlation with altered lipid peroxidation. Indian J. Exp. Biol., 30(9): 850–852.

NRA. 2000. National Registration Authority. The NRA Review of Chlorpyrifos, NRA Review Series 00.5. National Registration Authority for Agricultural and Veterinary Chemicals. Canberra, Australia.

Oncu M, Gultekin F, Karaoz E, Altuntas I, Delibas N. 2002. Nephrotoxicity in rats induced by chlorpyrifos-ethyl and ameliorating effects of antioxidants. *Human Experimen Toxicol* **21**: 223–230.

Qiao D, Seidler FJ, Slotkin TA. 2005. Oxidative mechanisms contributing to the developmental neurotoxicity of nicotine and chloropyrifos. Toxicol. Appl. Pharmacol., 206(1):17-26.

Ran Q, Liang H, Ikeno Y, Qi W, Prolla TA, Roberts LJ, Wolf N, Van Remmen H, Richardson A. 2007. Reduction in glutathione peroxidase four increases life span through increased sensitivity to apoptosis. J. Gerontol. A. Biol. Sci. Med. Sci., 62(9): 932–942.

Russo A, Troncoso N, Sanchez F, Garbarino JA, Vanella A. 2006. Propolis protects human spermatozoa from DNA damage caused by benzo[a]pyrene and exogenous reactive oxygen species. Life Sci., 78(13): 1401–1406.

Sarkar R, Mohanakumar KP, Choudhury M. 2000. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. J. Reprod. Fertil., 118(1): 29–38.

Sarkar MR, Chaudhury G, Chattopadhyay A, Biswas NM. 2003. Effect of sodium arsenate on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J. Androl., 5(1): 27–31.

Saulsbury MD, Heyliger SO, Wang K, Johnson DJ. 2009. Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. Toxicology, 259(1-2): 1–9.

Seth V, Banerjee BD, Chakravorty AK. 2001. Lipid peroxidation, free radical scavenging enzymes, and glutathione redox system in blood of rats exposed to propoxur. Pestic. Biochem. Phys., 71(3): 133–139.

Sies H. 1991. Oxidative stress: introduction, in: "Oxidative Stress: Oxidants and Antioxidants, (Sies H. Ed.)", Academic Press, San Diego, CA, USA, 23: 21–48.

U.S. Department of Health and Human Services. (1994). Preventing tobacco use among young people: A report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention.

EPA. 1986. Ambient water quality criteria for chlorpyrifos - 1986. U.S. Environ. Protection Agency Rep. 440/5-86-005. 64 pp.

US EPA. 1996. Guidelines for health risk assessment of chemical mixtures. US Environmental Protection Agency. Federal Registration 51: 34014–34025.

Verma RS, Srivastava N. 2003. Effects of chlorpyriofos on thiobarbi-turic acid reactive substances, scavenging enzymes and glutathione in rat tissues. Indian J. Biochem. Biophys., 40: 423–428.

Verma RS, Mnugya A, Srivastava N. 2007. *In vivo* chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pestic. Biochem. Phys., 88(2): 191–196.

Voellmy R. 1999. In: "Stress Inducible Cellular Response, (Feige U, Morimoto RI, Yahura I, Polla B. Eds)", Birkhauser, Basel, p. 121–137.

Wetscher GJ, Bagchi M, Bagchi D, Perdikis G, Hinder PR, Giaser K, Hinder RA. 1995. Free radicals production in nicotine-treated pancreatic tissue. Free Radical Biol. Med., 18(5): 877-882.

Xu JB, Yuan XF, Lang PZ. 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. Chinese Environ. Chem., 16: 73–76.

Fu JY, Xia Y, Zheng YY. 2004. Antimutagenicity of propolis against some mutagens *in vivo* and *in vitro*. Biomed. Environ. Sci., 17(4): 469-475.

Yousef MI, Salama AF. 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol., 47(6): 1168–1175.

Yu F, Wang Z, Ju B, Wang Y, Wang J, Bai D. 2008. Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamin C and E. Exp. Toxicol. Pathol., 59(6): 415–423.

تقييم التأثير الوقائى للبروبولس ضد الضرر التأكسدى والتحول الدهنى فى الخصية والعقم الناجم عن الكلوربيريفوس

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قد تسبب المبيدات الإجهاد الأكسدة والذي يؤدى إلى توليد نظام الشقوق (الشوارد الحرة) وإنزيمات مضادات الأكسدة وبديل شقوق الأكسجين الحرة. وقد أجريت هذه الدراسة لتقصى سمية الجرعة تحت المميتة عن طريق الفم لمبيد الكلوربيريفوس نحو ذكور الفئران و الإجهاد الأكسيدى على مستوى الأكسدة الدهنية ، الجلوتاثايون المختزل، ونشاط الأنزيمات المضادة للأكسدة. وأيضا التأثير الوقائي للبروبولس (50 مجم/كجم) سواء كان منفرد أو مع المبيد. وقد سببت جرعة المبيد (9مجم/كجم) عن طريق الفم إلى ارتفاع كبير في مستوى تأكسد الدهون . انخفض نشاط أنزيمات المضادة للأكسدة في نسيج الخصية. تناول البروبولس مع المبيد أدى إلى انخفاض مستوى تأكسد الدهون وزيادة نشاط إنزيمات المضادة للأكسدة في نسيج الخصية. في المجمل، البروبولس يقلل أو يخفف الإجهاد الأكسيدى الناجم عن سمية المبيد الكلوربيريفوس في خصي الفئران البالغة ويعزى هذا العلاج إلى الخواص الكامنة في البروبولس المضادة للأكسدة.

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