*Full Length Research Paper*

# Effect of propolis consumption on hepatotoxity and brain damage in male rats exposed to chlorpyrifos

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**This study was undertaken to determine the protective effect of propolis against the hepatotoxity and brain damage of chlorpyrifos (CPF) in male rats. Animals were assigned to one of four groups. The first group was used as control. Groups 2, 3 and 4 were treated with 6.8 mg CPF /kg BW (1/20 LD50); 50 mg propolis/kg BW; CPF (6.8 mg CPF/kg BW) plus propolis (50 mg propolis/kg BW). Rats were orally administered their respective doses daily for 28 days. Serum transaminases, glucose, lactate dehydrogenase, total proteins, albumin, reduced glutathione, thiobarbituric acid-reactive substances, superoxide dismutase and catalase were assessed. Also, total lipid, cholesterol, triglyceride and LDL-c were assayed. Oral treatment with CPF was found to elicit significant deterioration in all the tested parameters confirming its toxicity. The injury of liver tissues after CPF-treatment was confirmed by the histological changes. Also, CPF caused significant decrease in the activity of serum and brain cholinesterase but increased glial fibrillary acidic protein-expression and cause some histological changes in the brain tissues. While, oral treatment with propolis plus CPF could antagonize CPF-toxicity. These results suggest that propolis may become a promising tool for wide use in reducing the liver and brain damage during CPF-exposure.**

**Key words:** Chlorpyrifos, propolis, rats, lipid peroxidation, antioxidant enzymes, biochemical parameters.

## INTRODUCTION

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is an organophosphate (OPIs) pesticide used to control household, public health and stored product insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips and white flies in greenhouse, outdoor fruit and vegetable crops (Ambali, 2009). Chloropyrifos (CPF) induces toxicity through inhibition of acetyl cholinesterase (AChE) in addition to multiple other mechanisms (Slotkin et al., 2005). Pesticide chemicals can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity (Sharma et al., 2005). Exposure to CPF can differentially modify endogenous antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPX) and reduced glutathione (GSH), which can lead to the development of oxidative stress in some tissues (Bebe and Panemangalore, 2003). Also, it has been reported that CPF-intoxication causes a significant decrease in the reduced glutathione (GSH) and the activities of some antioxidant enzymes (Goel et al., 2005).

The mammalian cells reduced the adverse effect

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of lipid peroxidation via the utilization of both enzymatic and non-enzymatic antioxidants, which scavenge the free radicals in the living system. To control the level of reactive oxygen species (ROS) and to protect cells under stress conditions, mammalian tissues contain several enzymes that scavenge ROS such as catalase (CAT) and glutathione-S-transferase (GST). Therefore, during oxidative stress, an increase in the exogenous supply of antioxidants improves the capacity of the tissue to cope with high antioxidant demands such as propolis (Goel et al., 2009). Propolis or bee glue is a resinous hive product collected by honey bees from plant exudates and contains more than 160 constituents. Historically, it has been used for various purposes such as a medicine (Ghisalberti, 1979). Flavonoids are thought to be responsible for many of its biological and pharma-cological activities including anticancer (Valente et al., 2011), anti-inflammatory (Nirala and Bhadauria, 2008) and antioxidant effects (Yousef et al., 2004a).

Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals and thereby protecting lipids from being oxidized or destroyed during oxidative damage (Nieva Moreno et al., 2000). Propolis has gained popularity and used extensively in healthy drinks and foods to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer (Matsuno, 1995). The role of propolis against CPF-induced toxicity in rats has not so far been studied. Therefore, the present study was carried out to investigate: 1) the alterations in biochemical parameters, free radicals and antioxidant enzymes induced by CPF in the serum of male rats; 2) the histological alterations induced by CPF in the liver and brain tissues and 3) the protective effect of propolis against the possible toxicity caused by CPF-exposure.

## MATERIALS AND METHODS

### Chemicals

Chlorpyrifos (CPF) was applied as a commercial emulsified concentrate formulation containing 48% emulsifiable concentrate chlorpyrifos (EC) (Trade name pestban) active ingredient from Egychem a chemicals company (El-Watania Co), Egypt. The EC was emulsified in water just before use and orally administrated to animals by esophageal intubation, and propolis was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other used chemicals in our experiment were of analytical grade. The dose of chloropyrifos (CPF) was 6.8 mg CPF /kg BW (1/20 LD$_{50}$). The acute oral LD$_{50}$ of CPF was 136 mg /kg BW for rats (Suleiman et al., 2010). The dose of propolis was 50 mg/kg BW. This dose was used according to the previous study of Newairy et al. (2009).

### Experimental design

Forty male Wistar rats were obtained from the animal house, Faculty of Medicine, Alexandria University, Egypt. The weights of

rats were about 180 to 225 g. 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 caged in groups, each of five rats, and were given feed and water *ad libitum*. After one week of acclimatization, animals were divided into four equal groups. The first group was used as control. Groups 2, 3 and 4 were orally treated with CPF (6.8 mg/kg BW), the combination of CPF (6.8 mg/kg BW) and propolis (50 mg/kg BW) and propolis (50 mg/kg BW), respectively. Rats were orally administered their respective doses daily, for 28 days. Rats of each group were euthanized at the end of treatment period. Trunk blood samples were collected from the sacrificed animals and placed immediately on ice. Serum samples were obtained by centrifugation at 860 *g* for 20 min and stored at -60°C till measurements. Specimens of the liver and brain were fixed immediately in 10% buffered formalin for histological study.

### Biochemical parameters

The level of glucose was determined with kits from Biosystems, S.A. Costa Brava, 30-Barcelona (Spain). The extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured according to the method of Esterbauer and Cheeseman (1990). Tissue homogenate was mixed with 1 ml TCA (20%), 2 ml TBA (0.67%) and heated for 1 h at 100°C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. Glutathione content (GSH) was determined using commercial glutathione reduced kits (Biodiagnostic for diagnostic reagents: Dokki, Giza, Egypt) according to the method of Beutler et al. (1963). The activities of liver aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) were assayed by the kinetic methods of Bergmeyer et al. (1986). Determination of lactate dehydrogenase (LDH; EC 1.1.1.27) activity was carried out using kits from SENTINEL CH. (via principle Eugenio 5-20155 MILAN-ITALY). The catalase enzyme (CAT; EC 1.11.1.6) converts $H_2O_2$ into water. The CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H2O2; the substrate of the enzyme (Xu et al., 1997).

Super oxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture that contains the tissue supernatant. The change in extinction coefficient was followed at 480 nm in a Spectrophotometer. Plasma concentrations of total lipids (TL) and cholesterol were assayed by the method of Knight et al. (1972) and Carr et al. (1993), respectively. High density lipoprotein (HDL) and low density lipoprotein (LDL) were determined according to the methods of Warnick et al. (1983) and Bergmenyer (1985), respectively. The protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Acetyl cholinesterase (AChE; EC 3.1.1.7) activity was measured according to the method of Blawen et al. (1983).

### Immunohistological examination

Brain samples were fixed in 10% neutral-buffered formalin and embedded in the paraffin wax. Six micrometer thick sections were cut, placed on glass slides and incubated at 37°C overnight. Dewaxed sections were incubated with protein block for 30 min and then incubated overnight at 4°C with the polyclonal anti-glial fibrillary acidic protein (GFAP) antibody (1:1000). Sections were then incubated with biotinylated antirabbit IgG antibody (1:100) and

**Table 1.** The effect of CPF and/or propolis administration on the levels of glucose, total proteins and albumin and the specific activities of AST, ALT and LDH in the serum samples of male rats.

| Parameter | Control | CPF | CPF + Propolis | Propolis |
|---|---|---|---|---|
| Glucose (mg/dL) | 80.86±1.40 | 136.31± 1.26** | 81.10 ± 0.97 | 78.68 ± 1.06 |
| AST (U/L) | 37.05 ± 0.46 | 46.23± 1.09** | 39.13 ± 0.41 | 31.56 ± 0.45** |
| ALT (U/L) | 26.08 ± 0.33 | 54.35± 0.81** | 29.54± 0.28** | 22.82± 0.49** |
| LDH (U/L) | 737.02 ± 13.64 | 1153.45±21.39** | 775.37 ± 12.81 | 716.12 ± 15.07 |
| Total Proteins (g/L) | 8.25 ± 0.16 | 4.55± 0.18** | 8.0 ± 0.15 | 8.35 ± 0.23 |
| Albumin (g/L) | 3.75 ± 0.23 | 2.82± 0.27* | 3.20 ± 0.23 | 4.12 ± 0.24 |

Values are expressed as means ± SE; *significantly different at $P < 0.05$ compared to the control group; **, significantly different at $P < 0.01$ compared to the control group; CPF, chlorpyrifos; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase.

**Table 2.** The effect of CPF and/or propolis administration on the levels of GSH and TBARS and the specific activities of SOD and CAT in the serum samples of male rats.

| Parameter | Control | CPF | CPF + Propolis | Propolis |
|---|---|---|---|---|
| GSH (mg/dL) | 30.07 ± 0.57 | 16.40± 0.32** | 29.45 ± 0.57 | 30.97 ± 0.58 |
| TBARS (mg/dL) | 0.66 ± 0.13 | 1.36± 0.06** | 0.70 ± 0.01 | 0.62 ± 0.04 |
| SOD (U/mL) | 2.021 ± 0.07 | 1.23± 0.10** | 1.87 ± 0.12 | 3.01± 0.05** |
| CAT (U/mL) | 48.98 ± 1.0 | 30.42± 0.70** | 47.71 ± 1.04 | 49.12 ± 1.0 |

Values are expressed as means ± SE; *significantly different at $P < 0.05$ compared to the control group; **significantly different at $P < 0.01$ compared to the control group; CPF, chlorpyrifos, GSH: reduced glutathione; TBARS, thiobarbituric acid-reactive substances; SOD, superoxide dismutase; CAT: Catalase.

finally with the avidin-biotin-peroxidase reagent for 30 min at room temperature. The peroxidase reaction was visualized with DAB. Samples were counterstained with haematoxylin. To visualize neurons, sections were stained with Nissl staining according to the method of Malkiewicz et al. (2006).

**Histopathology**

Specimens of the liver and brain tissues were fixed immediately in 10% formalin for histological studies. Then the tissues were treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4 to 6 μm thickness. The sections were stained with Hematoxylin and Eosin (H and E) stain for studying the histopathological changes (Drury and Wallington, 1980).

**Statistical analysis**

Data were analyzed according to Steel and Torrie (1981). Statistical significance of the difference in values of control and treated animals was calculated by F test at 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS, 1986).

**RESULTS**

The effect of CPF and/or propolis on the levels of serum biochemical parameters is summarized in Table 1. Results from this study shows that the oral ingestion of CPF caused functional and structural damage of liver

tissue. This damage was indicated in part through highly significant increases in the levels of serum transaminases (ALT and AST). They increased from 26.08 ± 0.33 and 37.05 ± 0.46 to 54.35 ± 0.81 and 46.23 ± 1.09, respectively as shown in Table 1. Concurrent with this result, significant elevations in the activity of LDH from 737.02 ± 13.64 to 1153.45 ±21.39 and the level of glucose from 80.86±1.40 to 136.31 ± 1.26, respectively were recorded in the same group when compared to the control one. The functional damage caused by CPF-treatment was indicated by the significant decrease in the serum total protein (4.55 ± 0.18) and albumin (2.82 ± 0.27) concentrations compared to the control levels; 8.25 ± 0.16 and 3.75 ± 0.23, respectively (Table 1). This indicates impaired synthetic and excretory functions of the liver, respectively. The treatment with CPF plus propolis ameliorated the levels of the tested serum biochemical parameters compared to the control group as shown in Table 1.

The results of the reduced glutathione content are represented in Table 2. It is clear that, the level of reduced GSH in the serum (16.40 ± 0.32, $P < 0.05$) was greatly depleted by CPF-treatment compared to the control group (30.07 ± 0.57). In contrast, the level of TBARS was greatly elevated in CPF-treated group (1.36 ± 0.06, $P < 0.05$) compared to the control group (0.66 ± 0.13). The treatment with the CPF plus propolis could normalize the levels of reduced GSH (29.45 ± 0.57) and

**Table 3.** The changes in the serum lipids profile after oral administration of male rats with chlorpyrifos and/or propolis.

| Parameters (mg/dl) | Control | CPF | CPF +Propolis | Propolis |
|---|---|---|---|---|
| Total lipids | 460.39 ± 0.80 | 561.24[**]±3.44 | 519.13[**]±0.71 | 395.26[**]±1.81 |
| Total cholesterol | 109.98 ± 0.57 | 126.85[**]±1.57 | 110.10 ± 0.47 | 105.66[*]±0.81 |
| LDL-c | 97.10 ± 2.28 | 125.43[**]±1.25 | 101.05 ± 2.61 | 85.92 ± 1.80 |
| HDL-c | 31.42 ± 0.41 | 20.92[**]± 0.44 | 31.16 ± 0.48 | 41.03[**]± 0.66 |
| TG | 81.46 ± 0.97 | 92.10[**]± 0.95 | 82.20 ± 0.81 | 75.18[**]± 1.32 |

The data are presented as mean ± SE, (n = 10); *significantly different at P <0.05 compared to the control group; **significantly different at P <0.01 compared to the control group; CPF, chlorpyrifos; LDL-c, low density lipoprotein-cholesterol; HDL-c, high density lipoprotein-cholesterol Lipoprotein; TG, triglycerides.

TBARS (0.70 ± 0.01) compared to the control group (30.07 ± 0.57 and 0.66 ± 0.13, respectively). Table 2 shows also that CPF-treatment caused significant (P≤ 0.05) inhibition in the specific activities of catalase (30.42 ± 0.70) and super oxide dismutase (1.23 ± 0.10) compared to the control values; 48.98 ± 1.0 and 2.021 ± 0.07, respectively. On contrast, the specific activities of serum catalase and super oxide dismutase could be normalized after treatment with CPF plus propolis (47.71 ± 1.04 and 1.87 ± 0.12, respectively).

Table 3 shows the lipids profile of all the studied groups in this study. The results shows that the treatment of rats with CPF induced significant (p < 0.05) increases in the serum total lipids from 460.39 ± 0.80 to 561.24 ±3.44, total cholesterol from 109.98 ± 0.57 to 126.85 ±1.57, triglycerides from 81.46 ± 0.97 to 92.10 ± 0.95 and LDL-c from 97.10 ± 2.28 to 125.43 ±1.25 while HDL-c was significantly decreased compared to the control group from 31.42 ± 0.41 to 20.92 ± 0.44. On the other hand, treatment with CPF plus propolis showed amelioration in the levels of serum total lipids, total cholesterol, triglycerides, LDL-c and HDL-c (519.13 ±0.71, 110.10 ± 0.47, 82.20 ± 0.81, 101.05 ± 2.61 and 31.16 ± 0.48, respectively).

Light micrographs of the liver showed degenerated hepatocytes, dilatation and vascular congestion in sinusoids, cytoplasmic vacuolation and degenerated hepatocytes with pyknotic nuclei are shown in the CPF-treated rats, compared to central vein with normal hepatocytes architecture in the control group and propolis-treated group. Histological changes induced after CPF-treatments were markedly reduced in the combination group showing moderate improvement in the hepatic cells (Figure 1).

Furthermore, CPF-treatment caused significant (p < 0.05) decrease in the activity of serum and brain AChE (64.80 ± 0.55 and 5.49 ± 0.10, respectively) compared to the control group (71.22 ± 0.34 and 20.23 ± 0.42, respectively) as shown in Table 4. On contrast, the specific activity of AChE in the serum and brain extract could be normalized after treatment with CPF plus propolis (70.77 ± 0.38 and 19.74 ± 0.42, respectively). Histopathological examination of the brain showed mild neuronal degeneration in purkinje cells, neuronal shrinkage and nuclear pyknosis after CPF-treatment (Figure 2), characterizing CPF-induced neuronal necrosis as compared to the controls. The changes observed in GFAP immunostaining revealed longer and more numerous astrocytic processes in the brain tissues of CPF-treated group as compared to controls. Our study shows that the astrocytic density was raised in the CPF-treated group (Figure 3).

## DISCUSSION

### Chlorpyrifos

Chlorpyrifos is an extensively used organophosphorus pesticide having many urban and agricultural crop pest control uses. The present study is an attempt to protect against CPF-induced toxicity by propolis-treatment in male rats. The present data showed that the activities of serum AST, ALT and LDH were significantly (p<0.05) increased after CPF-treatment compared to the control group (Table 1). This fact is a conventional indicator of liver injury (Rao, 2006). Elevation of serum AST and ALT indicates liver damage (Akhtar et al., 2009). This agrees with the low ALT and AST activities in the liver extract of rats exposed to CPF in the previous findings of Zama et al., (2007). When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Also, the elevation in the activity of LDH suggests an increase in lysosomal mobilization, cell necrosis and liver damage due to pesticide toxicity (Etim et al., 2006). Chen et al. (2000) observed a significant rise in serum LDH activity after carbon tetrachloride-induced hepatotoxicity.

On the other hand, Table 1 indicates that the serum total proteins and albumin were significantly (p<0.05) decreased in the CPF-treated rats compared to the control group, which agrees with the report of Peeples et al. (2009). Normally, the reduction of serum proteins and albumin levels as shown in Table 1 indicates a liver disease. This reduction could be attributed to the changes in the metabolism and the synthesis of the
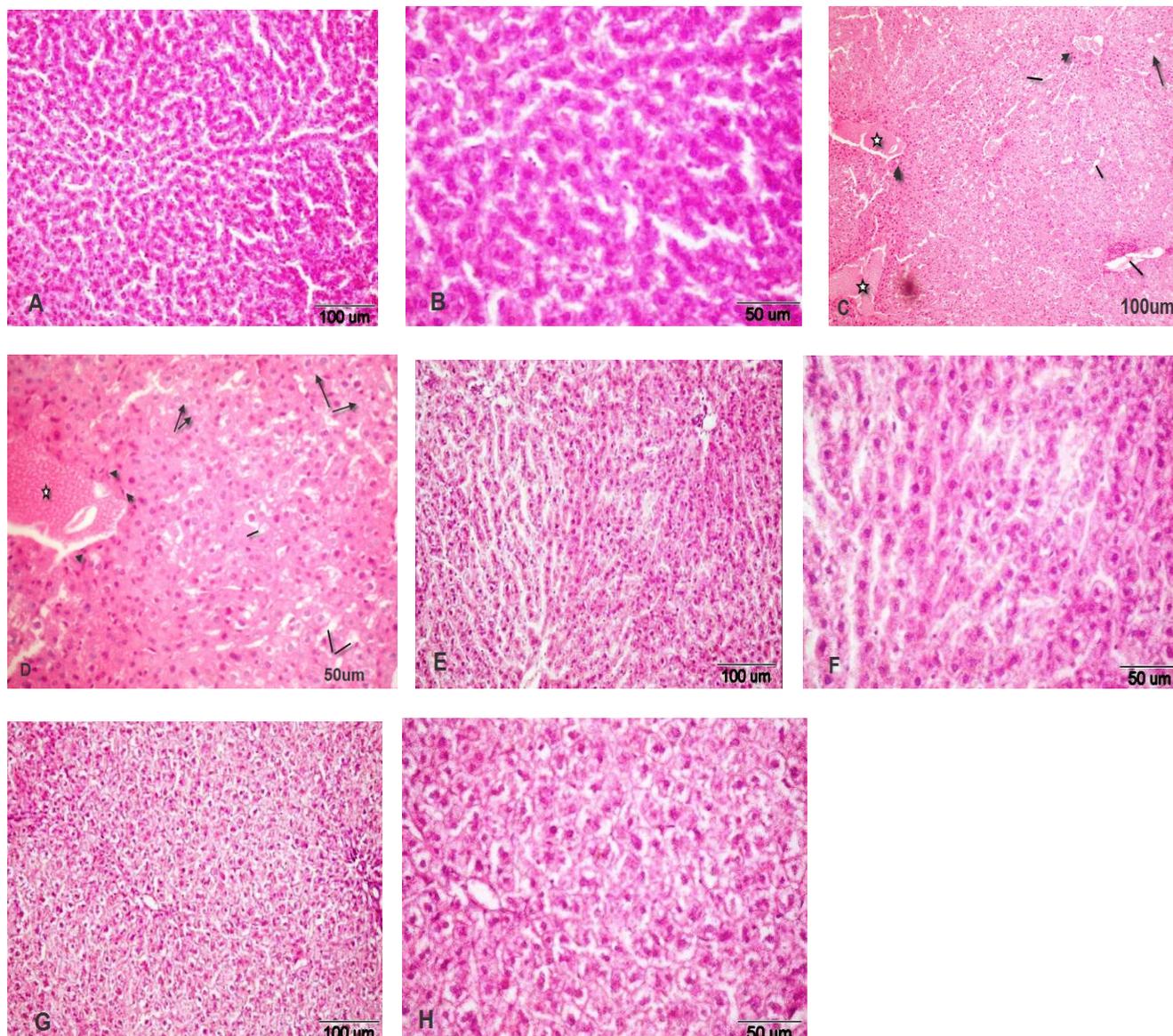
**Figure 1.** Light micrographs of the liver of rats treated: A and B, control; C and D, chlorpyrifos-treated rats; E and F, chlorpyrifos plus propolis-treated rats and G and H, propolis-treated group.

**Table 4.** The effect of CPF and/or propolis administration on the specific activity of AchE in the serum samples and brain extracts of male rats.

| Parameter | Control | CPF | CPF + Propolis | Propolis |
|---|---|---|---|---|
| AchE- serum (1) | 71.22 ± 0.34 | 64.80**± 0.55 | 70.77 ± 0.38 | 71.35 ± 0.37 |
| AchE- brain (2) | 20.23 ± 0.42 | 5.49**± 0.10 | 19.74 ± 0.42 | 20.98 ± 0.44 |

Values are expressed as means ± SE; **significantly different at $P < 0.01$ compared to the control group; 1, U/l; 2, n mol/min/mg protein; CPF, chlorpyrifos; AchE, acetylcholine esterase.

protein and free amino acid in the liver (Li et al., 2007). They suggested that albumin could be used as a biomarker of CPF-toxicity. Generation of oxidative stress and consequent lipid peroxidation by pesticides was reported in many species. Due to the high concentration of polyunsaturated fatty acids in cells, lipid peroxidation is
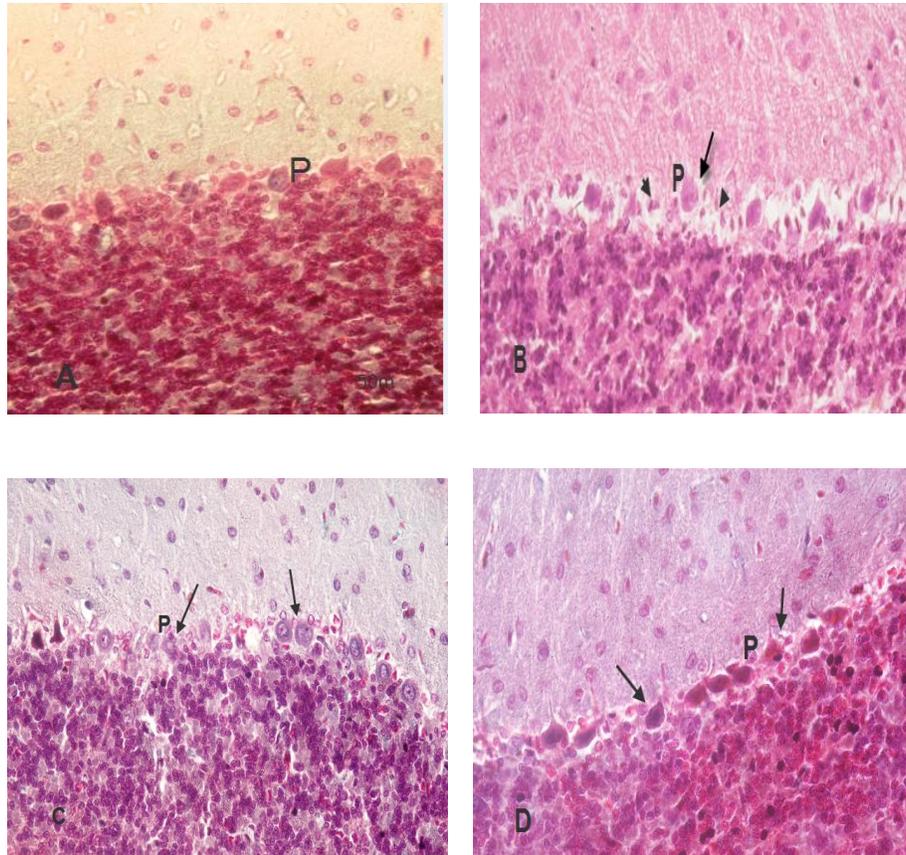
**Figure 2.** Light micrographs of cerebellar cortex in rats: Control group (A) and propolis-treated rats (D) showed normal histological structure of brain with dark-staining Purkinje cell (arrow). Chlorpyrifos-treated rats (B) showed contracted, dark-staining cell bodies described as "degenerating" Purkinje neurons (arrow) with loss of dendrites and loss of granules in granular layer. Chloropyrifos plus propolis-treated rats (C) revealed some amelioration of the neuron cells.

a major outcome of the free radical-mediated injury (Verma et al., 2007).

Results of the present study show that CPF-exposure caused significant decreases in the activities of serum SOD and CAT (Table 2). CPF has been shown to impair antioxidant enzyme activities either directly or through the induction of free radicals resulting in oxidative stress (El-Banna et al., 2009). Also, the decrease of antioxidant enzyme activities was observed after CPF-exposure in rats (El-Kashoury and Tag El-Din, 2010). Our results show also that CPF-exposure to male rats caused an increase in the lipid peroxidation, as evidenced by elevated level of TBARS and the decreased level of GSH (Table 2). These findings agree with the results obtained in a previous study (Saxena and Garg, 2010). They reported that tissue lipid peroxidation is a degradative phenomenon as a consequence of free radical chain production and propagation which affects mainly polyunsaturated fatty acids. Lipid peroxidation has been used as a measure of this xenobiotic-induced oxidative stress, which was originally defined as the disequilibrium

between prooxidants and antioxidants in the biological systems (Bebe and Panemangalore, 2003). Once this imbalance appears, cellular macromolecules may be damaged by the predominant free radicals. Lipid peroxidation was reported to be increased after exposure to various xenobiotics (Bebe and Panemangalore, 2003).

Thus, it is an important and necessary consequence of oxidative stress and is involved in the pathophysiology of the number of diseases and other natural degenerative conditions. The role of oxygen derived species in causing cell injury or death is increasingly recognized: superoxide and hydroxyl radicals are involved in a large number of degenerative changes often associated with an increase in peroxidative process and linked to low antioxidant concentration (Slotkin et al., 2005). Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidant defense mechanisms to minimize the cellular damage resulting from the interaction between cellular constituents and ROS (Goel et al., 2009). The enzymatic antioxidant defense mechanism contains various forms of superoxide dismutases, catalase and glutathione peroxi-
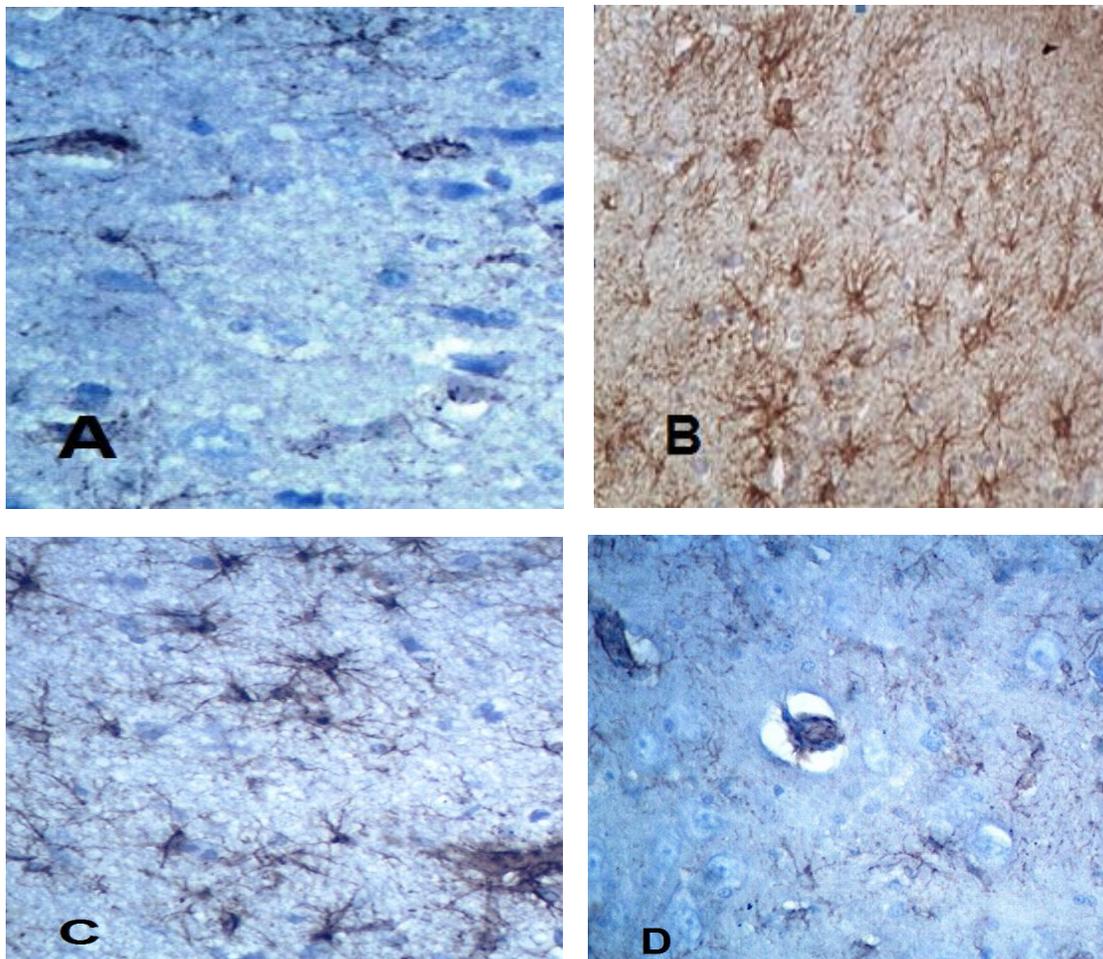
**Figure 3.** Photomicrograph showing the immunohistochemical staining of anti-glial fibrillary acidic protein (GFAP) expression in stratum moleculare-lacunosum of the cerebellar cortex in groups of rat at the end of experiment. Brown colour rounded cells with processes are the astrocytes. A, Control group; B, chlorpyrifos group; C, chlorpyrifos plus propolis group; D, propolis group (GFAP, 400×).

dase. Despite of the presence of these delicate cellular antioxidant systems, an overproduction of ROS in both intra- and extracellular spaces often occurs upon exposure of cells or individuals to certain chemicals (Goel et al., 2005).

An unbalanced production of ROS in localized compartments has been reported to play a role in the pathogenesis of a number of clinical disorders which illustrates the importance of antioxidant defense system in maintaining normal cellular physiology (El-sharaky et al., 2009). Also, Suleiman et al., (2010) have reported that lipid peroxidation was increased and the activities of antioxidant enzymes were decreased in rat erythrocytes on CPF-treatment which were restored to the normal values by vitamine C-treatment. GSH acts as reducing agent and a vital substance in detoxification and provides antioxidant protection in the aqueous phase of cellular systems (El-sharaky et al., 2009); its antioxidant activity is through the thiol group of its cysteine residue. Like

ascorbic acid, another important water soluble antioxidant, GSH can directly reduce a number of ROS and is oxidized to GSSG in this process. Intrahepatic glutathione was reported to afford protection against liver dysfunction by at least two ways:  1) as a substrate of GPx, GSH serves to reduce large variety of hydrope-roxides before they attack unsaturated lipids or convert the formed lipid hydroperoxides to the corresponding hydroxyl compounds and 2) as a substrate of glutathione-S-transferase, it enables the liver to detoxify foreign com-pounds or their metabolites and to excrete the products, preferably into the bile.

Relatively high ratios of GSI-I/GSSG are maintained intracellularly through the action of glutathione reductase (GR) and NADPH dependent reaction (El-Banna et al., 2009). Also, a similar decrease in the level of GSH and corresponding increase in GSSG has also been reported in different parts of brain on CPF-exposure (Suleiman et al., 2010). The present study clearly shows that CPF-

exposure generates oxidative stress in male rats.

Table 3 also shows that treatment of rats with CPF induced significant (p < 0.05) increases in serum total lipids, total cholesterol, triglycerides and LDL-c compared to the control group. The same trend was seen with other organophosphorus pesticide (Kalender et al., 2005). Previous studies demonstrated increases in the concentration of serum triglycerides in the experimental animals that were treated with different insecticides, including the organophosphate, dichlorvos (Ranjbar et al. 2002) and carbamate furadan (Ibrahim and El-Gamal, 2003). This elevation of serum or plasma triglycerides has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins (Goldberg et al., 1982). Also, our study showed that oral treatment with CPF induced significant decrease in serum HDL-c. HDL-c is mainly synthesized in the liver and intestinal cells. It plays an important role in cholesterol efflux from tissues and carries it back to the liver for removal as bile acids (Shakoori et al. 1988). It has been established that the reduced levels of HDL-c are associated with an increased risk for coronary artery disease (Stain, 1987).

In addition, the elevated cholesterol level in the present study due to CPF-treatment is consistent with the finding of Ashgar et al. (1994), who reported that total cholesterol was elevated in male rabbits treated by methyl parathion. The increase in the level of serum cholesterol may be due to an increased synthesis of cholesterol in the liver (Enan et al., 1987). The present study is in agreement with other investigations which reported that organophosphorus pesticide cause increases in the total cholesterol and the total lipid levels (Adham et al., 1997). The induced increase in serum cholesterol can be attributed to the effect of the pesticide on the permeability of liver cell membrane (Kalender et al., 2005). Also, the increase in serum total cholesterol level may be attributed to the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum (Aldana et al., 1998).

Also, Figure 1 demonstrates the degenerative changes in liver after CPF-treatment comprise fatty change, individual cell necrosis, cellular infiltration and areas of necrosis that are suggestive of acute hepatitis. Also, cellular infiltration and the presence of newly forming bile ducts suggest a persistent sub acute or chronic liver injury. The damage of the liver tissues in CPF-treated rats in the present study could be due to injured mitochondria (Goel et al., 2005). The present study suggests that the administration of CPF in rats induced pathological changes in the liver tissue.

Results of this study shows that CPF-treatment caused significant (p < 0.05) decrease in the activity of serum and brain AChE compared to the control group (Table 4). This is consistent with the study of Celik and Isik (2009). They reported that in animals intoxicated with methyl parathione and dichlorvos at sub lethal concentrations, AChE and butyl cholinesterase (BChE) activities are inhibited. They also reported that inhibition of AChE may be a better biomarker for the assessment of neurotoxic effects in the living.

Organophosphates toxicants generally elicit their effects by inhibition of acetyl cholinesterase, which lead to accumulation of the neurotransmitter acetylcholine in synapses; in the neuromuscular junction, over stimulation of postsynaptic cholinergic receptors leads to muscle fasciculation and eventual paralysis (Mileson et al., 1998)

Furthermore, our study showsthat the astrocytic density was raised in the CPF-treated group (Figure 3). One of the proteins associated with neuronal damage is glial fibrillary acidic protein (GFAP). GFAP is a cytoplasmic intermediate filament protein found in astrocytes.

They maintain the structural integrity of astrocytes, especially when these cells undergo hypertrophy and hyperplasia in response to a non-invasive CNS injury (Norenberg, 1994) whereby, expression of GFAP is upregulated (Abou-Donia et al., 2004). GFAP expression in the astrocytes was more prominent compared to the control groups. The astrocytic density of the groups receiving CPF was greater as compared to controls. This may be attributed to the neuroprotective effect of astrocytes limiting neuronal damage.

It has been suggested that the metabolites of CPF; trichloropyridinol (TCP) exert strong toxic effects on astrocytes, compromising their neuroprotective effects and thus increasing the neurotoxicity of CPF (Zurich et al., 2004). A characteristic feature of gliosis, GFAP upregulation often occurs in response to injury in the brain (Abou-Donia et al., 2004).

Ho et al. (2007) reported that GFAP is a sensitive and early biomarker of neurotoxicity, it's up-regulation preceding anatomically perceptible damages in the brain. Findings of Lim et al. (2011) suggest that GFAP expression is up regulated with dermal exposure to low dose of CPF.

## Propolis

Although certain compound has been tested for the detoxification of CPF (Suleiman et al., 2010), there are no previous studies that carried out to evaluate the curative effect of propolis against CPF-toxicity that cause dysfunction of the liver and brain. Treatment with chlorpyrifos plus propolis could normalize the serum glucose level compared to the control group (Table 1). This suggests that propolis can control blood glucose and modulate the metabolism of glucose (Fuliang et al. 2005). The present data indicated that treatment with propolis alone decreased the activities of AST, ALT and LDH in the serum samples (Table 1).

Also, the present data showed that the treatment with propolis alone caused reduction in TBARS level and increased the activities of SOD and CAT and the level of GSH in the serum (Table 2). These data are in agreement

with the results obtained by Jasprica et al., (2007) who reported that propolis caused reduction in the malondialdehyd (MDA) level and increased the activities of the antioxidant enzymes (SOD, GPx and CAT). Moreover, the present results show that propolis could normalize the levels of AST, ALT and LDH in the serum (Table 1), indicating that propolis tended to prevent the damage and suppressed the leakage of enzymes through cellular membranes. This result is in accordance with the findings that propolis induced reduction of the increased activity of AST and ALT concentrations in the plasma of galactoseamine-treated rats (Gonzalez et al., 1994). Our results indicate an increased TBARS levels in the serum of CPF-treated rats, implying the increased oxidative damage to the tissues. Propolis treatments returned the increased TBARS levels back to the control levels (Table 2). This result is in accordance with the findings that propolis extract induced reduction of the increased TBARS concentrations in serum of galactoseamine-treated rats (Gonzalez et al. 1994).

In the present work, CPF caused oxidative stress and consequently decreased the activities of the antioxidant enzymes (SOD and CAT). After treatment of rats with chlorpyrifos plus propolis, the activities of these antioxidant enzymes were normalized to their control values (Table 2). Jasprica et al. (2007) showed that propolis and related flavonoids exercise their activity through the scavenging of hydroxyl, superoxide free radicals and lipid peroxides. The antioxidant activities of propolis and its polyphenolic/flavonoid components are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, peroxyl radicals, hydroxyl radicals and peroxynitrite (Fonseca, 2011). Also, other previous studies of Yousef et al. (2004a) showed that the treatment with isoflavones as antioxidant in combination with cypermethrin minimized its hazardous effects.

Moreover, the results of Yousef et al. (2004a) concluded that isoflavone dosages (2.5 or 5 mg/kg body weight), which is more than two or four times to the amounts consumed (40 mg) in many Eastern nations, have beneficial effect on the level of plasma lipids and lipoproteins in rabbits. Fuliang et al. (2005) showed that propolis elevates GPX, SOD, GST and catalase activities. Therefore, the flavonoids of the propolis can increase the activities of the antioxidant enzymes and reduce the levels of the ROS. Kanbura et al. (2009) found decreases in the plasma and the tissues (liver, kidney and brain) malondialdehyde (MDA) levels and normalization in the antioxidant enzyme parameters (SOD, CAT and GSH-Px) of animals that were administered propolis in association with propetamphos, in comparison to the group that was administered propetamphos alone.

Moreover, Newairy et al. (2009) found decreased levels of the plasma and tissue (liver, kidney and brain) malondialdehyde and ameliorations in the antioxidant enzymes (SOD, CAT and GSH-Px) of animals that were administered propolis in association with aluminium chloride, in comparison to the group that was administered aluminium chloride alone. The primary mechanism of the effect of propolis may involve the scavenging of free radicals that cause lipid peroxidation. The other mechanism may comprise the inhibiting effect of propolis on the activity of xanthine oxidase, which is known to cause free radicals to be generated (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 found 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. Also, propolis was able to induce hepatoprotective effects on paracetamol induced liver damage in mice (Gonzalez et al.1994). Taken together, these findings constitute evidence that the antioxidative properties of the propolis contribute to the prevention of damage induced by CPF in rats.

This study shows that treatment of rats with CPF plus propolis normalized the total lipids, cholesterol, triglycerides and low-density lipoprotein-cholesterol (LDL-c) and increased high-density lipoprotein cholesterol (HDL-c) level compared to the control group (Table 3). These results are in agreement with those of Fuliang et al. (2005), who found that oral administration of propolis significantly lowered total cholesterol, triglycerides, LDL-c and very low-density lipoprotein cholesterol (VLDL-c) in the serum of rats while increased the serum level of HDL-c. Also, Kolankaya et al. (2002) found that propolis significantly decreased cholesterol and triglycerides. Some studies suggested that propolis can act in several ways to lower plasma LDL-bound cholesterol. First, uptake of cholesterol in the gastrointestinal tract could be inhibited. Second, LDL-c could be eliminated from the blood via LDL receptor and finally, the activity of cholesterol-degrading enzymes, namely cholesterol-7-hydroxylase could be increased. It has been suggested that propolis decreased total cholesterol and LDL-c while increased HDL-c due to absorption, degradation or elimination of cholesterol.

Other studies also show that propolis reduced cholesterol and increased HDL-c, indicating that it may mobilize cholesterol from extrahepatic tissues to the liver where it is catabolised (Fuliang et al.2005). Alves et al. (2008) reported that the hypocholesterolemic effect of propolis is the result of a direct effect on the liver or an indirect effect through the thyroid hormones, since thyroid hormones affect reactions in almost all the pathways of lipid metabolism. Furthermore, the specific activity of AChE in the serum and brain extract could be normalized after treatment with CPF plus propolis.

This study shows also that the astrocytic density was raised in the CPF-treated group (Figure 3). El-Masry et

al. (2011) reported that propolis increased brain vitamin C, vitamin E and P-SH levels in rat's brain that was treated with propolis alone/or in combination with Pb. They concluded that propolis has beneficial effects and could be able to antagonize Pb-induced neurotoxicity. The biological effects exhibited by propolis could be related to an overall effect of the phenolic compounds present in propolis. Caffeic acid phenethyl ester (CAPE) is an active component of propolis and has been used in traditional medicine to treat a number of diseases, CAPE treatment have been shown to protect tissues from ROS mediated oxidative stress and reduce lipid peroxidation in ischemia and toxic injuries. The antioxidant activity of CAPE is due to the presence of two hydroxyl groups in its structure (Sud'ina et al., 1993).

Twelve (12) different flavonoids, pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaemferol, a pigenin, myricetin and quercetin, two phenolic acids, cinnamic and caffeic acid are present in propolis (El-Masry et al., 2011). Propolis contains acid derivatives such as benzoic-4- hydroxy benzoic which improves the digestive utilization of calcium, phosphorus and magnesium (Haro et al., 2000). Furthermore, it has been reported that Chinese propolis contains many biologically active constituents expected to be useful for improvement of the neuropathological conditions in the injured spinal cord (Usia et al., 2002) and to protect the brain from ischemia-reperfusion injury (Khan et al., 2007). Therefore, CAPE is one of the most probable candidates that may act beneficially after traumatic spinal cord injury (Song et al., 2002). Chrysin, a flavonoid of highest concentration in Chinese propolis, potentially represents anti-oxidative capacity in neuronal cell death and exhibits an anti-inflammatory effect by inhibiting iNOS mRNA and NO production (Izuta et al., 2008).

## Conclusion

CPF has adverse effects on human health. Our results report that CPF induced oxidative damage and caused damage to the liver and brain of the male rats while, propolis administered in combination with chloropyrifos minimized its hazardous effect. In addition, propolis alone was proved to be beneficial in decreasing the levels of free radicals and lipids and increasing the activities of the antioxidant enzymes. Consequently, the exposure to CPF should be reduced. Also, the intake of propolis as supplement could be a beneficial way to overcome the toxicity of CPF-exposure.

## ACKNOWLEDGEMENT

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