

**Antioxidant effect of aqueous extract of propolis on hepatotoxicity induced  
by octylphenol in male rats**  
**Efecto antioxidante del extracto acuoso de propóleos sobre la hepatotoxicidad inducida  
por el octilfenol en ratas macho**

Saleh, Eman M.

Department of Biochemistry, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt 11566. Phone number: +202 26828641, Mobile: +2 (0100) 1648959, Fax: +202 26853561

[e\\_ahmed@sci.asu.edu.eg](mailto:e_ahmed@sci.asu.edu.eg)

Recibido: 10 de mayo de 2012  
Aceptado: 26 de agosto de 2012

**Abstract.** 4-tertiary-octylphenol (4-tert-OP) is an alkylphenol that affects human health by stimulating free radical production. Aqueous propolis extract is a natural product rich in flavonoids that have antioxidant activity. This study was designed to investigate the ability of aqueous propolis extract to reduce the hepatotoxicity induced by 4-tert-OP in male rats. Animals were assigned to 5 groups and treated for 6 weeks. Group 1: control; group 2: 100 mg 4-tert-OP/kg b.wt./day; group 3: 100 mg aqueous propolis extract /kg b.wt./day; group 4: 100mg 4-tert-OP/kg b.wt./day plus 100 mg aqueous propolis extract /kg b.wt./day; group 5: 100 mg 4-tert-OP/kg b.wt./day for 6 weeks followed by 100 mg aqueous propolis extract /kg b.wt./day for 6 weeks. Group 4-tert-OP significantly elevated AST, ALT, ALP, GGT, bilirubin, creatinine, urea, total lipids, total cholesterol, triglycerides, LDL-C and MDA with a significant decrease in total proteins, albumin, globulin, HDL-C, total antioxidant capacity, SOD, CAT and GST compared to control group. Administration of aqueous propolis extract either alone or combined with 4-tert-OP ameliorated the hepatotoxicity induced by 4-tert-OP. DNA fragmentation supported the deleterious effect of 4-tert-OP and the ameliorative effect of propolis on liver cellular proteins and enzymes. Histopathological findings revealed the hepatotoxicity induced by 4-tert-OP and the protective effect of aqueous propolis extract. In conclusion, aqueous propolis extract could reduce the damage and toxicity effects on liver cells induced by 4-tert-OP.

**Keywords:** 4-tertiary-octylphenol (4-tert-OP); Aqueous propolis extract; Hepatotoxicity; Antioxidant enzymes.

**Resumen.** El 4-terc-octilfenol (4-terc-OP) es un alquilfenol que afecta a la salud humana mediante la estimulación de la producción de radicales libres. El extracto acuoso de propóleos es un producto natural rico en flavonoides que tienen actividad antioxidante. Este estudio fue diseñado para investigar la capacidad del extracto de propóleos de reducir la hepatotoxicidad inducida por el 4-terc-OP en ratas macho. Los animales fueron asignados a 5 grupos y tratados durante 6 semanas. Grupo 1: control; grupo 2: 100 mg de 4-terc-OP/kg/día; grupo 3: 100 mg de extracto de propóleos/kg/día; grupo 4: 100 mg de 4-terc-OP/kg/día más 100 mg de extracto de propóleos/kg/día, grupo 5: 100 mg de 4-terc-OP/kg/día durante 6 semanas, seguidos de 100 mg de extracto de propóleos/kg/día durante 6 semanas. El grupo 4-terc-OP mostró niveles significativamente elevados de AST, ALT, ALP, GGT, bilirrubina, creatinina, urea, lípidos totales, colesterol total, triglicéridos, LDL-C y MDA, con una disminución significativa de proteínas totales, albúmina, globulina, HDL-C, la capacidad antioxidante total, SOD, CAT y GST, en comparación con el grupo control. La administración de extracto de propóleos, ya sea solo o combinado con 4-terc-OP redujo la hepatotoxicidad inducida por 4-terc-OP. Los estudios de fragmentación del ADN apoyan el efecto deletéreo observado por el tratamiento con 4-terc-OP y el efecto protector del extracto de propóleos, sobre las proteínas y las enzimas celulares hepáticas. Los resultados histopatológicos revelaron la hepatotoxicidad por 4-terc-OP y efecto protector inducido por el extracto de propóleos. En conclusión, el extracto de propóleos podría reducir el daño hepático y los efectos celulares de toxicidad en las células del hígado inducidos por 4-terc-OP.

## INTRODUCTION

Many reports suggest that different types of man-made chemicals have become widespread as environmental contaminants affecting the health of human and wildlife populations (Longnecker *et al.* 2000). Xenosteroids are a large and structurally diverse group of chemicals that affect human health. The sug-

gested mechanism of action of xenosteroids involves binding to estrogen and androgen receptors (Bulayeva and Watson 2004) but many recent studies explained the effect of these chemicals on different organs and systems (Karafakioglu and Aslan 2010). Two xenoestrogens that are currently produced in large volumes are alkylphenols (APs) and bisphenol A

(BPA). Alkylphenols, such as nonylphenol (NP) and 4-tertiary-octylphenol (4-tert-OP) have been found in sewage effluent, groundwater and drinking water (Céspedes *et al.* 2008). Exposure to 4-tert-OP may occur from contact with personal care products, detergents, water, and food containing 4-tert-OP (Calafat *et al.* 2008).

Normal cellular function depends on a balance between the reactive oxygen species (ROS) produced and the antioxidant defense mechanisms available for the cell. This equilibrium is hampered by the ROS upsurge that culminates in oxidative stress (Fidan and Dundar 2008). ROS arise as by-products of normal cellular metabolism or as a consequence of exposure to certain chemicals (Krieger and Loch-Carusio 2001). These electrophilic metabolites or radicals can readily interact with essential biomolecules, including DNA, proteins and lipids, leading to oxidative modification, hence, structural and functional alterations (Fernandez *et al.* 2003).

Propolis is considered as one of the most promising natural products presenting not only therapeutic action, but also a preventive one (Galvao *et al.* 2007). It contains more than 300 compounds from different groups. It contains mostly a mixture of polyphenols, flavonoids (major ingredients), phenolic acids and their esters, caffeic acid and their esters, phenolic aldehydes and ketones; moreover, proteins, amino acids, vitamins (A, B1, B2, B3 and biotin), minerals (calcium, phosphorous, magnesium, manganese, iron, zinc, silicon, potassium, cobalt and copper). Flavonoids and esters of phenolic acids in propolis have been recognized as antiseptic, cytostatic, antimicrobial, antibiotic, antiviral, antifungal, antibacterial and hepatoprotective (Najafi *et al.* 2007). General medicinal uses of propolis have also been described; they include treatment of cardiovascular, blood system and respiratory disorders, and cancer (Kimoto *et al.* 1999), digestive tract disorders and immune system support (Ansorge *et al.* 2003) and dermatological disorders (Hausen *et al.* 1992). Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation and collagen formation (El-Kott and Owayss 2008). Most of these effects have been related to the antioxidant and free radical scavenging properties of propolis (Wang *et al.* 2004). The aqueous propolis extract contains about 90% of carbohydrates and 80% of total flavonoids

found in crude propolis. In aqueous propolis extract, most of flavonoids, terpenes, vitamins, amino acids, caffeic acid phenyl esters and other water-soluble compounds were released and remain free of wax and resin (Najafi *et al.* 2007). Recently, this nontoxic natural product was reported to show multiple pharmacological effects represented as antiviral, antibacterial, antifungal, anticancer, anti-inflammatory and antioxidant properties (Kumazawa *et al.* 2004).

Octylphenol has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals. Moreover, it has been shown that, both *in vivo* and in cultured hepatocytes, in parallel to liver damage, octylphenol may also determine a recognizable fragmentation of nuclear DNA and karyolysis (Kim *et al.* 2006). On the other hand, propolis may be useful for ameliorating toxic effects of different substances (Aso *et al.* 2004).

Therefore, the present study was conducted to investigate: (1) the biochemical alterations, oxidative stress and DNA fragmentation induced in liver from male albino rats treated with 4-tert-OP, (2) the role of propolis as a natural curative antioxidant product in alleviating that negative effect of 4-tert-OP, and (3) the effect of propolis alone on the tested biochemical parameters, as a protective supplement.

## MATERIALS AND METHODS

### Chemicals

Octylphenol was purchased from Sigma-Aldrich Company (Los Angeles, USA). Crude propolis was obtained from honey bee (*Apis mellifera carnica*) colonies situated at the apiary of Faculty of Agriculture at Fayoum, Egypt. All other chemicals used in the experiment were of analytical grade.

### Preparation of 4-tert-OP

4-tert-OP was suspended in corn oil, sonicated for 60 seconds and kept in refrigerator during the experiment period. The resulting suspension was administered to animals by gavage at doses of 100 mg/kg b.wt. (Barles and Aydogan 2008).

### Preparation of aqueous propolis extract

Propolis was kept dry and frozen (-40°C) until used. Propolis samples were mixed with distilled water, heated gently and filtered through

Whatman No:1 filter paper. Propolis was freshly prepared and administered to animals by gavage at dose of 100 mg/kg b.wt. (El-Khayat *et al.* 2009).

### **Animals**

Fifty adult male Swiss albino rats of the same age (6 weeks) and weighing 120-150 g were used throughout this study. Animals were obtained from the Egyptian Holding Company for Biological Products and Vaccines, Cairo, Egypt. Animals were maintained under standard conditions of ventilation, temperature ( $25\pm 2^\circ\text{C}$ ), humidity (60-70%) and light/dark condition (12/12h). The rats were housed in stainless steel cages and provided with free access to food and drinking water *ad libitum*. After two weeks of acclimatization, animals were divided into 5 groups (n=10 each). Rats were orally administered their respective doses by gavage every day throughout the study. The local committee approved the design of the experiments, and the protocol complied with the guidelines of the National Institutes of Health (NIH, USA).

### **Experimental design**

Animals were divided into 5 groups of 10 animals each. Group 1 (control group); rats were orally administered with corn oil once per day for 6 weeks and for 12 weeks. Group 2 (4-tert-OP group); rats were orally administered with 4-tert-OP (100 mg/kg b.wt.) for 6 weeks. Group 3 (Propolis group); rats were daily administered with aqueous propolis extract (100 mg/kg b.wt.) for 6 weeks. Group 4; rats were orally administered with a combination of 4-tert-OP (100 mg/kg b.wt.) and aqueous propolis extract (100 mg/kg b.wt.) for 6 weeks. Group 5; rats were orally administered with 4-tert-OP (100 mg/kg b.wt.) for 6 weeks followed by a combination of 4-tert-OP (100 mg/kg b.wt.) and aqueous propolis extract (100 mg/kg b.wt.) for another 6 weeks.

### **Biochemical analysis**

At the end of the experiment, animals were sacrificed using ether anesthesia and decapitation after a 24 hours fasting period from the final administration. Blood samples were collected, left to clot and then centrifuged at 3000 rpm for 15 minutes. Serum was stored at  $-80^\circ\text{C}$  for evaluating the biochemical parameters. Another blood samples were collected immediately on heparin to obtain plasma samples. Tubes

with heparinized blood were centrifuged at 3000 rpm for 10 minutes and stored at  $-80^\circ\text{C}$ .

### **Liver tissue**

#### *Antioxidant parameters*

Liver tissue was minced and homogenized (10% w/v) in tris-sucrose buffer (50mM Tris-HCl, 0.25M sucrose, pH 7.2-7.4) by using a tissue homogenizer (Mechanika precyzyjna Warszawa MPW-309, Poland). The homogenate was centrifuged at 5000 rpm for 20 minutes at  $4^\circ\text{C}$ , and the supernatant was used for determination of biochemical parameters, assay of different antioxidant enzymes and thiobarbituric acid reactive substances (TBARS).

#### *Histopathological studies*

Liver were immediately excised, washed using chilled saline solution, blotted, weighed and processed for biochemical studies. A small piece of each was immediately fixed in 10% formalin. These formalin-fixed tissues were embedded in paraffin, sectioned (5mm), stained with hematoxylin and eosin (H&E), and examined under a light microscope for histopathological assessment.

### **Blood parameters**

Serum was used to estimate the following liver enzymes, alanine transaminase (ALT; EC 2.6.1.2) and aspartate transaminase (AST; EC 2.6.1.1) (Reitman and Frankel 1957), alkaline phosphatase (ALP; EC 3.1.3.1) (Bessey *et al.* 1946) and gamma glutamyl transferase (GGT; EC 2.3.2.2) (Rosalki 1975) using BioMed kit (Hannover, Germany). Total and direct bilirubin determinations (Pearlman and Lee 1974) were performed by using Diamond Diagnostics kit (Hannover, Germany). Serum and liver total protein contents (Lowry *et al.* 1951), albumin concentration (Doumas *et al.* 1971), urea and creatinine (Henry 1974; Patton and Crouch 1977), total lipids (Knight *et al.* 1972), total cholesterol (Allain *et al.* 1974), triglycerides (Fossati and Prencipe 1982) and HDL-cholesterol (Castelli *et al.* 1977) were quantified using BioMed kit (Hannover, Germany). Finally, serum LDL-cholesterol was calculated using the equation of [total cholesterol-(triglycerides/5)-HDL-cholesterol]. Total antioxidant capacity was measured in plasma using the commercial Biodiagnostic Company Kit according to the previously described method (Koracevic *et al.* 2001). Hepatic malondialdehyde (MDA) level was estimated

(Esterbauer and Cheeseman 1990) in terms of thiobarbituric acid reactive substances formation. The activity of glutathione S-transferase (GST; EC 2.5.1.18) was measured according to the method of Habig *et al.* (1974). The activity of catalase enzyme (CAT; EC 1.11.1.6) in tissue supernatant was measured according to the method of Xu *et al.* (1997). Total (Cu-Zn and Mn) superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to a previous described method (Minami and Yoshikawa 1979).

### DNA fragmentation

Liver samples were collected immediately after sacrificing the animals. The damage of DNA was tested by using the diphenylamine assay according to Gibb *et al.* (1997).

### Statistical analysis

Data were expressed as mean  $\pm$  standard deviation (S.D.) of ten replicate determinations. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among different groups (Sokal and Rohlf, 1969). The results are considered to be significant when  $P < 0.05$ . All statisti-

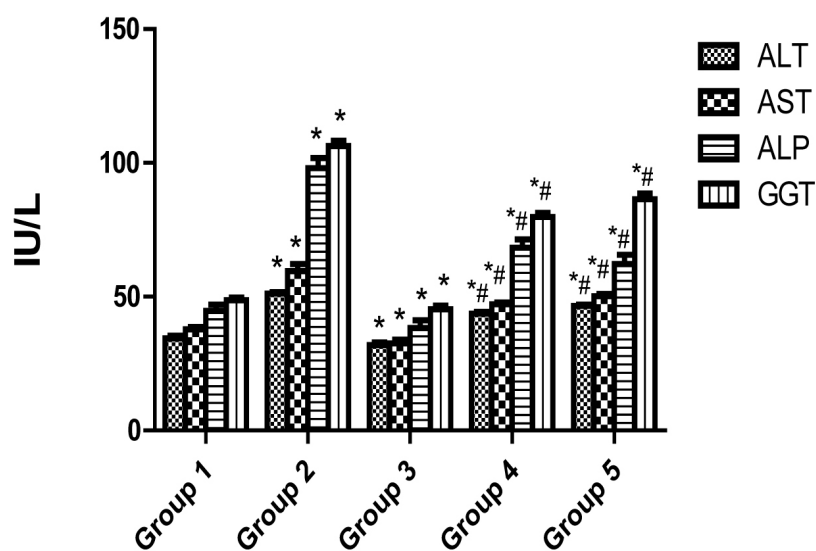
cal analyses were performed using SPSS software program version 16 (SPSS® Inc., USA).

### RESULTS

No mortality was seen in animals during the study. The dose of propolis did not initiate any side effects for the animals, whereas many side effects were observed in animals treated with 4-tert-OP such as yellowish body hair, loosing of body weight, general weakness (completely loss of activity), abdominal edema and enlargement of testes.

### Blood parameters

The data summarized in *Figure 1* indicates that, AST, ALT, ALP and GGT activities were significantly increased ( $P < 0.05$ ) in rats receiving 4-tert-OP either alone (group 2) or in combination with aqueous propolis extract (groups 4 and 5) when compared with the control group (group 1). However, the same parameters in rats receiving aqueous propolis extract alone (group 3) were significantly lower ( $P < 0.05$ ) than the control group. Control group of 12 weeks presented the same characteristics of the 6 weeks control group.



**Figure 1.** Changes in the activities of serum ALT (IU/l), AST (IU/l), ALP (IU/l) and GGT (IU/l) of male albino rats treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

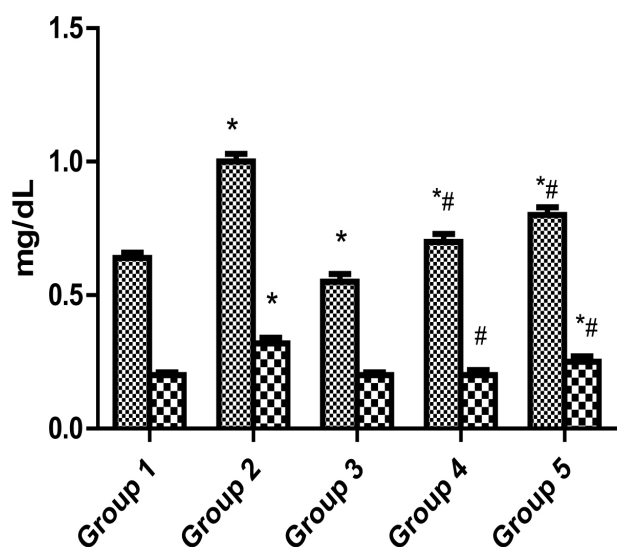
Values are expressed as means  $\pm$  SD; n=10 for each treatment group.

Significant difference from the control group at \* $P < 0.05$ .

Significant difference from the octylphenol group at # $P < 0.05$ .

Figure 2 shows the significant increase ( $P<0.05$ ) in total bilirubin level in groups treated with 4-tert-OP either alone or combined with aqueous propolis extract compared to control group. Meanwhile, there was a significant decrease ( $P<0.05$ ) in the level of the same parameter in propolis group compared to the control group. In addition, in Figure 2, it is demonstrated the positive effect of aqueous propolis extract in groups 4 and 5, as detected by the significant decrease ( $P<0.05$ ) in the level of bilirubin of those groups compared to the 4-tert-OP group. On the other hand, no significant changes were detected in the level of direct bilirubin of propolis group although there was a significant increase ( $P<0.05$ ) in the same parameter of 4-tert-OP group compared to control group. In groups 4 and 5, a sig-

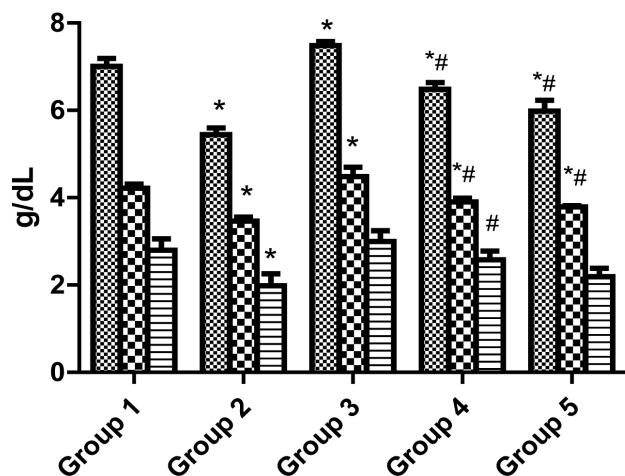
nificant decrease was detected in direct bilirubin level compared to 4-tert-OP group. Total proteins content and albumin were significantly decreased ( $P<0.05$ ) only in rats receiving 4-tert-OP alone when compared to the control group. However, the same parameters were significantly increased ( $P<0.05$ ) and significantly decreased ( $P<0.001$ ) when compared to rats receiving aqueous propolis extract alone (group 3) or rats receiving a combination of both 4-tert-OP and aqueous propolis extract (groups 4 and 5) in relation to control group, respectively. In addition, aqueous propolis extract treatment significantly attenuated the 4-tert-OP-mediated decrease in serum total proteins, albumin and globulin concentrations as shown in Figure 3.



█ Total Bilirubin  
 █ Direct Bilirubin

**Figure 2.** Concentration of total and direct bilirubin (mg/dl) of male albino rats treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

Values are expressed as means±SD; n=10 for each treatment group. Significant difference from the control group at \* $P<0.05$ . Significant difference from the octylphenol group at # $P<0.05$ .



█ Total Proteins  
 █ Albumin  
 █ Globulins

**Figure 3.** Serum level of total proteins (g/dl), albumin (g/dl) and globulin (g/dl) of male albino rats treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

Values are expressed as means±SD; n=10 for each treatment group. Significant difference from the control group at \* $P<0.05$ . Significant difference from the octylphenol group at # $P<0.05$ .

Further, 4-tert-OP treatment led to a significant increase ( $P<0.05$ ) in the creatinine and urea levels, while aqueous propolis extract showed a significant recovery in these parameters ( $P<0.05$ ) by reducing their concentrations in serum. Treatment with 4-tert-OP plus aqueous propolis extract significantly alleviated the undesirable increased levels of creatinine and urea when compared to with 4-tert-OP-treated animals (Table 1). Total serum lipids, total cholesterol, triglycerides and LDL-C levels were significantly in-

creased ( $P<0.05$ ) while HDL-C was significantly lowered ( $P<0.05$ ) in 4-tert-OP group when compared to the normal control rats (Table 2). Aqueous propolis extract could significantly ( $P<0.05$ ) modulate the lipid contents in rats by decreasing total lipids, total cholesterol, triglycerides and LDL-C levels with significantly favorable increase ( $P<0.05$ ) in HDL-C level. In addition, propolis extract reversed the alterations induced by 4-tert-OP in all lipid contents as shown from the results of groups 4 and 5 (Table 2).

**Table 1.** Serum level of creatinine (mg/dl) and urea (mg/dl) of male albino rats treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

Group Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Creatinine	0.57±0.02	0.81±0.03*	0.54±0.02*	0.69±0.03*#	0.74±0.03*#
Urea	22.48±0.17	30.18±0.46*	20.94±0.46*	26.64±0.54*#	28.23±0.32*#

Values are expressed as meansSD; n=10 for each treatment group.  
Significant difference from the control group at \* $P<0.05$ .  
Significant difference from the octylphenol group at # $P<0.05$ .

**Table 2.** Changes in the concentrations of serum total lipids (mg/dl), total cholesterol (mg/dl), triglycerides (mg/dl), HDL (mg/dl) and LDL (mg/dl) of male albino rats treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

Group Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Total lipids	436.57±3.03	838.83±6.76*	400.275±10.50*	548.91±12.03*#	514.75±11.42*#
Total cholesterol	119.46±1.69	174.64±0.60*	99.31±1.16*	138.60±1.21*#	121.72±1.65#
Triglycerides	89.44±1.64	129.42±1.91*	50.20±2.42*	104.06±1.82*#	94.91±1.98*#
HDL	37.80±0.80	16.71±0.82*	55.34±0.90*	31.60±0.95*#	34.79±0.85*#
LDL	63.77±1.77	132.10±1.69*	34.00±0.90*	86.19±2.11*#	67.96±1.86*#

Values are expressed as meansSD; n=10 for each treatment group.  
Significant difference from the control group at \* $P<0.05$ .  
Significant difference from the octylphenol group at # $P<0.05$ .

### Hepatic oxidative stress

Plasma total antioxidant enzymes concentration and GST, SOD and CAT activities were significantly decreased ( $P<0.05$ ) in 4-tert-OP group compared to control group. However, the concentrations and activities of total antioxidant enzymes, GST, SOD and CAT were significantly increased ( $P<0.05$ ) when aqueous propolis extract was administered in both, groups 4 and 5, compared to 4-tert-OP group. Administration of aqueous propolis extract alone induced a significant increase in total antioxidant capacity, GST, SOD

and CAT compared to control group (Table 3). Animals treated with 4-tert-OP had a significant increase ( $P<0.05$ ) in the level of MDA concentration compared to control group (Table 3). Simultaneous treatment with aqueous propolis extract (groups 4 and 5) significantly abolished the enhancing effect of 4-tert-OP on hepatic lipid peroxidation, fact that was expressed as a lower level of MDA in hepatocytes ( $P<0.05$ ). In group 3, treated with aqueous propolis extract only, no effect on lipid peroxidation in the liver was observed (Table 3).

**Table 3.** Changes in the levels of plasma total antioxidant capacity (mM/L) and liver MDA (n mole/g tissue), SOD (units/g tissue), CAT (mole/min/g tissue), and GST (units/g tissue) of male albino rat treated with 4-tert-OP (group 2), aqueous propolis extract (group 3) and combination of both (groups 4 and 5).

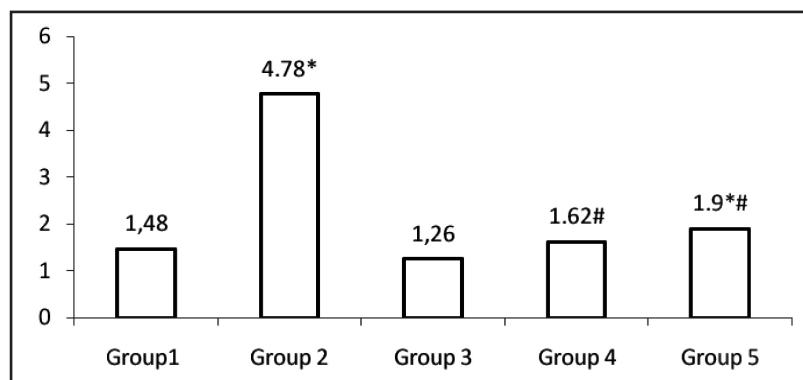
Group Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Total antioxidant capacity	1.09±0.06	0.59±0.03*	1.19±0.02*	0.83±0.01**	0.76±0.02**
MDA	158.81±11.23	658.96±20.46*	138.56±7.92	227.86±8.56**	352.24±7.89**
SOD	59.43±1.30	23.77±2.39*	65.70±3.19*	40.23±1.75**	31.91±1.62**
CAT	0.77±0.02	0.41±0.01*	0.91±0.01*	0.61±0.01**	0.51±0.01**
GST	2.04±0.11	1.06±0.06*	2.44±0.10*	1.80±0.08**	1.49±0.11**

Values are expressed as means±SD; n=10 for each treatment group.  
Significant difference from the control group at \* $P<0.05$ .  
Significant difference from the octylphenol group at # $P<0.05$ .

### DNA fragmentation

The results of the diphenylamine assay (Figure 4) showed that DNA of liver cells from 4-tert-OP treated animals was significantly degraded ( $P<0.05$ ), compared to control group. Groups

4 and 5 showed a significant improvement ( $P<0.05$ ) in the level of DNA fragmentation of liver cells, compared to 4-tert-OP treated group.



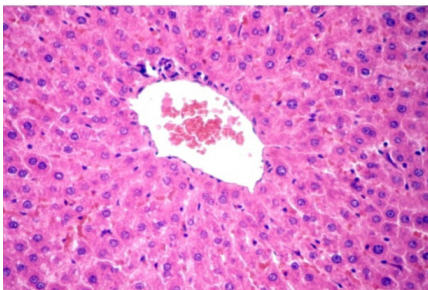
**Figure 4.** DNA fragmentation as a measure of soluble DNA released from apoptotic nuclei into the cytoplasm of male albino rat treated with 4-tert-OP, aqueous propolis extract and combination of both.

Values are expressed as means±SD; n=10 for each treatment group.  
Significant difference from the control group at \* $p<0.05$ .  
Significant difference from the octylphenol group at # $p<0.05$ .

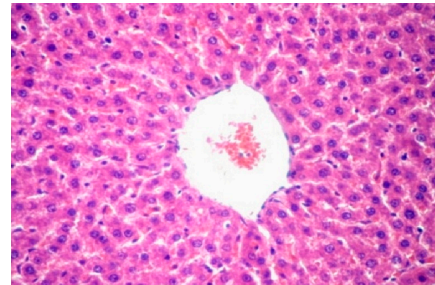
### Histopathology

Histopathological examination of liver of control group revealed normal hepatic architecture, cellular and nuclear configurations (Figure 5). Histopathological alterations observed in 4-tert-OP treated group were variable. Liver section of rats treated with 4-tert-OP showed sinusoidal dilatation and atrophy of hepatocytes (Figure 6A) and multifocal areas of coagulative necrosis (Figure 6B). Sections of liver from rats treated with aqueous propolis extract alone, showed to be similar

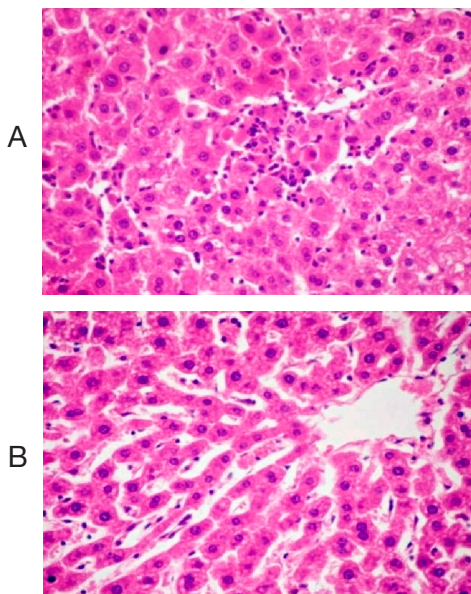
to the control group sections (Figure 7). Livers from rats treated with 4-tert-OP plus aqueous propolis extract did not reveal any necrosis area and regained their normal structure (Figure 8A&B). Liver section from rats treated with 4-tert-OP followed by aqueous propolis extract showed moderate dilatation of portal blood vessels and vacuolar degeneration of hepatocytes (Figure 9A) and multifocal areas of minute aggregations of lymphocytes scattered here and there in the hepatic parenchyma (Figure 9B).



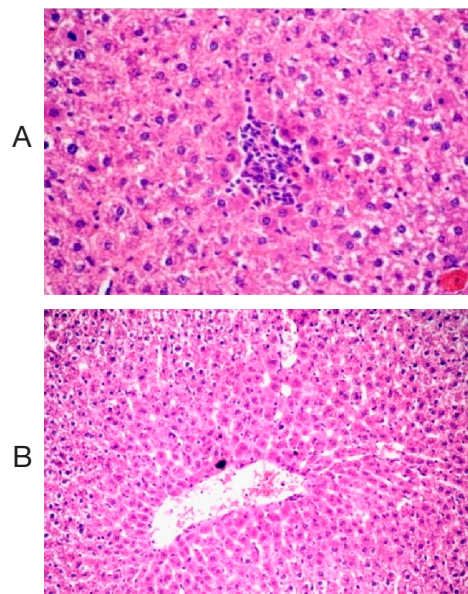
**Figure 5.** Photomicrograph of control liver section showing normal hepatic architecture. H&E stain (400x).



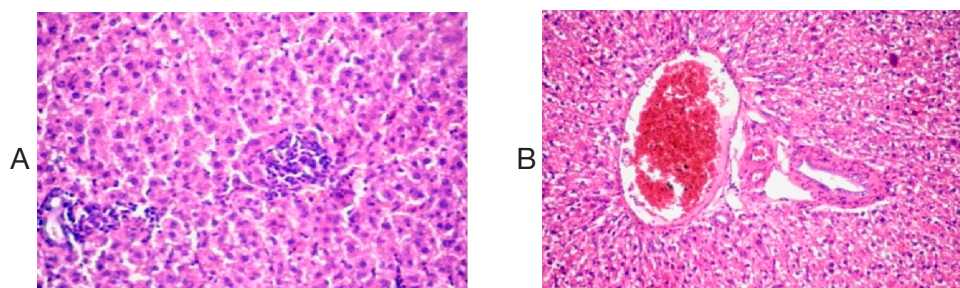
**Figure 7.** Photomicrograph of liver section of rats treated with aqueous propolis extract showing almost normal hepatic structure. H&E stain (400x).



**Figure 6.** Photomicrograph of liver section of rats treated with 4-tert-OP showing sinusoidal dilatation and atrophy of hepatocytes (A) and multifocal areas of coagulative necrosis (B). H&E stain (400x).



**Figure 8.** Photomicrograph of liver section of rats treated with 4-tert-OP plus aqueous propolis extract showing central veins were mildly dilated with mild peripheral vacuolar degeneration of hepatocytes and Kupffer cell activation (A) and minute foci of mononuclear cell infiltrate were found scattered in the hepatic parenchyma (B). No areas of necrosis were observed in the livers of such group. H&E stain (400x).



**Figure 9.** Photomicrograph of liver section of rats treated with 4-tert-OP followed by aqueous propolis extract showing moderate dilatation of portal blood vessels and vacuolar degeneration of hepatocytes (A) and multifocal areas of minute aggregations of lymphocytes scattered here and there in the hepatic parenchyma (B). H&E stain (400x).

## DISCUSSION

The multi-generation studies in rats showed toxic effects of xenoestrogens not only on reproductive organs but also on non-reproductive targets such as kidney and liver (Nagao *et al.* 2001). The present study was performed to investigate the toxic effects of 4-tert-OP on liver and the ameliorating effect of aqueous propolis extract as a natural product able to reduce the induced oxidative damage in hepatocytes of male albino rats. Although few compounds have been tested for the detoxification of 4-tert-OP, there is no previous study carried out with propolis (Korkmaz *et al.* 2010). The adverse effects of a large number of xenoestrogens depend on their production of free radicals which initiate the process of lipid peroxidation in the cell membrane. It has been suggested that therapeutic activities of propolis depend mainly on the presence of flavonoids. These flavonoids are known for their strong scavenging effect on free radicals and may also be able to suppress the formation of free radicals by binding to heavy metal ions which are known to catalyze many processes leading to the generation of free radicals (Cavallini *et al.* 1978). The aqueous propolis extract was shown to have a protective effect on hepatocytes against carbon tetrachloride (CCl<sub>4</sub>)-induced injury *in vitro* (Mahran *et al.* 1996) and *in vivo* (El-Khatib *et al.* 2002).

The current results showed a pronounced decrease in the activities of serum ALT, AST, ALP and GGT after oral treatment with aqueous propolis extract in both group 4 and 5 compared to 4-tert-OP group. These results indicate hepato-protection induced by propolis. This protective effect may be due to the antioxidant effect of propolis which was previously confirmed (Almaraz-Abarca *et al.* 2007).

Another study (Kolankaya *et al.* 2002) reported that the treatment with propolis significantly prevented the release of transaminases and significantly enhanced protein towards control, suggesting its hepatoprotective potential. The induction of AST, ALT and ALP reflects liver injury associated with necrosis, whereas GGT points to hepatic cholestasis (Rodriguez-Garay 2003). As shown from the present results, elevated levels of these liver enzymes plus total and direct bilirubin in blood circulation were observed in 4-tert-OP group compared to control group indicating hepatic injury. This is in agreement to previous results, showing that increased bilirubin levels indicated diffused harm to the liver (El-Kott and Owayss 2008). The rise in both AST and ALT levels is one of the most familiar indicators of hepatocellular damage. These observations are similar to the previously reported data (Barles and Aydogan 2008) which indicated that the exposure of male and female rats to 4-tert-OP induced potential toxic effects on liver, kidney and spleen tissues. The present data also indicated that treatment with aqueous propolis extract alone decreased the activities of AST, ALT, ALP and GGT enzymes and total bilirubin in serum compared to control group.

The present study indicated hepatocellular toxicity in 4-tert-OP treated rats as evidenced by decreased levels of protein content compared to control animals. These results are in agreement with other studies (Lotfy 2006). The anabolic effect of propolis was suggested by the increased level of serum total proteins and globulin contents in propolis treated group compared to control. This effect of propolis is in agreement with other study (Nirala *et al.* 2008) which stated that propolis significantly improved the total proteins content of the

liver and kidney and showed more profound therapeutic effects. Cellular recovery was also evident through the improvement in total proteins, albumin and globulin contents in both group 4 and 5 compared to 4-tert-OP group. This is in agreement to previous results that showed intracellular glutathione regulated protein turnover after treatment with propolis (Demasi and Davies 2003).

In this study, nephrotoxicity was manifested by inhibition of kidney function as indicated by increased serum creatinine and urea levels in 4-tert-OP group compared to control group. These results are supported by similar findings (Ferreira-Leach and Hill 2001). 4-tert-OP acts essentially in the proximal renal tubules, inhibiting the enzyme phosphoenol-pyruvate carboxylase, and it alters the structural and functional renal ability to metabolize calcium (Betina 1989). Significant reduction of serum urea and creatinine levels was noticed after administration of aqueous propolis extract in both group 4 and 5 compared to 4-tert-OP group. These results may indicate that propolis can attenuate renal damage by decreasing the concentrations of urea and creatinine as previously reported (Abo-salem *et al.* 2009) on STZ-induced diabetic rats. This effect is probably due to the antioxidant protective effect of propolis which could have accumulated in the cells of the proximal convoluted tubule of the kidney where propolis was reported to be collected and secreted (Sun *et al.* 2000). Caffeic acid phenethyl ester (CAPE), a biological active component of propolis was found to improve renal function tests in a rat model with lithium-induced renal tubular damage and oxidative stress (Oktem *et al.* 2005).

In the current study, a marked increase in the concentrations of serum total lipids, total cholesterol, triglycerides, LDL-C with a decrease in the level of HDL-C was found in the 4-tert-OP treated group compared with control group, which may reflect the impairment of liver function, particularly on lipid metabolism. Different studies indicated that propolis alleviated too high blood lipids, high total cholesterol and arteriosclerosis (Castaldo and Capasso 2002). This result is in agreement to the present data in both group 4 and 5 compared to 4-tert-OP group and represents the powerful influence of propolis to reduce the risk of hyperlipidemia as a result of some toxicant. Several studies are in agreement with the present study (Nirala *et al.* 2008) which proved the modulating effect

of propolis on total cholesterol and triglycerides levels with a significant increase in total proteins content after beryllium toxicity and the improvement of serum level of HDL-C by propolis in a dose-dependent manner.

Malondialdehyde (MDA), the stable end product of lipid peroxidation, was elevated suggesting the generation of free radicals in the metabolism of 4-tert-OP. Since, membrane phospholipids are major targets of oxidative damage, lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Lipid peroxidation produces a progressive loss of cell membrane integrity, impairment in membrane transport function and disruption of cellular ion homeostasis (Bano and Bhatt 2007). Previous studies suggested that the toxicity of 4-tert-OP may be the result of three major effects: inhibition of ATP synthesis, inhibition of protein synthesis and enhanced lipid peroxidation (Marquardt and Frohlich 1992). In the present study, aqueous propolis extract attenuated the 4-tert-OP induced MDA formation possibly due to its intrinsic antioxidant properties, thus aqueous propolis extract may prevent peroxidative changes in liver tissue. This observation demonstrates the scavenging effect of propolis on free radicals produced by liver in response to 4-tert-OP toxicity. These results are in agreement with another study (Abdel-Wahhab *et al.* 2005), in which the protecting effect of propolis on ochratoxin A toxicity was demonstrated.

Improvement of lipid profile, MDA and SOD activity in mice by propolis treatment was demonstrated (Luan *et al.* 2000). Furthermore, propolis was found to modulate antioxidant enzymes and decrease lipid peroxidation processes in plasma, liver, lungs, and brain of mice in a dose- and tissue-dependent manner (Shinohara *et al.* 2002). Propolis is a rich source of essential elements, including Zn<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup> and Ca<sup>2+</sup> (Haro *et al.* 2002), that might also be responsible for reactivating antioxidant enzymes by providing optimum trace elements.

The present study showed significant improvement in SOD activity after treatment with aqueous propolis extract. This improvement can play an important role in the cellular defense against the oxidative stress induced by 4-tert-OP. Previous study (Benguedouar *et al.* 2008) proved the decreasing of superoxide anion radicals and inhibition of the lipid

peroxidation in animals receiving 100 mg/kg propolis extract. In the present study, the increase of antioxidant enzyme activities such as SOD, CAT and GST may be considered as a protective mechanism against free radical production and lipid peroxidation. The results also showed that SOD, CAT and GST activities significantly decreased in rat blood by 4-tert-OP intoxication compared to control animals. It is possible that the observed insufficiency in antioxidant power could be due to direct modification of the antioxidant defenses by 4-tert-OP.

The dramatic effects of 4-tert-OP on protein level and the activity of liver enzymes and antioxidant enzymes are supported by the results on DNA fragmentation. The liver DNA in 4-tert-OP treated animals was found to be greatly degraded compared to control animals, which may explain the deleterious effect of 4-tert-OP on the level of cellular proteins and enzymes. This result is in agreement with previous results of Kim *et al.* (2006). The ameliorative effect of aqueous propolis extract on DNA fragmentation and cell apoptosis was confirmed by the results of group 4 and 5 compared to 4-tert-OP group, and this result is supported by similar findings of Aso *et al.* (2004).

## CONCLUSION

The present study demonstrates that 4-tert-OP induced oxidative stress in rat blood by decreasing the activities of antioxidant enzymes and protein content with an increase in liver enzymes activities. Propolis could improve liver function and serum lipid profile, and diminish the generation of free radicals by inducing antioxidant defense mechanism, hence, minimizing the damage of cell membranes. In addition, it had an anabolic effect. These results are confirmed by measuring the level of DNA fragmentation. These results were observed when aqueous propolis extract was administered alone compared to control group and also in combination with 4-tert-OP compared to 4-tert-OP group either in group 4 and 5. Based on these promising findings, it might be anticipated that propolis treatment may be beneficial in the severely hepatotoxic ailments. Consequently, propolis could be used as a potential antioxidant against 4-tert-OP intoxication as it restore the normal liver functions.

## ACKNOWLEDGMENTS

The author thanks Biochemistry Department, Ain Shams Univer-

sity-Faculty of Science for laboratory facilities and instrumental support of this work. I also thank Professor Elshazly M.O. (professor of Pathology-Faculty of veterinary medicine-Cairo University) for his help in histopathologic examination.

## REFERENCES

Abdel-Wahhab M.A., Abdel-Galil M.M., El-Lithey M. Melatonin counteracts oxidative stress in rats fed an ochratoxin A contaminated diet. *J. Pineal Res.* 2005;38:130-135.

Abo-salem O., El-edel R.H., Harisa G.E., El-halawany N., Ghonaim M.M. Experimental diabetic nephropathy can be prevented by propolis: effect on metabolic disturbances and renal oxidative parameters. *Pak. J. Pharm. Sci.* 2009;22(2):205-210.

Allain C.C., Poon L.S., Chan C.S., Richmond W., Fu P.C. Enzymatic determination of the total serum cholesterol. *J. Clin. Chem.* 1974;20:470-475.

Almaraz-Abarca N., Campos M.G., Avila-Reyes J.A. Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite. *Journal of Food Composition and Analysis.* 2007;20:119-124.

Ansorge S., Reinhold D., Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-beta1 production of human immune cells. *Zeitschrift für Naturforschung. C, Journal of biosciences.* 2003;58(7-8):580-589.

Aso K., Kanno S., Tadano T., Satoh S., Ishikawa M. Inhibitory Effect of Propolis on the Growth of Human Leukemia U937. *Biol. Pharm. Bull.* 2004;27(5):727-730.

Bano M., Bhatt D.K. Neuroprotective role of a novel combination of certain antioxidants on lindane ( $\gamma$ -HCH) induced toxicity in cerebrum of mice. *Res. J. Agric. Biol. Sci.* 2007;3(6):664-669.

Barles N., Aydogan M. Histopathological effects of maternal 4-tert-octylphenol exposure on liver, kidney and spleen of rats at adulthood. *Archives of toxicology.* 2008;83(4):341-349.

Benguedouar L., Boussenane H.N., Kebsa W., Alyane M., Rouibah H., Lahouel M. Efficiency

of propolis extract against mitochondrial stress induced by antineoplastic agents (doxorubicin and vinblastin) in rats. *Indian Journal of Experimental Biology*. 2008;46:112-119.

Bessey O.A., Lowry O.H., Brock M.J. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.* 1946;164:321-329.

Betina V. *Mycotoxins-chemical, biological and environmental aspects*. Amsterdam (NL): Elsevier. 1989;325-421.

Bulayeva N.N., Watson C.S. Xenoestrogen-induced ERK-1 and ERK-2 activation via multiple membrane-initiated signaling pathways. *Environmental Health Perspectives*. 2004;112(15):1481-1487.

Céspedes R., Lacorte S., Ginebreda A., Barceló D. Occurrence and fate of alkylphenols and alkylphenol ethoxylates in sewage treatment plants and impact on receiving waters along the Ter River (Catalonia, NE Spain). *Environmental Pollution*. 2008;153(2):384-392.

Calafat A.M., Ye X., Wong L.Y., Reidy J.A., Needham L.L. Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol: 2003–2004. *Environmental Health Perspectives*. 2008;116(1):39-44.

Castaldo S., Capasso F. Propolis an old remedy used in modern medicine. *Fitoterapia*. 2002;73:1-6.

Castelli W.P., Doyle J.T., Gordon T., Hames C.G., Hjortland M.C., Hulley S.B., Kagan A., Zukel W.J. HDL Cholesterol and Other Lipids in Coronary Heart Disease: The cooperative Lipoprotein Phenotyping Study. *Circulation*. 1977;55(5):767-772.

Cavallini L., Bindoli A., Siliprandi N. Comparative evaluation of antiperoxidative action of silymarin and other flavonoids. *Pharmac. Res. Commun.* 1978;10:133-136.

Demasi M., Davies K.J. Proteasome inhibitors induce intracellular protein aggregation and cell death by an oxygen dependent mechanism. *FEBS Letters*. 2003;542:89–94.

Doumas B.T., Watson W.A., Biggs H.G. Albu-

min standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta*. 1971;31:87-96.

El-Khatib A.S., Agha A.M., Mahran L.G., Khayyal M.T. Prophylactic Effect of Aqueous Propolis Extract against Acute Experimental Hepatotoxicity in vivo. *Z. Naturforsch.* 2002;57c:379-385.

El-Khayat Z., Ezzat A.R., Arbid M.S., Rasheed W.I., Elias T.R. Potential effects of bee honey and propolis against the toxicity of ochratoxin a in rats. *Macedonian Journal of Medical Sciences*. 2009;2(4):1-8.

El-Kott A.F., Owayss A.A. Protective effects of propolis against the amitraz hepatotoxicity in mice. *American Journal of Pharmacology and Toxicology* 2008;3(5):402-408.

Esterbauer H., Cheeseman K.H. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymology*. 1990;186:407-421.

Fernandez V., Massa L., Quinones L., Simon-Giavarotti K.A., Giavarotti L., D'Almeida V., Azzalis L.A., Junqueira V.B., Videla L.A. Effects of gamma-hexachlorocyclohexane and L-3,3',5-triiodothyronine on rat liver cytochrome P450E1-dependent activity and content in relation to microsomal superoxide radical generation. *Biological Research* 2003;36(3-4):359-365.

Ferreira-Leach A.M., Hill E.M. Bioconcentration and distribution of 4-tert-octylphenol residues in tissues of the rainbow trout (*Oncorhynchus mykiss*). *Mar. Environ. Res.* 2001;51:75-89.

Fidan A.F., Dundar Y. The effects of *Yucca schidigera* and *Quillaja saponaria* on DNA damage, protein oxidation, lipid peroxidation and some biochemical parameters in streptozotocin-induced diabetic rats. *Journal of Diabetes and its complications* 2008;22(5):348-356.

Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *J. Clin. Chem.* 1982;28(10):2077-2080.

Galvao J., Abreu J.A., Cruz T., Machado G.A.S., Niraldo P., Daugsch A., Moraes C.S.,

Fort P., Park Y.K. Biological therapy using propolis as nutritional supplement in cancer treatment. *International Journal of Cancer Research*. 2007;3(1):43-53.

Gibb R.I., Taylor D.D., Wan T., O'Connor D.M., Doeriog D.L., GerHel-Taylor T. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. *Gynecology. Oncol.* 1997;65:13-22.

Habig W.H., Pabst M.J., Jakoby W.B. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 1974;249:7130-7139.

Haro A., Lopez-Aliaga I., Lisbona F., Barrionuevo M., Alferez M.J.M., Campos M.S. Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. *Journal of Agricultural Food Chemistry*. 2002;48:5715-5722.

Hausen B.M., Evers P., Stuwe H.T., Konig W.A., Wollenweber E. Propolis allergy (IV) Studies with further sensitizers from propolis and constituents common to propolis, poplar buds and balsam of Peru. *Contact Dermatitis*. 1992;26(1):34-44.

Henry R.J. *Clinical chemistry: principles and techniques*. 2<sup>nd</sup> edition, Harper and Row, New York, USA, 1974.

Karafakioglu Y.S., Aslan R. Taurine prevents nonylphenol-induced oxidative stress in rats. *Journal of Animal and Veterinary Advances*. 2010;9(1):37-43.

Kim S.K., Kim B.K., Shim J.H., Gil J.E., Yoon Y.D., Kim J.H. Nonylphenol and octylphenol-induced apoptosis in human embryonic stem cells is related to Fas-Fas ligand pathway. *Toxicological Sciences*. 2006;94(2):310-321.

Kimoto N., Hirose M., Kawabe M., Satoh T., Miyataka H., Shirai T. Post-initiation effects of a super critical extract of propolis in a rat two-stage carcinogenesis model in female F344 rats. *Cancer Lett.* 1999;147:221-227.

Knight J.A., Anderson S., Rawle J.M. Chemical basis of the sulfo-phosphovanillin reaction for the estimation of total serum lipids. *J. Clin.*

*Chem.* 1972;18(3):199-202.

Kolankaya D., Selmanoglu G., Sorkun K., Salih B. Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. *Food Chem.* 2002;78:213-217.

Koracevic D., Koracevic G., Djordjevic V., Andrejevic S., Cosic V. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.* 2001;54:356-361.

Korkmaz A., Ahbab M.A., Kolankaya D., Barlas N. Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Food Chem. Toxicol.* 2010;48(10):2865-2871.

Krieger T.R., Loch-Carusio R. Antioxidants prevent gamma-hexachlorocyclohexane-induced inhibition of rta myometrial gap functions and contractions. *Biology of Reproduction*. 2001;64(2):537-547.

Kumazawa S., Hamasaka T., Nakayama T. Antioxidant activity of propolis of various geographic origins. *Food Chem.* 2004;84:329-339.

Longnecker M.P., Gladen B.C., Patterson D.G., Rogan W.J. Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. *Epidemiology*. 2000;11:249-254.

Lotfy M. Biological activity of bee propolis in health and disease- a review. *Asian Pac. J. Cancer Prev.* 2006;7:22-31.

Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951;193:265-272.

Luan J., Wang N., Tian L. Study on the pharmacologic effect of propolis. *Zhong Yao Cai.* 2000;23(6):346-348.

Mahran L.G., El-Khatib A.S., Agha A.M., Khayyal M.T. The protective effect of aqueous propolis extract on isolated rat hepatocytes against carbon tetrachloride toxicity. *Drugs Exp. Clin. Res.* 1996;22:309-316.

Marquardt R.R., Frohlich A.A. A Review of Recent Advances in Understanding Och-

ratoxicosis. *Journal of Animal Science*. 1992;70:3968-3988.

Minami M., Yoshikawa H. A simplified assay method of superoxide dismutase. *Clinica Acta*. 1979;92:337.

Nagao T., Yoshimura S., Saito Y., Nakagomi M., Usumi K., Ono H. Reproductive effects in male and female rats from neonatal exposure to p-octylphenol. *Reprod. Toxicol*. 2001;15:683-692.

Najafi M.F., Vahedy F., Seyyedini M., Jomehzadeh H.R., Bozary K. Effect of the water extracts of propolis on stimulation and inhibition of different cells. *Cytotechnology* 2007;54(1):49-56.

Nirala S.K., Bhadauria M., Mathur R., Mathur A. Influence of  $\alpha$ -tocopherol, propolis and piperine on therapeutic potential of tiferron against beryllium induced toxic manifestations. *Journal of Applied Toxicology*. 2008;28: 44-54.

Oktem F., Ozguner F., Sulak O., Olgar S., Akturk O., Yilmaz H., Altuntas I. Lithium-induced renal toxicity in rats: protection by a novel antioxidant caffeic acid phenethyl ester. *Mol. Cell. Biochem*. 2005;277(1-2):109-115.

Patton C.J., Crouch S.R. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem*. 1977;49:464-469.

Pearlman F.C., Lee R.T. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. *Clin. Chem*. 1974;20:447-453.

Reitman S., Frankel S. Colorimetric determination of GOT and GPT activity. *Amer. J. Clin. Pathol*. 1957;28:56-63.

Rodriguez-Garay E.A. Cholestasis: human disease and experimental animal models. *Ann. Hepatol*. 2003;2:150-158.

Rosalki S.B. Gamma-glutamyltranspeptidase. In: *Advances in clinical chemistry*. Bodanski, O., and Latner, A. L. (eds.), Vol. 17, New York, London: Academic Press, 1975. p: 53-107.

Shinohara R., Ohta Y., Hayashi T., Ikeno T. Evaluation of antilipid peroxidative action of propolis ethanol extract. *Phytother Res*. 2002;16(4):340-347.

Sokal R.R., Rohlf F.J. *The principles and practice of statistics in biological research*. 1st edn. Freeman, W.H. and company, San Francisco, USA, 1969.

Sun F., Hayami S., Haruna S., Ogiri Y., Tanaka K., Yamada Y. In vivo antioxidative activity of propolis evaluated by the interaction with vitamin C and E and the level of lipid hydroperoxides in rats. *Journal of Agricultural Food Chemistry*. 2000;48(5):1462-1465.

Wang B-J., Lien Y-H., Yu Z-R. Supercritical fluid extractive fractionation: study of the antioxidant activities of propolis. *Food Chemistry*. 2004;86:237-243.

Xu J.B., Yuan X.F., Lang P.Z. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. *Chinese Environ. Chem*. 1997;16:73-76.