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Original Article

Role of propolis in improving hematological, biochemical and histopathological changes in rat liver after subchronic exposure to formaldehyde

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ABSTRACT

Exposure to formaldehyde by injection, ingestion, or inhalation causes toxic effects in the respiratory tract, nervous and urogenital systems and liver as well as inflammation in the brain and kidney in many species including humans. In traditional medicine honeybee products, such as honey, propolis, royal jelly, beeswax, and also bee venom, have long been used as a scavenging agent. The aim of the present study is to evaluate the protective effect of propolis against the hepatotoxic effects of formaldehyde (FA) in rats. 80 adult albino rats (200-250 gm) were divided into four groups. Group 1 was considered as the control group and was treated with water, Group II received propolis (50 mg/kg bw), and Group III received FA (25 mg/kg body weight/day). Group IV received FA (25 mg/kg body weight/day) prior to treatment with propolis (50 mg/kg bw). All rats were administered formaldehyde (FA) and propolis by means of gastric gavage for 2 months. Blood and liver were sampled and prepared for hematological, biochemical, histopathological and ultrastructural examinations. Hematological and histochemical variations detected in Group III were greater in comparison with Groups I & II. Histopathological examination of the liver of rats of Group III showed pyknosis of hepatocyte nuclei, fibrosis, cytoplasmic vacuolation and dilatation of central vein. Ultrastructural examination of the liver of rats of Group III showed damages to the nuclear membrane, with shrinking nucleus and a less prominent nucleolus, swollen and vacuolated mitochondria, large fat droplets, and cytoplasmic vacuoles. The endoplasmic reticuli were small in numbers and enlarged and swollen in shape; also there were degenerated Golgi apparatus and some degenerated hepatocytes. On the other hand, Group IV showed partial and gradual improvement in the pathological changes and the damage of the liver. Our results demonstrate an ameliorative effect of propolis on the FA-induced pathological effects in the liver of rats.

KEYWORDS: propolis, formaldehyde, liver, histopathology, rat.

1. INTRODUCTION

Formaldehyde (FA) is widely used in manufacturing disinfectants that are used for disinfecting houses and equipments and in the treatment of seeds of the rape plant, as well as soybean oil, and groundnut meals used for feeding large animals [1]. FA is used as a preservative of foods (e.g. seafood, fruits, honey and vegetables) and added in some drugs. FA is also used as an antibacterial against Salmonella in chicken food [2]. FA is broadly used in the production of insulation material, carpets, and wood-pressed products [3]. FA is used in the manufacture of plastics which can tolerate heat, including those used in kitchen tools, filters and whiteboards [4].

But FA is toxic to the respiratory system, urinary system, genital system and nervous system [2, 5]. Honey bees produce a substance which has a sticky texture, called propolis or bee glue, which is produced by mixing their own waxes with resins collected from plant exudates. It has been found to have many biological and pharmacological effects including antibacterial [6], hepatoprotective [7], anticancer [8, 9], immunomodulatory [6], antioxidative [10] and anti-inflammatory effects [11]. Propolis also contains more than 200 components including polyphenols, phenolic aldehydes, coumarins, sequiterpene quinines, amino acids, steroids, and inorganic compounds [12]. The antioxidant activity of propolis, which is mainly due to its phenolic components such as phenolic acids and flavonoids, has a major scavenging effect. Moreover, propolis also inhibits the generation of superoxide anion [13, 14]. Also, propolis could reverse the consumption of glutathione which is synthesized in the liver and has radical scavenging activity [15].

2. MATERIALS AND METHODS

2.1. Chemicals and propolis sample

FA (40% solution) was bought from El Gomhouria Co. (Cairo, Egypt). It was diluted in distilled water to working stock concentrations to be used in this experiment [16].

Propolis from the Al-Shafa region situated 20 km from Taif, Saudi Arabia with different dominant floras was used. The sample was collected from different sites inside the beehive during March 2018 and was kept in the dark in a freezer [17].

2.2. Animals

According to the guidelines of the Ethics Committee of Animal Experiments of University of Taif, Saudi Arabia, the recommendations in the Guide for Care and Use of Laboratory Animals were followed carefully. 80 male and female rats (200-250 gm), were obtained from Taif laboratory. Animals were housed in plastic cages and offered water and balanced diet *ad libitum* and acclimated to laboratory conditions.

2.3. Experimental design

One week before the experiment, the rats were acclimatized. 80 adult albino rats (200-250 gm) were equally divided into four groups and each group was housed in separate plastic cages.

Group I was considered as the control group and was treated with water; Group II was treated with propolis (50 mg/kg bw) [18, 19]. Group III received FA (25 mg/kg b.wt/day) [20]. Group IV received FA (25 mg/kg body weight/day) [20], followed by propolis (50 mg/kg bw). Rats were given FA and propolis by gastric gavage for 2 months.

2.4. Sample collection and tissue samples

At the end of the 2nd month, the rats were anesthetized by ether inhalation. Blood samples were collected by intracardiac puncture from each rat and divided into two. The first sample was kept in an ethylenediaminetetraacetic acid (EDTA) tube (heparinized tube) for determination of hematological parameters and the second sample was left to clot at 37 °C and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was collected and stored at -20 °C for biochemical analysis. Livers of rats were excised and divided into small slices, cleaned, washed with normal saline and then divided into three groups. The first group was kept at -80 °C until used, the second group was used for histological examination and the third group was processed for ultrastructure examination.

2.5. Hematological studies

The first sample in the EDTA tube (heparinized tube) was analyzed for the number of red blood cells (RBCs), white blood cells (WBCs), hemoglobin concentration (Hb %), and the differential count of polymorphs and lymphocytes according to standard methods using an Animal Blood Counter-ABC vet (Horiba ABX, France).

2.6. Biochemical assays

2.6.1. Liver biomarker assessment

The second sample was left to clot at 37 °C and centrifuged at 3000 rpm for 15 min and the serum (supernatant) was collected and stored at -20 °C for biochemical analysis of levels of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany) [21].

Serum albumin was determined by using a commercial kit and total serum bilirubin (TSB) was assayed according to the method described in [22].

2.6.2. Liver samples

The liver samples were dissected and put in Petri dishes. After washing with physiological saline (0.9% NaCl), part of these samples was taken for histopathological investigations and the remaining part was kept at -80 °C until used. The collected tissues were ground with liquid nitrogen in a mortar. The ground tissues (0.5 g) were then homogenized in 2 mL 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA and 1% PVP (Polyvinylpyrrolidone). The homogenate was centrifuged under cooling at 15,000 xg for 20 min, and the supernatant was stored at -80 °C until its use for the determination of catalase (CAT) according to [23], the total amount of glutathione (GSH), the content of malondialdehyde (MDA) and superoxide dismutase (SOD) [24].

2.7. Histological studies

Specimens from each liver were fixed in 10% buffered formalin solution for 24 h, dehydrated, cleared and embedded in paraffin blocks. Serial sections (3-5 micrometer) were cut using a microtome. The sections were left in the oven for de-waxing. Thereafter, the sections were stained with hematoxylin & eosin for general histological feature determination, Masson's trichrome stain for connective tissue staining, and Periodic acid-Schiff (PAS) stain to demonstrate that mucopolysaccharides are PAS-positive materials [25].

2.8. Electron microscopy

Livers were cut into small pieces of 1 mm thick and fixed in phosphate buffer solution (pH 7.2) for 3 h at 4 °C, after which the tissues were removed and post-fixed in buffered 2% OsO_4 for one hour at 4 °C. Post-fixed tissues were rinsed in the buffer and dehydrated at 4 °C through a graded series of ethanol, then embedded in epon-araldite mixture in labeled beam capsules. Ultrathin sections (50 nm thick) were cut, collected on naked copper-mesh grids and stained with uranyl acetate for 1/2 hour and lead citrate for 20-30 min [26].

2.9. Statistical analyses

Results were expressed as mean ± SEM. Comparison of means was done by the Student's t-test (One-way analysis of variance (ANOVA)) and the Mann–Whitney U test. Values of $p < 0.05$ were considered statistically significant. Statistical evaluation was conducted using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Hematological findings

Data shown in Table 1 reveal that rats treated with FA, in Group III, showed significant reduction in red blood cell count and hemoglobin and decrease in lymphocytes (lymphocytopenia), and significant increase in WBCs, significant increase in neutrophils and monocytes in comparison with the Groups I & II. Whereas rats treated with FA then with propolis in Group IV showed amelioration in the toxic effects of FA on hematological parameters. The red blood cell count, and levels of hemoglobin and lymphocytes were significantly increased, while

Table 1. Effect of FA used alone or with propolis on the mean ± SD of the blood count of rats.

Parameters	Group I (control)	Group II (propolis)	Group III (FA)	Group IV (propolis + FA)
RBCs (x 10^6/µL)	5.72 ± 0.23	5.64 ± 0.25	3.97 ± 0.82*	5.12 ± 0.23**
Hb (g/dl)	12.12 ± 0.01	12.06 ± 0.07	9.41 ± 0.34*	12.01 ± 0.04**
WBCs (x 10^3/ul)	6.06 ± 0.12	6.04 ± 0.45	8.17 ± 0.37*	6.02 ± 0.11**
Lymphocytes (%)	35.12 ± 0.34	35.67 ± 0.56	26.44 ± 0.69*	35.12 ± 0.51**
Eosinophils (%)	0.01 ± 0.02	0.02 ± 0.03	0.02 ± 0.06	0.02 ± 0.02
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Neutrophils (%)	53.05 ± 0.51	52.97 ± 0.42	61.45 ± 0.67*	52.34 ± 0.67**
Monocytes (%)	6.23 ± 0.34	6.03 ± 0.02	7.42 ± 0.21*	6.12 ± 0.23**

Number per group = 20. SD = standard deviation.
* = $p < 0.05$ (significant difference in comparison with control group).
** = $p < 0.05$ (significant difference in comparison with second group).

there was significant decrease in WBCs, (significant decrease of neutrophils and monocytes) in Group IV compared to Group III.

3.2. Liver biomarkers of rats

Data shown in Table 2 reveal that rats in Group III treated with FA showed toxic effects of FA on liver of rats; also there was a significant increase in serum AST, ALT and TSB levels and significant reduction in serum albumin compared to Groups I & II. Whereas rats in Group IV treated with FA prior to propolis showed amelioration in the toxic effects of FA on liver functions, as it caused significant reduction in serum AST, ALT and TSB levels and significant increase in serum albumin compared to Group III.

3.3. Antioxidants and lipid peroxidation

Data shown in Table 3 reveal rats in Group III treated with FA showed toxic effects of FA on liver of rats as evidenced by significant reduction in SOD, CAT and GSH levels but significant increase in MDA level compared to Groups I & II was detected. Whereas rats in Group IV treated with FA prior to propolis showed amelioration in the toxic effects of FA on liver as evidenced by significant increase in SOD, CAT and GSH levels and significant reduction in MDA level compared to Group III.

3.4. Light microscopic observations

The liver of rats of Groups I & II showed a normal structure of liver cells which have a large spherical

Table 2. Effect of FA used alone or with propolis on the mean ± SD of the liver function of rats.

Parameters	Group I (control)	Group II (propolis)	Group III (FA)	Group IV (propolis + FA)
AST (U/L)	54.12 ± 0.23	53.92 ± 0.89	70.23 ± 0.45*	53.84 ± 0.98**
ALT (U/L)	74.78 ± 0.87	74.78 ± 0.87	132.27 ± 0.82*	74.12 ± 0.54**
TSB (mg/dl)	1.01 ± 0.01	1.02 ± 0.03	2.86 ± 0.02*	1.03 ± 0.04**
Albumin (mg/dl)	4.12 ± 0.04	4.23 ± 0.45	2.85 ± 0.07*	4.17 ± 0.12**

Number per group = 20. SD = standard deviation.
* = $p < 0.05$ (significant difference in comparison with control group).
** = $p < 0.05$ (significant difference in comparison with second group).
TSB: total serum bilirubin; AST: aspartate amino transferase enzyme;
ALT: alanine amino transferase enzyme.

Table 3. Effect of FA used alone or with propolis on the mean ± SD of the hepatic antioxidant enzymes in rats.

Parameters	Group I (control)	Group II (propolis)	Group III (FA)	Group IV (propolis + FA)
SOD	8.12 ± 0.23	8.34 ± 0.12	6.02 ± 0.67*	8.09 ± 0.78**
CAT	12.98 ± 0.34	12.38 ± 0.09	8.01 ± 0.82*	12.99 ± 0.68**
GSH	20.34 ± 0.56	19.84 ± 0.24	12.97 ± 0.06*	19.34 ± 0.23**
MDA	38.12 ± 0.78	37.92 ± 0.12	59.28 ± 0.14*	38.05 ± 0.67**

Number per group = 20. SD = standard deviation.
All were given by gastric gavage for 2 months.
* = $p < 0.05$ (significant difference in comparison with control group).
** = $p < 0.05$ (significant difference in comparison with second group).
GSH :Glutasthione.
SOD: Superoxide dismutase.
CAT: Catalase.
MDA: Malon dialdehyde.

nucleus with marked nucleolus and were arranged into hepatic cords running radiantly from the central vein and the spaces between the cell cords called blood sinusoids were lined by Kupffer cells (Figures 1 & 2).

Light microscopic examination of the liver of rats of Group III, which were treated with FA, showed that most of the hepatocytes had degenerative changes in the form of enlarged and foamy vacuolated cytoplasm and dilated blood sinusoids

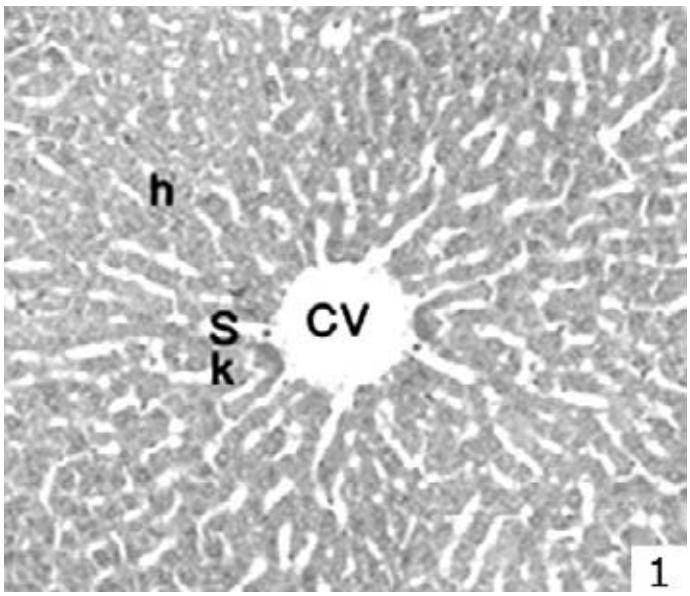

Figure 1. Light photomicrography of the liver of the rat of Groups I & II showing hexagonal hepatocytes (h), arranged into hepatic cords running radiantly from the central vein (CV), separated by adjacent blood sinusoids (S) containing Kupffer cells (k) (H&E, x400).

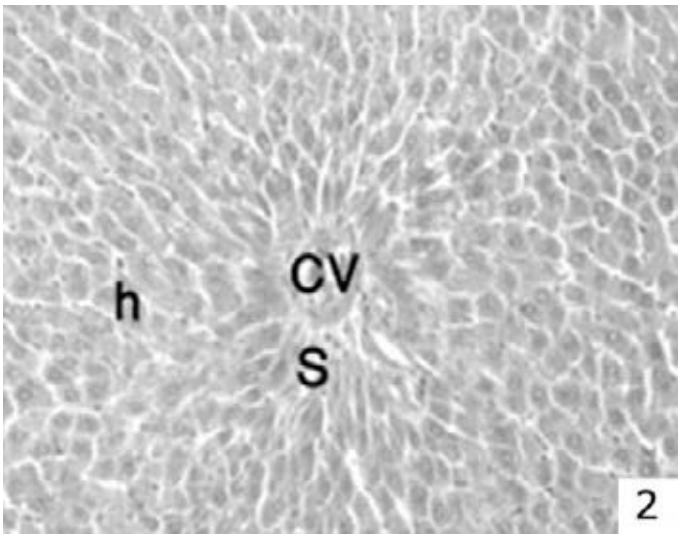

Figure 2. Light photomicrography of the liver of the rat of Groups I & II showing hepatocytes (h) arranged into hepatic cords running radiantly from the central vein (CV), separated by adjacent blood sinusoids (S) (Mallory's stain, x400).

with numerous Kupffer cells. In a few liver areas, FA caused necrotic changes in most of the hepatocytes which had small pyknotic nuclei with condensed chromatin, and absence of nucleolus; there was also portal fibrosis (Figures 3 and 4).

Propolis in combination with FA in the rats of Group IV showed amelioration of toxic effects of FA on the liver cells in the form of nearly normal hepatic cells in most areas of liver, decrease in sinusoidal dilation, and decrease in portal fibrosis.

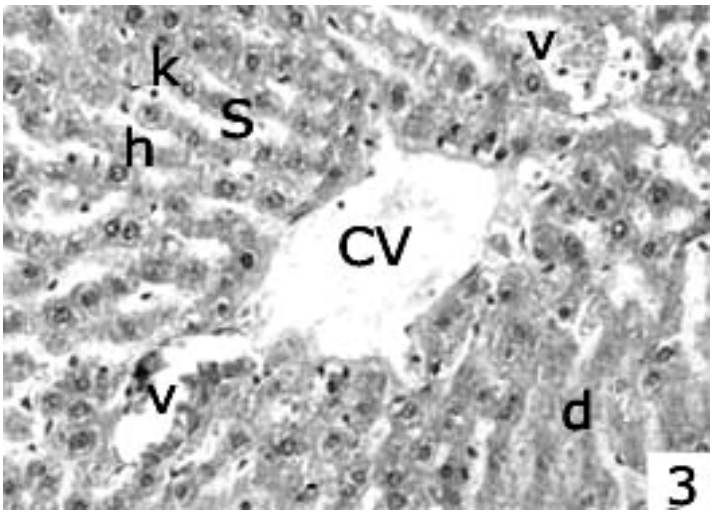

Figure 3. Light photomicrography of the liver of a rat of Group III after eight weeks of exposure to FA showing the shrunken hepatocytes (h) around the central vein (CV) with vacuolated cytoplasm of hepatocytes (v), necrosis of some hepatocytes and the contracted nuclei, pycnotic degenerated areas (d) and widening of blood sinusoids (S). The sinusoid walls show numerous Kupffer cells (K) (H&E, x400).

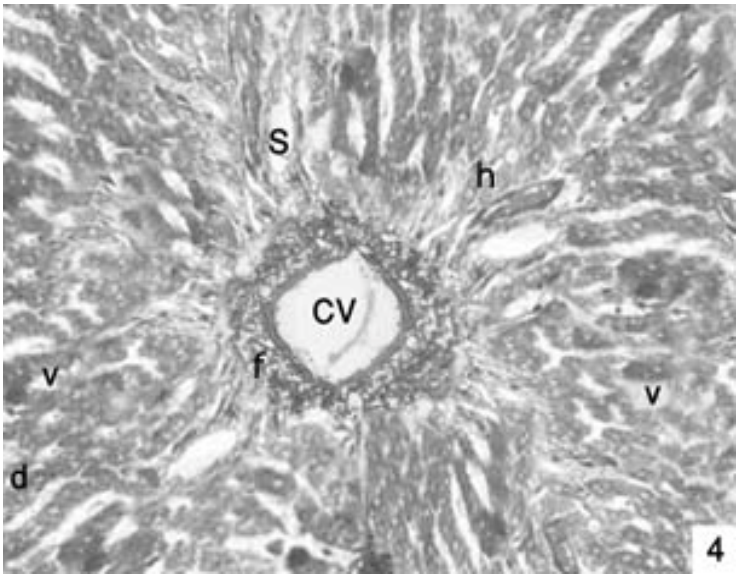

Figure 4. Light photomicrography of the liver of a rat after eight weeks of exposure to FA (Group III), showing degenerated areas (d), vaculated cytoplasm (v), degenerated hepatic cells (h) within degenerated areas (d), widening of central vein (CV) and sinusoidal spaces (S) surrounded by fibrosis (F) (Mallory's stain, x400).

We also noticed the presence of rare inflammatory sites in the sinusoids (Figure 5 and 6).

3.5. Histochemical observations

The light microscopic observations revealed that the liver tissues of the Groups I & II showed strong positive PAS reaction in the cells' cytoplasm (Figure 7). Liver tissues of rats in Group III exposed to FA alone showed a marked reduction in PAS reaction in the cytoplasm of liver cells (Figure 8).

The PAS reaction in liver tissues of the rats of Group IV, which were exposed to propolis in

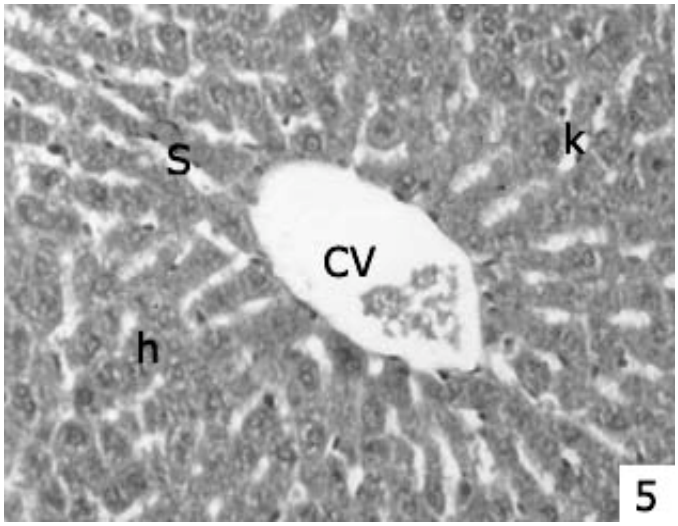

Figure 5. Light photomicrography of the liver of a rat after eight weeks of exposure to propolis in combination with FA (Group IV), showing nearly normal hepatocytes (h) arranged around the central vein (CV), nearly normal-sized blood sinusoids (S) containing Kupffer cells (k), and nearly normal hepatocyte nuclei (H&E, x400).

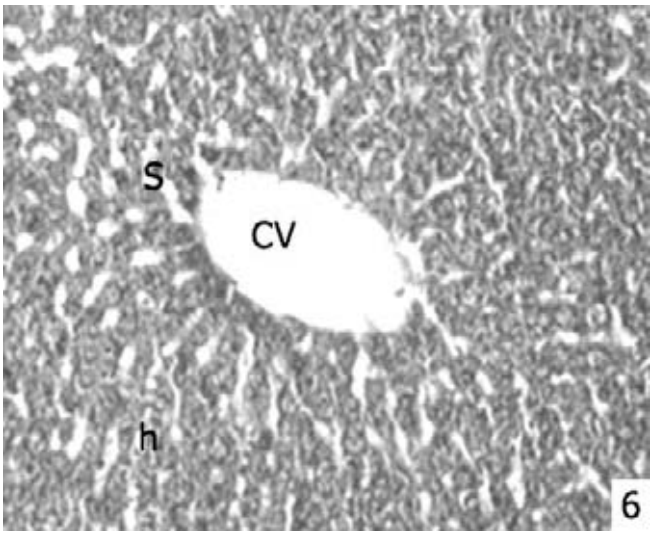

Figure 6. Light photomicrography of the liver of a rat after eight weeks of exposure to propolis in combination with FA (Group IV), showing hepatic cells (h), normal-sized central vein (CV) and sinusoidal spaces (S) with marked absence of portal fibrosis around the central vein (Mallory's stain, x400).

combination with FA, appeared to have a moderate increase in intensity of PAS-positive reaction in the cytoplasm of liver cells (Figure 9).

3.6. Transmission electron microscopic observations

The liver cells of the rats of Groups I & II showed normal ultrastructure in the form of hepatocytes with normal euchromatic nucleus, normal cell organelles, normal nucleolus, many normal mitochondria, numerous rough endoplasmic reticuli and Golgi apparatus (Figure 10).

In the experimental Group III which was exposed to FA alone the transmission electron microscopic observation of the liver cells showed damages in the nuclear membrane with shrinking of the nucleus

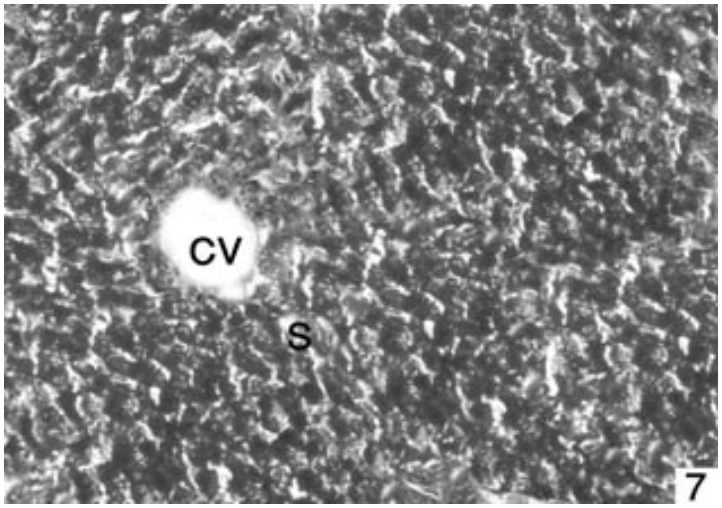

Figure 7. Light photomicrography of the liver of the rat of Groups I & II showing, a strong positive reaction of PAS in all its components particularly the hepatocytes, central vein (CV) and blood sinusoids (S) (Periodic acid-Schiff's stain, x400).

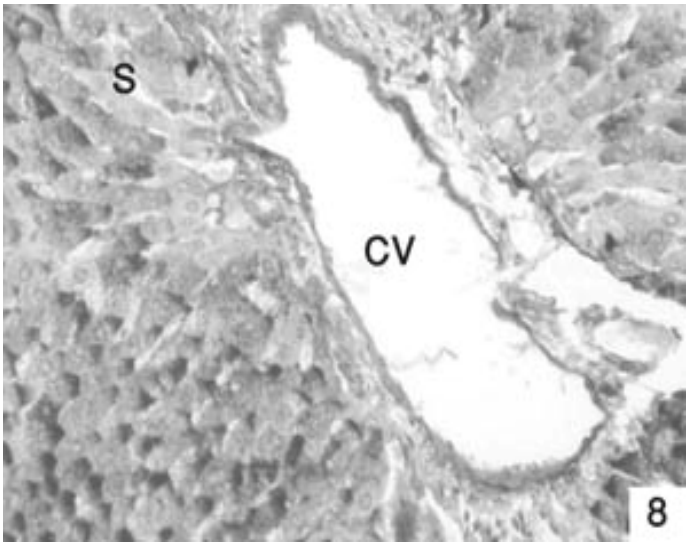

Figure 8. Light photomicrography of the liver of a rat after eight weeks of exposure to FA, of Group III, showing marked decrease in PAS reactions in all areas of the liver tissues, particularly inside the hepatocytes and around the central vein (CV) and blood sinusoids (S) due to depletion of glycogen in hepatocytes (Periodic acid-Schiff's stain, x400).

and less prominent nucleolus, swollen, enlarged and vacuolated mitochondria, large fat droplets, many cytoplasmic vacuoles, decreased and swollen rough endoplasmic reticuli, degenerated Golgi apparatus and some degenerated hepatocytes (Figure 11).

In the rats of experimental Group IV which was exposed to propolis in combination with FA, there were rare sites of ultrastructural pathological changes in the blood sinusoids and hepatocytes (Figure 12).

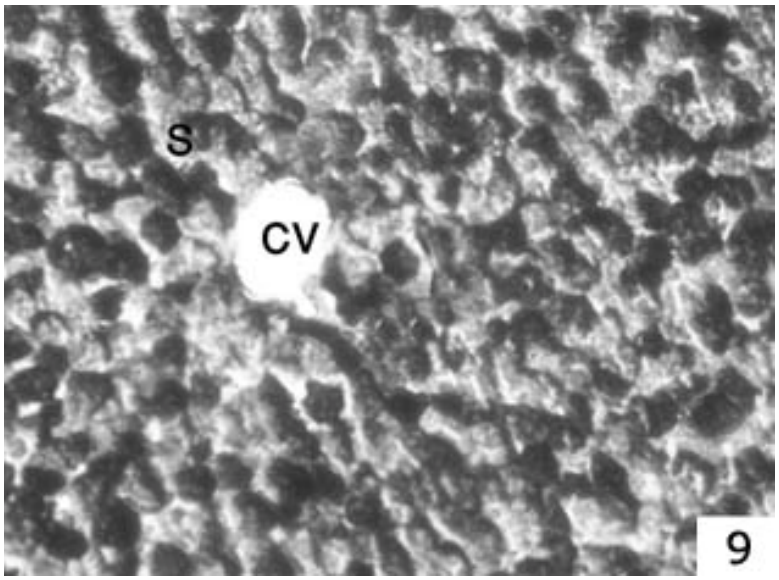

Figure 9. Light photomicrography of the liver of a rat after eight weeks of exposure to propolis in combination with FA (Group IV), showing marked increase in PAS reactions in liver, particularly in all its components like the hepatocytes, the central vein (CV) and blood sinusoids (S) (Periodic acid-Schiff's stain, x400).

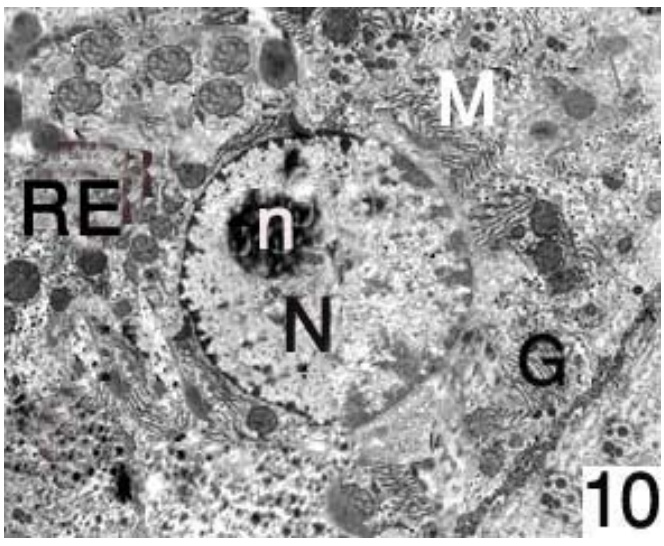

Figure 10. Electron micrographs of hepatocytes of the liver of the rat of Groups I & II showing normal euchromatic nucleus (N) with prominent nucleolus (n) and Golgi apparatus (G), rough endoplasmic reticulum (RE) and many mitochondria (M) (TEM mag. = 10000X).

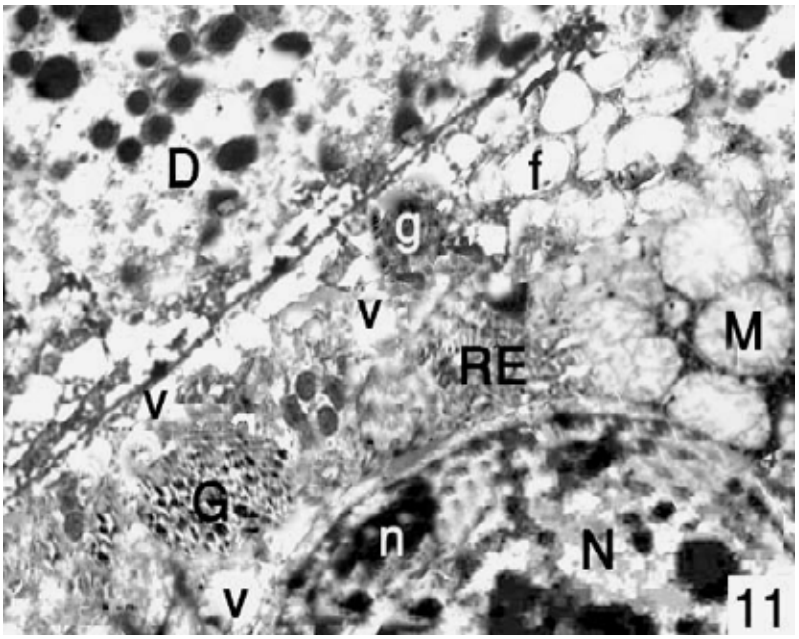

Figure 11. Electron micrographs of hepatocytes of a rat treated with FA for 8 weeks (Group III), showing damages of nuclear membrane, with shrinkage of the nucleus (N) and less prominent nucleolus (n), swollen and vacuolated mitochondria (M), large fat droplets (f), decrease in many cytoplasmic vacuoles (V), swollen rough endoplasmic reticuli (RE), degenerated Golgi apparatus (G) and degeneration of some hepatocytes (D) (TEM mag. = 12000X).

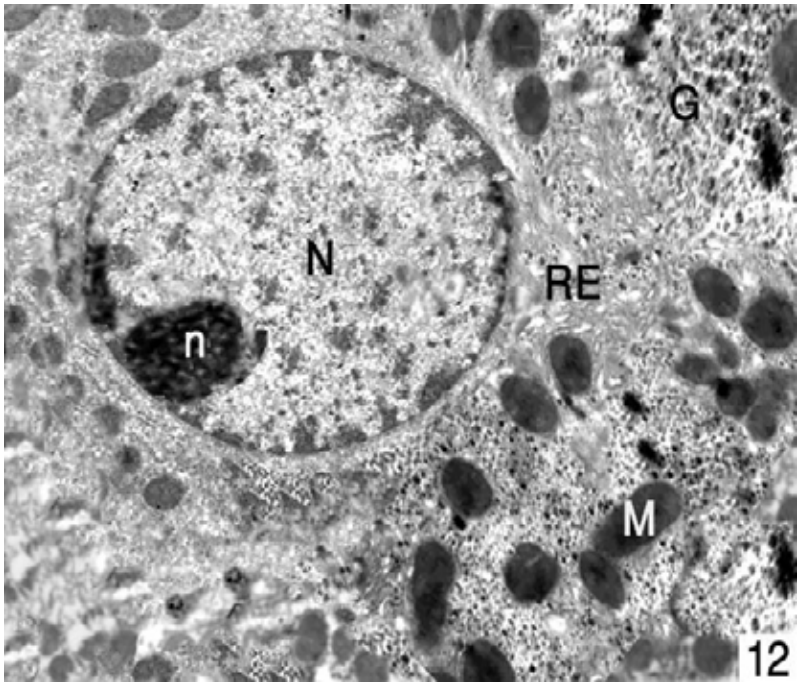

Figure 12. Electron micrographs of hepatocytes of a rat treated with propolis in combination with FA for 8 weeks (Group IV), showing marked recovery of the hepatocyte nucleus (N) and prominent nucleolus (n), Golgi apparatus (G) and rough endoplasmic reticulum (RE) and numerous mitochondria (M) (TEM mag. = 14000X).

4. DISCUSSION

4.1. Blood examination

The present study revealed that rats administrated with FA (Group III) showed significant reduction in red blood cell count and hemoglobin while significant increase in WBCs was detected in comparison with the Groups I & II. These findings were in agreement with those reported by Khan *et al.* [27] who reported that FA administration not only reduced erythrocyte numbers and both Hb and mean corpuscular hemoglobin concentration (MCHC) values, but also led to an increase in host mean corpuscular volume (MCV) and packed cell volume (PCV) values. These results are parallel with the results of Zhang *et al*. [28] who evaluated that these changes might be due to an inhibitory effect of FA on cell multiplication in bone marrow. The present study also revealed that FA administrated to rats caused significant decrease in the number of peripheral blood lymphocytes. These findings were in agreement with those reported by Abd-Elhakim *et al*. [16] who reported that FA caused reduction in the number of peripheral blood lymphocytes in mice. Also, they said that the reduction in lymphocytes might be due to the toxic effect of FA on lymphoid organs. The present study also showed that there was a marked reduction in the total leukocytes in FA-only-treated rats; these results are in line with the reports of Kuo *et al*. [29] who reported that occupational FA exposure led to decreased peripheral blood lymphocytes.

4.2. Liver function

The AST and ALT are considered as biomarker enzymes used in the evaluation of the function and integrity of liver cells. Both enzymes are present mainly in the cytoplasm of hepatocytes [30]. In the present study, the rats treated with FA (Group III) showed elevation in serum levels of ALT, AST, and TSB and reduction in serum level of albumin compared with Groups I & II. Based on the histopathological findings of the present study it could be concluded that the elevation in the serum liver enzymes and the decrease in serum albumin level might be due to necrotic lesions in the liver cells or might be due to impairment in both synthetic and excretory activities of liver cells, which is parallel with the results of Afrin *et al*. [31] who reported that elevation in serum levels of ALT, AST, TSB and reduction in serum level of albumin might be due to necrotic lesions in the liver cells. Also our results are parallel with the results of Mukaddes *et al*. [32] who reported that FA caused increases in ALT, AST and ALP and significant decreases in total protein (TP) and albumin (ALB). Also Euphoria *et al*. [33] added that FA toxicity increased the levels of AST, ALT, and ALP in FA-exposed persons.

4.3. Antioxidants and lipid peroxidation

The rats treated with FA (Group III) showed reduction in serum levels of SOD, CAT activities and GSH level in the liver tissue and increase in the level of MDA compared to Groups I & II. These results are in agreement with the results of [32] which reported that FA treatment led to decrease in superoxide dismutase (SOD) and catalase (CAT) activities in liver tissues while causing significant increase in the levels of tissue malondialdehyde (MDA).

Our findings revealed that co-administration of propolis with FA ameliorated the toxic effects of FA on rat hematology and blood biochemistry. Mani *et al*. [13] reported that flavonoids of propolis had biological effects, including antibacterial, antiviral, antiinflammatory, antiallergic, antioxidant and vasodilatory activities. Euphoria *et al*. [33] added that propolis was used as a protective agent in the prevention of hematopoietic organ injuries and other degenerative diseases.

4.4. Histopathological changes

In the present study liver cells of rats treated with FA showed degenerated hepatic tissues, necrosis, massive fatty changes and broad infiltration of the lymphocytes; these changes were parallel with an increase in the release of the liver enzyme in the blood stream. These results are parallel with the reports of Beall *et al*. [34] who reported that exposure to formaldehyde can cause focal cellular necrosis and centrilobular vacuolization in the liver. Ergün *et al*. [35] added that FA caused degenerated hepatic tissues, necrosis, massive fatty changes, and broad infiltration of the lymphocytes of the liver of rats. In addition to that, Afrin *et al*. [31] added that mice treated with 10 mg/kg FA showed centrilobular necrosis and degeneration of parenchymatous cells, and dilated sinusoidal spaces were accompanied by

vasculitis; this vascular reactivity was characterized by scattered aggregation of lymphocytes.

The present study revealed that FA caused negative PAS; these results are parallel with the results of Zararsız *et al*. [36] who found negative PAS, which means that there was no glycogen presence. Also our results are in agreement with those of Ahmed *et al*. [37] who reported that the livers of the FA-exposed group showed dilation of central vein, enlargement of sinusoids, cloudy swelling of hepatocytes, an ovoid nucleus with slightly irregular profile, appearance of many lipid droplets in cytoplasm and a lot of swollen mitochondria.

The present histopathological examination revealed that there was marked recovery and restoration to almost normal hepatic cells by propolis therapy in contrast to FA; these results are parallel with the findings of Abdul-Hamid *et al*. [38] who reported that propolis therapy changed the toxicity caused by cypermethrin. Our results are also parallel with those of Gomaa *et al*. [39] who reported that when propolis was co-administered with cypermethrin, it would result in normal hepatic cells; however, mild sinusoidal dilation with inflammatory cell infiltration was also present. Our results revealed that administration of propolis with FA ameliorated the toxic side effects of FA through its antioxidant, radical-scavenging and antiperoxidative activities; these results are in agreement with those of Bouayed *et al*. [40] who reported that propolis has antioxidant components which regulated antioxidant enzymes, inhibited lipid peroxidation, and reduced hepatic damage. El-Masry *et al*. [41] reported that antioxidants may play an important role in protecting the cells against oxidative stress and damage caused by free radicals; they also showed that propolis has twelve different flavonoids, and two phenolic acids, cinnamic and caffeic acid. They also added that propolis is an excellent source of essential elements, including Mg^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Ni^{2+} and Mn^{2+}.

5. CONCLUSION

The present study revealed that administration of FA caused hepatotoxic effects in the form of alterations to hematological parameters of liver biomarkers, antioxidant enzymes including CAT, SOD, and GSH, and lipid peroxidation biomarker (MDA), and histological and ultrastructure toxicities. In contrast the pre-treatment with propolis had a beneficial role in reversing these FA-induced changes through its antioxidant and free radical scavenging activities. Thus, we can conclude that propolis might be considered as a useful dietary supplementary compound for patients exposed to FA. This provides a cheap protective strategy in the management of acute hepatotoxicity of FA.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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