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Hepatoprotective Effects Exerted by Propolis against Doxorubicin-induced Rat Liver Toxicity: A Biochemical and Histopathological Study

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Abstract Doxorubicin (DOX) is a currently used broad spectrum anticancer agent used to treat many cancer types. DOX belongs to anthracycline group of cytotoxic drugs. Unfortunately, DOX induces many side effects e.g. cardiotoxicity, hepatotoxicity and nephrotoxicity. In this experimental study, we assessed the protective potential of honey bee propolis against DOX-induced side effects. Thirty Sprague Dawley male rats (weighing about 200-220 g) were included in our study and divided into three equal experimental groups: group I (untreated control), group II (DOX-treated rats) that received 25 mg/kg DOX by intraperitoneal (i.p) injection for three consecutive days and group III where animals received both DOX and propolis oral propolis (250 mg/kg for 30 consecutive days). By the end of experimental time, all animals were sacrificed on the 30th day where blood samples and tissue sections were collected for detection of the biochemical and histopathological changes. Our data revealed that propolis evidently resulted in hepatoprotective effects against DOX-induced toxicity in rats liver. It can be concluded that propolis provides partial protection against DOX-induced hepatotoxicity.

Keywords: *Doxorubicin, Liver toxicity, anthracyclines, Bee propolis, Chemotherapy*

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1. Introduction

Doxorubicin (DOX) is an anthracycline-derived chemotherapeutic agent that is isolated from cultures of Streptomyces peucetius var. DOX exerts its anticancer effects through binding to cell's nucleic acids (DNA double helix). DOX is clinically used to treat a diverse range of malignant tumors [1]. DOX is well-known to induce cardiotoxicity, hepatotoxicity and nephrotoxicity [2,3]. DOX-induced dose-dependent cardiotoxicity may result in heart failure [3]. DOX-induced toxicity may be due to inflammatory changes in the affected tissues that may result in increased capillary permeability and glomerular atrophy [4]. DOX-induced hepatotoxicity may also occur due to oxidative stress damage [5]. Honey bee propolis contains many antioxidant compounds e.g. polyphenols, aglycones, flavonoids, phenolic acids and phenolic esters, phenolic aldehydes, sterols, vitamins, ketones, terpenes, and amino acids that may guard against DOX-induced oxidative stress [6,7,8]. DOX-induced and anthracycline-induced free radicals and toxic intermediates may be reduced by nutritional supplements

and exogenous antioxidants [9,10]. In this work, we aimed to examine a possible protective role of propolis on the hepatotoxic effects in DOX-treated male albino rats.

2. Materials and Methods

2.1. Chemicals

DOX was purchased as DOX hydrochloride (50mg/vial) from Pfizer Pharma, Egypt. ALT and AST assay kits were obtained from Randox, Egypt. Albumin assay kits were obtained from Diamond, Egypt.

2.2. Animals

Male Sprague Dawley rats (200-220 g) were purchased from the central animal facility of Assiut University, Egypt. Animals were maintained in pathogen-free conditions at the central animal house of Assiut University, Egypt. Animal maintenance was in their polypropylene cages in a 12 h light/dark schedule at a temperature of 22 ± 3ºC. Animals were fed per mouth using standard laboratory pellet chow diet and had free open access to water source during the whole period of the study. Animals' weight was estimated before the experimental work. All animal experimental procedures were done in agreement with the laboratory animals' principles of the European Community Guidelines. The study experimental design and protocol were approved by the ethical committee of the animal facility house in Assiut University and Sohag Faculty of Medicine, Egypt.

2.3. Sample Preparation

Animals were allowed to fast overnight. Blood samples for assessing clinical chemistry (from each rat) were collected from the tail vein. To get serum sample, collected blood samples were incubated (for 10 minutes) at room temperature until blood forms blood clots. Samples were then centrifuged at 3000 rpm for 10 min. where serum was was separated and stored through keeping in sterile plastic vials (kept frozen at –30°C) until the time of analysis.

2.4. Biochemical Analyses

Serum ALT and AST activities were assayed using commercially available kits (Randox, Egypt) as reported previously [11]. Albumin and total proteins concentration in serum was assayed using commercially available kits (Diamond, Egypt) as previously reported [12].

2.5. Extract Preparation

Ethanolic extract of propolis was prepared as previously reported [13]. Propolis collected from bee hives was confirmed to be pure (wax-free, paint-free and wood-free) and was ground into fine pieces. Simply, 30 grams of propolis were added to 100 ml of 70% ethanol, put in a sterile container with sealing container's tip followed by moderate shaking. Resulting proplois extract was kept for 2 weeks at room temperature. Just before experimental work, propolis extract was filtered twice using suitable filters. Proplis was dried out and stored for future use in tightly closed bottles and kept at 4°C. The dried propolis extract was dissolved in water just before oral administration according to the decided dosage.

2.6. Experimental design

Control group (G1): Animals in this group were given normal 0.9% saline i.p. twice daily for thirty successive days.

DOX-treated group (G2): Animals were given 25 mg/kg DOX i.p. on the 1st, 2nd and 3rd days of the experiment.

Propolis-DOX group (G3): Propolis extract (250 mg/kg bw/day for 30 days) and DOX (during the 1st three consecutive days) were given to the animals.

2.7. Histological Studies

At the end of the previously decided study time (30th day), all rats in the study were euthanized using i.p. sodium pentobarbital. All animals were subjected to complete necropsy. Histological liver specimens underwent further processing for paraffin sections and subsequent staining using haematoxylin and eosin for general morphology evaluation. Mallory's trichrome was used for staining for the collagen fibers. Staining was done to detect any structural changes using a bright field microscope.

2.8. Statistical Analysis

Data obtained in the study were shown as mean ± SEM. Statistical analysis was done using one way analysis of variant, ANOVA (using SPSS statistical version 16 software package (SPSS® Inc., USA) to examine the presence of significant differences among the animals in treatment groups vs. negative control. The cut-off for statistical significance was set at $p<0.05$ for the biochemical data.

3. Results

Effects of DOX treatment on body weight: Body weights were measured weekly during the whole 30 days of the experiment and results were analyzed (Table 1). In DOX-treated animals, there was a decreased weight gain percentage compared to controls indicating retarded growth. In animals treated with DOX and bee propolis, weight gain significantly improved.

Table 1. Change in the body weight of the animals of the three experimental groups

Animal groups	Group I	Group II	Group III
Body Weight (g)	215±12	172±61	196±34

Data are expressed as mean ± SEM.

Biochemical observations (Table 2): Liver function tests e.g. AST, ALT, albumin and total proteins were measured in animals' serum to investigate the magnitude of liver toxicity. In DOX-treated group, both AST and ALT were significantly elevated but on the contrary, there was a significant decrease ($p<0.001$) in serum levels of albumin and total proteins compared to control animals indicating hepatic dysfunction. In DOX-propolis-treated rats, both AST and ALT significantly decreased ($p< 0.01$) with near normal levels in serum levels of albumin and total proteins compared to GII.

Table 2. Changes in the serum AST (U/l), ALT (U/l), albumin (g/dl) and total protein (g/dl) levels in different groups of the experiment

Animal groups	GI	GII	GIII
AST	74.3 ± 2.20	171.1 ± 0.19	98.32 ± 1.14
ALT	65.2 ± 3.31	121.2 ±1.6	100.5 ±0.60
Albumin	5.48 ± 1.12	3.61 ±1.16	3.67 ± 1.16
Total proteins	6.76 ±1.31	5.63 ±1.15	6.60 ±1.15

Data are expressed as mean ± SEM.

3.1. Histological Observations

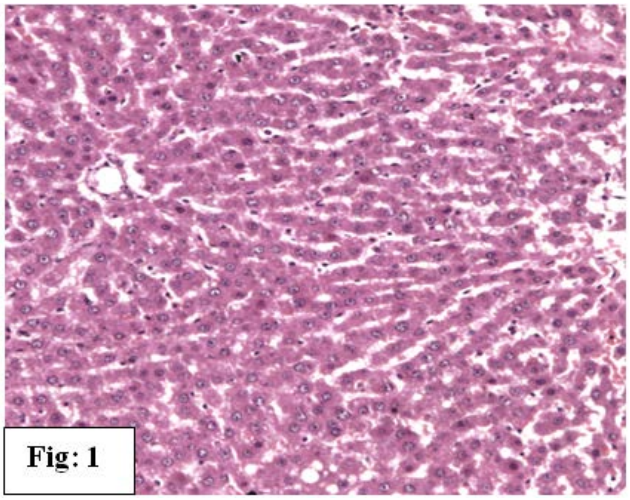

Figure 1. Normal liver microscopic structure. A photomicrograph of rat liver section in group I (control group) animals shows normal criteria of hepatic microscopic structure. It shows the general morphology of the classic hepatic lobule that is nearly hexagonal in shape. At the lobule center, there is a central vein. Hepatocytes are organized into anastomosing cords or plates that are separated by anastomosing hepatic sinusoids. The plates of hepatocytes and hepatic sinusoids radiate from the central vein to the periphery of the lobule. **(H&E, X200)**

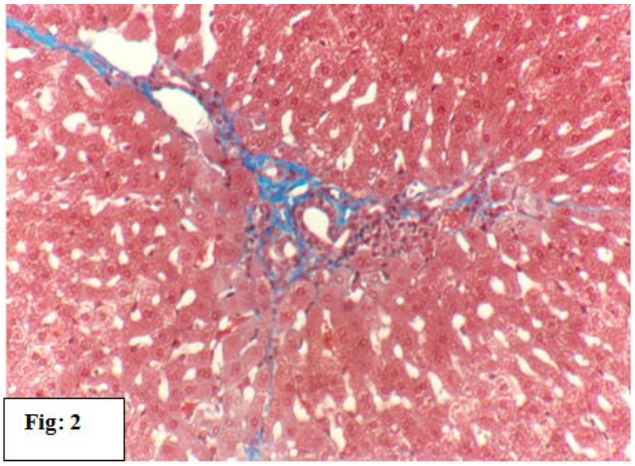

Figure 2. Normal collagen fiber distribution in liver sections of control rats. Liver section of group I (control group) shows normal distribution of collagen fibers. Collagen fibers are stained blue and are located around a portal tract containing a terminal portal venule, a terminal hepatic arteriole, an interlobular bile ductule and fine lymph vessels. **(**Masson's trichrome, X200)

Light microscopic examination denoted that hepatic tissue of the control animals showed normal criteria of normal liver tissue i.e. large polyhedral cells with eosinophilic cytoplasm and apparently round nuclei. There was a few numbers of variable-sized spaced hepatic sinusoids that are situated in between the hepatic cords. Kupffer cells were evident (Figure 1 & Figure 2). In contrast, groups receiving DOX only (25mg/kg of body weight) exhibited pronounced liver toxicity manifested histologically in the form of dissolved hepatic cords (appearing as empty vacuoles surrounded by chains of dead hepatocytes). Liver sections exhibited the presence of dense focal inflammatory cells together with membrane changes of endothelial lining cells. There were also hepatic inflammatory signs in the form of periportal fibrosis, marked atrophy of the hepatic cords, and increased incidence of vacuolar and cellular degeneration together with increased apoptotic cell death.

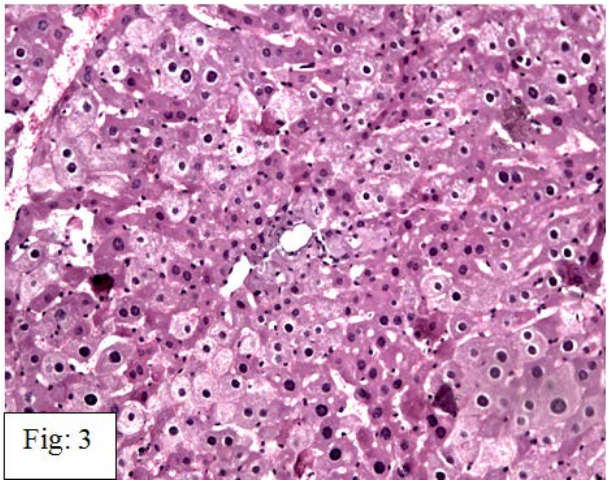

Figure 3: DOX induces hepatotoxic effects. Photomicrograph of rat liver sections of group II (DOX-treated group) shows complete loss of the normal hepatic architecture, marked massive damage of the hepatocytes and strongly marked hepatocellular vacuolation together with focal areas of necrosis where the cell boundaries were lost with distortion of the normal hepatic architecture **(H&E, X200)**

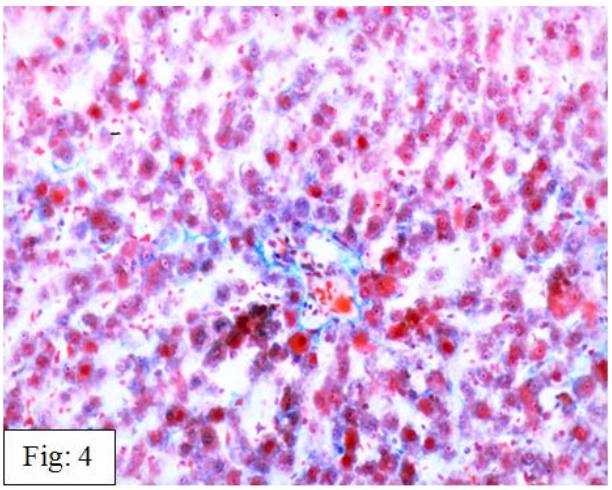

Figure 4. DOX results in evident hepatic fibrosis. A photomicrograph of rats' liver sections exhibiting minimal increase in collagen fibers (C) around the blood vessels in the portal tract **(**Masson's Trichrome, X200)

In addition, liver fibrosis existed as evidenced by the occurrence of many areas of locally dispersed cellular granulomatous lesions associated with a minimal increase in the number of collagen fibers around the blood vessels in the portal tract (Figure 3 & Figure 4).

However, in propolis-DOX treated group, hepatocytes improved better and showed many vacuolated hepatocytes having mild congestion in central veins. Restoration of normal hepatic structure was observed more than in GII

animals but –in the same time- less than animals in the control group. Collagen fibers were normally distributed around the blood vessels in propolis-DOX treated group animals.

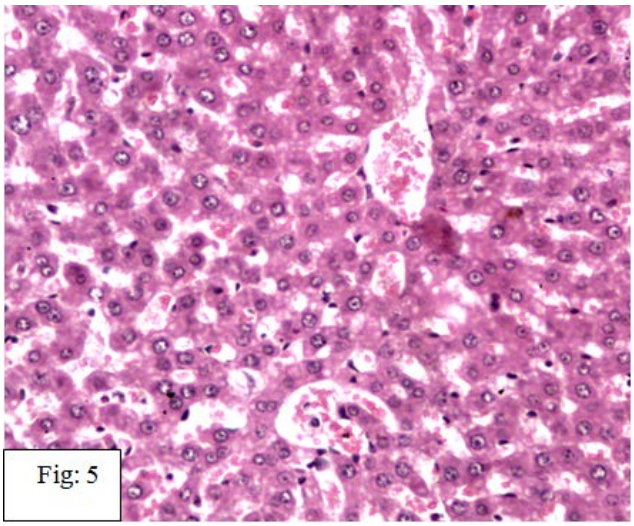

Figure 5. Hepatoprotective effects of propolis against DOX-induced toxic effects. Photomicrograph of rats' liver section of group III (Propolis-DOX treated group) exhibits moderate degree of improvement in the treated animals hepatocytes. Few vacuolated hepatocytes exist with mild congestion in the central veins

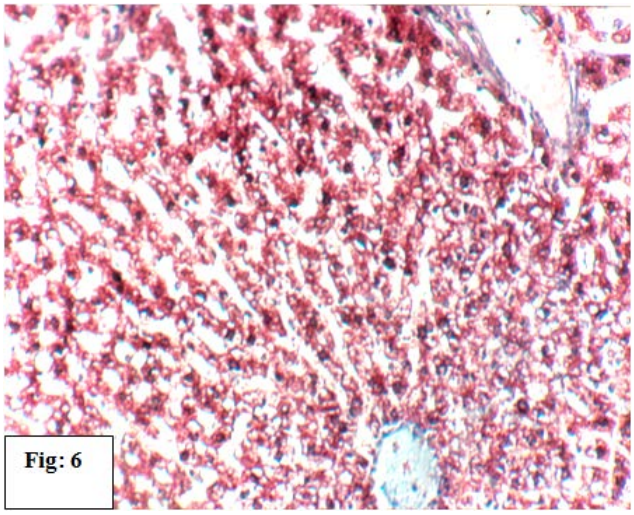

Figure 6. Hepatoprotective effects of propolis against DOX-induced hepatic fibrosis. Photomicrograph of rat liver section of group III (Propolis-DOX treated group) shows normally distributed of collagen fibers around the blood vessels **(**Masson's trichrome, X 200)

4. Discussion

Most of the injected DOX reaches the liver through the portal vein where it ends finally in the terminal portal venules in the portal tracts. Thus, the peripheral hepatocytes become exposed to a higher concentration of the drug more than the central cells. Normally, the direction of blood flow in the hepatic cords proceeds from the periphery of the hepatic lobule towards the central vein i.e. the flow of blood is centripetal. At the periphery of the lobule, the inlet venules (portal vein) and inlet arterioles (hepatic artery) pierce the periportal hepatocytes limiting plate to open into the sinusoids. Blood passes within the sinusoids to the central vein is exposed to the activities of the hepatocytes around the sinusoids [14].

Plasma flows freely through the sinusoidal wall into the perisinusoidal spaces where it is exposed to the various activities of the hepatocytes, and then flows back into the bloodstream. Plasma remaining in the perisinusoidal space shares in lymph formation. Polyphenols are well-known to exert antioxidant effects that are protectants against drug-induced oxidative stress tissue damage. Propolis has arisen as a promising agent due to its reported antioxidant effects. DOX-induced cytotoxicity has been reported previously [15]. Mechanism of DOX-induced toxicity is through production of free radical and their capability to induce apoptosis by inducing the formation of free radicals e.g. reactive oxygen and nitrogen species, DNA intercalation, lipid membrane peroxidation and inducing cell membrane damage [16].

In the present experimental work, statistically significant differences were noticed in the serum levels of the investigated biochemical parameters in male rats treated with DOX compared to the non-treated control group rats. Moreover, statistically significant alleviation of DOX-induced toxicity was observed in DOX-propolis treated group compared to DOX alone. Our results strongly suggest that propolis exerts hepatoprotective effects (against DOX-induced hepatotoxicity) when administered at the indicated doses and for the given time period to experimental animals. Bee propolis caused protective influence on the investigated parameters. Propolis was reported to evidently exert some tissue protective effect against cypermethrin-induced toxicity [16]. A recent study showed that the endogenous antioxidant system of the whole body was damaged by adriamycin [7]. In our results, it is clearly evident that ingestion of propolis in animals exerts antioxidant and hepatoprotective effects against DOX-induced hepatotoxicity.

Moreover, propolis was recently reported to exert potent anti-apoptotic effects through increasing the anti-apoptotic Bcl-2 mRNA expression and decreasing the expression of the apoptotic proteins caspase-3 and Bax. This strongly suggests adding propolis as a daily food supplement to benefit stroke patients [17].

It is thus concluded that propolis may be regarded as a nutritional therapy that protects against DOX-induced hepatotoxicity in rats as evidenced by improved serum biochemical parameters and microscopic tissue architecture.

5. Conclusion

Bee propolis evidently exerts tissue protective effect against DOX-induced hepatotoxicity in treated rats' livers. Propolis brought the hepatocytes and liver functions to near normal status though it was still not normalized. Bee propolis may be promising in exerting hepatoprotection against anthracycline-induced hepatotoxicity.

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