**Propolis Attenuates Doxorubicin-Induced Testicular Toxicity in Rats**

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**A B S T R A C T**

Doxorubicin (Dox), an effective anticancer agent, can impair testicular function leading to infertility. The present study aimed to explore the protective effect of propolis extract on Dox-induced testicular injury. Rats were divided into four groups (n = 10). Group I (normal control), group II received propolis extract (200 mg kg\(^{-1}\); p.o.), for 3 weeks. Group III received 18 mg kg\(^{-1}\) total cumulative dose of Dox i.p. Group IV received Dox and propolis extract. Serum and testicular samples were collected 48 h after the last treatment. In addition, the effects of propolis extract and Dox on the growth of solid Ehrlich carcinoma in mice were investigated. Dox reduced sperm count, markers of testicular function, steroidogenesis and gene expression of testicular 3\(\beta\)-hydroxysteroid dehydrogenase (3\(\beta\)-HSD), 17\(\beta\)-hydroxysteroid dehydrogenase (17\(\beta\)-HSD) and steroidogenic acute regulatory protein (STAR). In addition, it increased testicular oxidative stress, inflammatory and apoptotic markers. Morphometric and histopathologic studies supported the biochemical findings. Treatment with propolis extract prevented Dox-induced changes without reducing its antitumor activity. Besides, administration of propolis extract to normal rats increased serum testosterone level coupled by increased activities and gene expression of 3\(\beta\)-HSD and 17\(\beta\)-HSD. Propolis extract may protect the testis from Dox-induced toxicity without reducing its anticancer potential.

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**1. Introduction**

The anthracycline doxorubicin (Dox), or Adriamycin, is the anticancer drug of choice in the treatment of many solid tumors. Its application, however, carries the risk of serious dose-dependent toxicity to other non-target tissues including the testis (Malekinejad et al., 2012; Ahmed et al., 2013). Dox could strikingly impede spermatogenesis leading eventually to infertility (Howell and Shalet, 2005; Yeh et al., 2007). The mechanism responsible for Dox-induced testicular injury is not yet completely clear, but findings from several studies strongly suggest oxidative stress, lipid peroxidation (Atessahin et al., 2006; Yeh et al., 2007) and cellular apoptosis (Shinoda et al., 1999) as major causes. Based on this concept, a variety of antioxidant or anti-apoptotic agents have been employed to counteract Dox-induced testicular damage (Jahnukainen et al., 2001; Hou et al., 2005; Malekinejad et al., 2012). Nevertheless, so far there is still no single agent proven effective enough to prevent or reverse this adverse effect.

Propolis is an adhesive, resinous substance collected, transformed and used by honeybees to seal holes in their honeycombs, smooth out the internal walls, and protect the entrance of intruders. Honeybees collect the resin from cracks in the bark of trees and leaf buds. They bring propolis back to the hive, where it is modified and mixed with other substances, including bees’ own wax and salivary secretions (Ghislalberti, 1979). Propolis has been used in folk medicine all over the world. It has anti-inflammatory, immunoregulatory, bacteriostatic, and antibacterial activities (Tosi et al., 2007; Bueno-Silva et al., 2013). Propolis extract presents low toxicity to experimental animals, with LD50 higher than 7 g/kg for mice (Dantas et al., 2006). It has strong cytoprotective effect against different exogenous toxic stimuli (Bhaduria and Niral, 2009; Boutabet et al., 2011). Egyptian propolis is rich in aromatic acids, aromatic acid esters, flavonoids, and some triterpenoids (Abd El Hady and Hegazi, 2002). Many of the reported pharmacological effects of propolis extract reside in its high content of caffeic acid, caffeic acid phenethyl ester (CAPE) and polyphenolic compounds (Ozturk et al., 2012; Ak yol et al., 2013; Búf alo et al., 2013; Szliszka and Krol, 2013).

To determine whether propolis extract could attenuate Dox-induced apoptosis and oxidative stress in testicular tissue, the present study was designed to examine the effects of propolis extract...
on pathological and molecular biological abnormalities induced by Dox in rat testis. The study was extended to examine the antican-
cer effects of Dox when combined with propolis extract using solid Ehrlich carcinoma (SEC) model in mice. The results of the current study could clarify the role of propolis extract in prevention of this serious side effect associated with Dox treatment.

2. Materials and methods

2.1. Animals

Male Wistar albino rats weighing 160–180 g and female Swiss albino mice weighing 20–22 g were provided by the breeding unit of the Egyptian organization for biological product and vaccines (Hewan, Egypt). They were housed under controlled conditions (25 ± 1 °C constant temperature, 55% relative humidity, 12 h lighting cycle), and received standard pelleted diet and water ad libitum during the study period. The study complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

2.2. Preparation of propolis extract

Fifty grams of the resinous material of Egyptian propolis (obtained from Dakahlia Governorate, Egypt) was cut into small pieces and extracted with 600 ml of 80% (v/v) ethanol at 60 °C for 30 min. After extraction, the mixture was centrifuged and the supernatant was evaporated to complete dryness under vacuum at 40 °C (Francli et al., 2012). The yield of dried residue was about 61.1% (w/w) and it was kept at 4 °C for further use. Aqueous suspension of propolis was prepared in 15% acacia suspension, and orally administered to the animals for 3 weeks in a dose of 200 mg kg−1 (Bhadouria and Nirala, 2009).

2.3. Experimental design and protocol

Forty male Wistar rats were divided into four groups consisting of ten animals in each group. Group I: animals received distilled water as a vehicle for 21 days and normal saline (i.p.) on 8th, 10th, 12th, 15th, 17th and 19th day (normal control). Group II: animals received propolis extract (200 mg kg−1, p.o.) 5 days/week for 21 days. Group III: control Dox received 18 mg kg−1 total cumulative dose of Dox on six di-

2.4. Analysis of blood samples

Blood samples were collected, and the sera were separated and kept at −20 °C till determination of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Serum level of testosterone was assayed using automated chemiluminescence's immunoassay system (ADVIA Centaur; Bayer Vital, Fernwald, Germany), while serum LH and FSH were assayed using rat LH and FSH ELISA kits (Shibayagi Co., Japan) according to manufacturer instructions.

2.5. Analysis of tissue samples

The testes and the epididymis were removed and cleared of adhering connec-
tive tissue. The left side of testis was rinsed in ice-cold saline, weighed and homog-

2.5.1. Determination of testicular marker enzymes

The first portion of the testicular homogenate was mixed with 0.1 M Tris buffer (pH 8.1) and centrifuged at 105,000g for 4 °C for 45 min using Dupont Sorvall Comm-

2.5.2. Determination of testicular oxidative stress markers

Malondialdehyde (MDA), as an index for lipid peroxidation, was determined in testicular homogenate by detecting the absorbance of thiobarbituric acid reactive substances at 532 nm (Mihara and Uchiyama, 1978). Another aliquot of the testicu-
lar homogenate was mixed with equal volume with 10% metaphosphoric acid and centrifuged at 1000g. The resulting protein-free supernatant was used for the deter-
nation of reduced glutathione (GSH) according to the method described by Beut-
tier et al. (1963).

2.5.3. Determination of the extent of Dox-induced hemorrhage

Another portion of the tests from each animal was homogenized in 10 ml of 5 mM Tris HCl (pH 7.0), containing 1 mM MgCl2 and 100 mM CaCl2. The homoge-

2.5.4. Measurement of interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF-α) contents and myeloperoxidase (MPO) activity in the rat testis

Testicular homogenate was ultracentrifuged at 105,000g for 1 h, and the super-
натant was stored at −80 °C until assay. Tissue contents of IL-4 and TNF-α were as-

2.5.5. Determination of testicular 3α-hydroxysteroid dehydrogenase (3α-HSD) and 17β-
hydroxy steroid dehydrogenase (17β-HSD) activities

Testicular 3α-HSD and 17β-HSD activities were measured according to the methods of Talalay (1962) and Jarabak et al. (1962). An aliquot of the testicular tis-
ue homogenate was mixed with equal volume of homogenizing fluid containing 30% 

2.5.6. Determination of epididymal sperm count

Epididymal sperm count was measured according to the method of Talalay (1962) and Jarabak et al. (1962). The yield of dried residue was about 61.1% (w/w) and it was kept at

2.6.2. Real-Time PCR.

Gene expressions were analyzed by quantitative RT-PCR using the SYBR Green PCR Master Mix (Applied Biosystems, California, USA) with the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) using the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the house-
keeping gene. The sense and anti-sense primers were as follows: for rat 3α-HSD, 5′-
CCAGTCTAGAAGGATGAGGTC-GC-3′ (sense) and 5′-CACTTCTGTCGAGGGGTC-3′ (antisense); for rat 17β-HSD, 5′-TCTGAGAGGATTCCACGG-3′ (sense) and 5′-ACA-
CTACCTGCGGCTTCT-3′ (antisense); for rat STAR, 5′-ATGCTGACCAAAGG

2.6. Determination of epididymal sperm count

Spermatozoa in the epididymis were counted according to the method of Yokoi et al. (2003). Briefly, the epididymis was minced with anatomical scissors in 5 ml of physiological saline, placed in a rocker for 10 min, and incubated at room temper-

2.6.3. Data analysis.

Gene expression was normalized to the endogenous control GAPDH, and fold changes in the genes of interest were determined using the com-
parative threshold cycle (Ct) method (Pfaffl, 2001).

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5 g sodium bicarbonate, 1 ml formalin (35%) and 25 mg eosin per 100 ml of distilled water. Total sperm number was determined with a hemocytometer. Approximately 10 μl of the diluted sperm suspension was transferred to each counting chamber and was allowed to stand for 5 min for counting under a light microscope at 200× magnification.

2.7. Histological studies

The testis were removed and kept in 10% formal saline for 24 h, dehydrated in ethanol and embedded in paraffin. Sections were cut at 5 micron thickness, mounted on slides and stained with hematoxylin and eosin (for histological and morphometric studies). Some sections were mounted on positive charged slides for immunohistochemical studies.

2.8. Immunohistochemistry staining for Fas-L, and caspase-3

The pro-apoptotic factor Fas-L is a TNF related type II membrane protein and is expressed in various tissues and cells. Fas-L binds to its receptor (Fas) thus inducing apoptosis (Galvao et al., 2010). Immunohistochemistry to active caspase-3 is commonly used for apoptosis detection (Bressonot et al., 2009). Tissue sections were deparaffinized, rehydrated and treated with 10% hydrogen peroxide to reduce endogenous peroxidase. For antigen retrieval tissue sections were boiled in citrate buffer pH 6.0 then cooling was allowed. Sections were incubated with the primary antibodies; Fas L; a rabbit polyclonal antibody (Neo Markers Laboratories, Westinghouse, USA) and caspase-3; a rabbit polyclonal antibody (Neo Markers Laboratories, Westinghouse, USA). Then sections were incubated with the secondary antibody a biotinylated goat anti-rabbit immunoglobulin followed by streptavidin biotin complex. The site of the reaction was visualized by adding diaminobenzidine HCl which is converted into brown precipitate by peroxidase. Counterstaining was performed using Meyer's hematoxylin.

2.9. Morphometric measurements

Using the computer assisted software Leica Qwin 500 LTD image analyzer, the following parameters were measured:

1. Mean diameters of seminiferous tubules in hematoxylin and eosin stained sections were measured using the interactive measuring menu. They were measured in 10 non-overlapping fields at a magnification 400× for each specimen.

2. Mean area percent of Fas-L and caspase-3 immunoreactivities were measured in immunostained sections using the color detect menu. They were measured in 10 non-overlapping fields for each specimen of all groups at a magnification 400× in relation to a standard measuring frame.

2.10. Effects on the growth of SEC in mice

In this experiment, 2 × 10⁶ Ehrlich ascites carcinoma cells were transplanted subcutaneously in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumor mass that developed within 7 days after implantation were divided into four groups, 6 animals each, and received the same doses of propolis extract and Dox, previously mentioned, for 2 weeks. The change in tumor size was measured every 3 days using a Vernier caliper and was calculated using the formula adapted from Osman et al. (1993).

\[ \text{Tumor volume (mm}^3\text{)} = 0.52 AB^2 \]

where A is the minor tumor axis and B is the major axis.

2.11. Statistical analysis

Data are expressed as mean ± SE. Comparisons between different groups were done using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test using the software GraphPad InStat. A probability level of less than 0.05 was accepted as statistically significant.

3. Results

3.1. Effects on markers of testicular function

Administration of Dox was associated with reduction in testicular activities of LDH, SDH, ACP and G6PD by 39.55%, 31.52%, 37.28 and 30.23%, respectively parallel to increased activities of ALT and AST by 26.83% and 41.97%, respectively as compared to the normal control group. Treatment of rats by propolis extract attenuated Dox-induced changes in the aforementioned parameters and normalized testicular function markers (Table 1).

3.2. Effects on steroidogenesis

Dox resulted in marked decrease in serum testosterone, FSH and LH levels by 83.37%, 52.38% and 45.56%, respectively as compared to normal control group (Fig. 1). To ascertain the possible mechanism of the suppressed testosterone production following Dox administration, the expressions of testicular key androgenic enzymes like 3ß-HSD and 17ß-HSD along with StAR, a prime regulatory protein for testosterone biosynthesis in tests were studied. Dox decreased the activities of 3ß-HSD and 17ß-HSD by 76.53% and 84.25%, respectively parallel to decreased gene expression of 3ß-HSD, 17ß-HSD and StAR by 67.06%, 71.30% and 57.45%, respectively as compared to the normal control group (Fig. 2). Administration of propolis extract prevented Dox-induced decreases in serum levels of testosterone, FSH and LH as well as activities or gene expression of testicular 3ß-HSD, 17ß-HSD and StAR indicating the protective role of propolis on testicular androgenic disorders of Dox exposure (Figs. 1 and 2). Moreover, administration of propolis extract to normal rats significantly increased serum testosterone level by 28.59 (Fig. 1A) coupled by increased activities of testicular 3ß-HSD and 17ß-HSD by 46.68% and 43.41%, respectively (Fig. 2A) and increased gene expression of both enzymes by 36.11% and 49.89% (Fig. 2B), respectively as compared to the normal control group.

3.3. Sperm count and morphometric results

Parallel to the observed decrease in testicular and steroidogenesis markers, Dox reduced sperm count and the diameter of seminiferous tubules by 52.51% and 28.87%, respectively (Fig. 3A–B) and increased testicular hemorrhage by 1.62 folds (Fig. 3C) as compared to normal control group. Such changes were largely attenuated in the group receiving propolis extract where sperm count and the diameter of seminiferous tubules were normalized and testicular hemorrhage was reduced by 38.24% (Fig. 3A–C). Moreover, administration of propolis extract to normal rats increased sperm count by 31.20% as compared to normal control group (Fig. 3A).

3.4. Hematoxylin and eosin stained sections

Examination of testicular sections of control group showed the normal structure of seminiferous tubules. They were lined with a thick layer of uniformly arranged spermatogenic cells in variable stages of maturation; spermatogonia, spermatocytes, spermatids and sperms in addition to Sertoli cells. The interstitium contained small scattered groups of Leydig cells (Fig. 4A). Testicular sections of rats which received propolis extract showed normal seminiferous tubules lined with spermatogenic cells at different stages of maturation (Fig. 4B).

In Dox-treated rats, sections of the testes showed disrupted spermatogenic structure in many seminiferous tubules, shedding and degeneration of many spermatogenic cells (Fig. 4C). Many tubules showed detached primary spermatocytes with dark pyknotic condensed nuclei and increased cytoplasmic eosinophilia, few elongated spermatids and many apoptotic spermatids shedded inside the lumen. Sertoli cells appeared disrupted (Fig. 4D).

Sections of rat testis which received Dox and propolis extract showed few tubules exhibiting detachment of germ cells from the basement membrane (Fig. 4E). Most of the seminiferous tubules showed organized epithelium containing various cell types, 1ry spermatocyte, spermatids, and sperms. Germinal epithelium exhibited detachment in some areas. Few spermatocytes showed pyknotic nuclei (Fig. 4F).
3.5. Effect on oxidative stress markers

Administration of Dox, in the current study, increased testicular MDA content by 81.93% parallel to reduced testicular GSH content by 46.20% as compared to normal rats (Fig. 5). Pretreatment with propolis extract prevented Dox-induced changes in both MDA and GSH contents (Fig. 5).

3.6. Effect on MPO, TNF-α and IL-4

Dox administration resulted in marked increase in testicular MPO activity and TNF-α content by 61.10% and 115.76%, respectively (Fig. 6A–B) parallel to decreased content of IL-4 by 38.37% (Fig. 6C) as compared to normal control group. Such changes were prevented by pretreatment with propolis extract (Fig. 6A–C).

3.7. Effect on caspase-3 and Fas-L

Moreover, Dox increased testicular activities of caspase-3 and Fas-L by 7.19 and 17.91 folds, respectively as compared to normal control group (Fig. 7). Pretreatment of rats by propolis extract decreased testicular activities of caspase-3 and Fas-L by 51.15% and 68.58%, respectively as compared to doxorubicin control group (Fig. 7).

3.8. Fas-L immunostained sections

In control and propolis extract treated groups, sections showed negative to weak immunostaining for Fas-L (Fig. 8A and B). In Dox-treated group strong positive immunostaining for Fas-L could be detected in the cytoplasm of spermatogenic cells. Sertoli and myoid cells showed negative staining (Fig. 8C). Section of testis from a rat that received Dox and propolis extract showed reduced immunostaining for Fas-L as few spermatogenic cells exhibited positive reaction (Fig. 8D).

3.9. Caspase-3 immunostained sections

In control and propolis extract treated groups, sections showed weak immunostaining for caspase-3 in some spermatogenic cells (Fig. 9A and B). In Dox-treated group strong positive immunostaining for caspase-3 could be detected in the cytoplasm of spermatogenic cells. Sertoli and myoid cells showed negative staining (Fig. 9C). Section of testis from a rat treated with Dox and propolis...
extract showed reduced immunostaining for caspase-3 as few spermatogenic cells exhibited positive reaction (Fig. 9D).

3.10. Effects on growth of SEC in mice

The results in Fig. 10 showed that the size of tumors was significantly reduced by Dox to 16.50% of control values at day 20 from the start of experiment. Moreover, pretreatment with propolis extract did not significantly affect the efficiency of Dox in reducing the growth of SEC. In other words, there was no significant difference among groups receiving Dox, propolis extract or their combination.

4. Discussion

Male germ cells are known to be one of the tissues vulnerable to Dox toxicity (Hou et al., 2005; Ahmed et al., 2013). The potentially
infertility-causing complication renders protection of testicular tissue a critical issue whenever Dox is employed in cancer chemotherapy.

In the present study, we measured several biochemical parameters related to testicular toxicity and oxidative stress in the testis tissue to evaluate the protective effect of propolis extract on Dox toxicity. In testis, ALP is associated with the division of spermatogenic cells and the transportation of glucose to spermatogenic cells. ACP is one of the markers of dyszoospermia associated with the denaturation of seminiferous epithelium and phagocytosis of Sertoli cells (Samarth and Samarth, 2009). In the present study, the decreased activities of ALP and ACP were notable manifestations of Dox-induced toxicity. However, treatment with propolis extract maintained the activities of these enzymes almost close to normal.

LDH and SDH are associated with the maturation of spermatogenic cells, testis and spermatozoa as well as the energy metabolism of spermatozoa. Hence, the decline in their activities following Dox administration suggests a regression in testicular development that was reversed by pretreatment with propolis extract.

Similarly, the activities of AST and ALT increase when the membrane of spermatozoa is damaged, and the rate of intact acrosome spermatozoa decrease (Yang et al., 2010). The present results showed that Dox-induced elevated activities of AST and ALT in testis were obviously decreased by propolis extract treatment.

In the present study, propolis extract administration significantly activated G6PD enzyme in normal rats and antagonized its decrease in Dox-treated rats. G6PD is another key enzyme of the testicular tissue (Prasad et al., 1995) that provides reducing equivalents for the hydroxylation of steroids.

In the present study, a significant decline in serum testosterone, LH and FSH was recorded. In addition disturbance in the diameter of seminiferous tubules was also recorded in Dox-treated rats. High level of testosterone in testis is essential for the normal spermatogenesis as well as for the maintenance of the structural morphology and the normal physiology of seminiferous tubule (Sharpe et al., 1992). To ascertain the possible mechanism of the suppressed testosterone production following Dox administration, the activities and the mRNA expressions of 3β-HSD and 17β-HSD, the key enzymes for testicular androgenesis (Jana et al., 2006) as well as StAR, the rate-limiting step in steroidogenesis (Stocco,
In the present study, Dox treated group showed reduced sperm count in addition to degeneration of spermatogenic cells in many tubules. Many apoptotic spermatids appeared within the lumen, few spermatozoa, disrupted Sertoli cells could be observed. Meistrich et al. (1990) reported that single dose of Dox (5 mg/kg) resulted in disruption of spermatogenic cells maturation, alterations of spermatogonia DNA and sperm morphology. Other investigators suggested that Dox reduced the number of Sertoli cells and motility of sperms in a dose-dependent manner (Takahashi et al., 2011; Brilhante et al., 2012). In addition, Dox administration caused significant reduction in the diameter of seminiferous tubules. This could be regarded as a consequence of massive germ cell death which is usually followed by a sharp decline in testicular morphometric parameters (Vendramini et al., 2010).

The deleterious effects of Dox result chiefly from its inherent tendency to generate free radicals and suppress antioxidant enzymes in various tissues (Atessahin et al., 2006). The current study confirmed that testicular lipid peroxidation was increased by Dox as reflected by the increased content of testicular MDA parallel to reduced GSH content. This phenomenon could be at least partially attributable to the structure of the male germ cell membrane, which is rich in polyunsaturated fatty acids and is thereby particularly prone to lipid peroxidation (Lenzi et al., 2002). The present decrease in G6PD activity in testis by Dox, further increases the risk of oxidative stress in this tissue as G6PD is directly associated with GSH metabolism (Prasad et al., 1995). The inherent deficiency of a potent component of the superoxide dismutase (SOD) family (SOD2) in testicular tissues, also contributes to the vulnerability of this tissue to Dox-induced oxidative stress (Kasahara et al., 2002). Treatment with propolis extract reduced Dox-induced alterations in testicular oxidant balance. The antioxidant effects of propolis extract were previously reported (Russo et al., 2006; Capucho et al., 2012; Kamiya et al., 2012).

Bien et al. (2007) found a strong association between oxidative stress and inflammatory response including cytokine release after Dox treatment. Our data showed that Dox causes imbalance in immune regulation leading to skewing ratio towards Th1-type cytokines (high TNF-α and low IL-4) with a concomitant increase in testicular MPO activity compared to the controls. Pretreatment with propolis extract reduced Dox-induced inflammatory response in the testis suggesting that alongside antioxidative mechanisms, propolis may also possess anti-inflammatory properties. Indeed, CAPE, the main component in propolis, decreased the release of the inflammatory cytokines from the inflammatory cells and in the same time stimulated production of anti-inflammatory cytokines like IL-10 and IL-4 (Korish and Arafa, 2011; Büfalo et al., 2013).

Apoptosis plays a causative role to the development of Dox toxicity in various tissues (Shinoda et al., 1999; Nakamura et al., 2000). In the present study, Dox caused significant increase in the immunoreactivity of Fas-L and caspase-3 in the cytoplasm of spermatogenic cells that was confirmed by morphometric measurements. Previous studies implicated the Fas system as a possible regulator of germ cell apoptosis in the rat testis especially under some pathological conditions such as thermal stress, hormone deprivation or environmental toxicants (Nair and Shaha, 2003; Mishra and Shaha, 2005). Moreover, Lizama et al. (2007) suggested that the germ cells with high levels of Fas could trigger caspase activation and apoptosis. The current study also elucidated the effective suppression of apoptosis and morphometric changes by propolis extract in testis exposed to Dox. This anti-apoptotic effect of propolis has been reported by other investigators (Szliszka and Krol, 2013; Tsuchiya et al., 2013). Moreover, treatment with propolis extract prevented Dox-induced decrease in sperm count and increased sperm count of normal rats. In accordance with the present results, Capucho et al. (2012) reported that propolis extract can increase sperm count of rats.

The observed antioxidant and anti-apoptotic effects of propolis extract raised an important question regarding its influence on Dox antitumor activity. Therefore, the effects of propolis and Dox as well as their combination were studied on the growth of SEC in mice. The present experiments showed that Dox antitumor...
The present findings can be explained based on the reported anti-cancer effects of propolis extract (Ozturk et al., 2012; Akyol et al., 2013). Phenolic compounds isolated from propolis extract were reported to enhance tumor necrosis factor-related apoptosis inducing ligand (TRAIL), a naturally occurring anticancer agent that preferentially induces apoptosis in cancer cells and is not toxic to normal cells (Szliszka and Krol, 2013). Moreover, CAPE, the main constituent of propolis extract, was shown to enhance docetaxel and paclitaxel cytotoxicity in prostate cancer cells (Tolba et al., 2013).

In conclusion, the present study provided evidence that pretreatment with propolis could effectively alleviate Dox-induced toxicity to testis without affecting its antitumor efficiency. These findings raised the possibility that propolis may be an adjuvant therapy, potentially protecting the testis from oxidative and apoptotic actions related to Dox, and might help, ultimately, to prevent this devastating adverse effect of Dox in clinical practice. Further studies are needed to verify the role of propolis extract when used curatively in management of Dox-induced testicular toxicity.
Fig. 8. (A) Photomicrograph of a section of control rat testis showing negative immunostaining for Fas-L in the cytoplasm of spermatogenic cells, (B) photomicrograph of a section of testis from propolis extract-treated rat showing weak immunostaining for Fas-L in the cytoplasm of some spermatogenic cells (arrows), (C) photomicrograph of a section of rat testis from doxorubicin control group showing strong positive immunostaining for Fas-L in the cytoplasm of spermatogenic cells (arrows). Note negative staining of Sertoli and myoid cells and (D) photomicrograph of a section of testis from rat treated with propolis extract and doxorubicin showing reduced immunostaining for Fas-L as few spermatogenic cells exhibit positive reaction (arrows) (Fas-L immunostain 400×).

Fig. 9. (A) Photomicrograph of a section of control rat testis showing weak immunostaining for caspase-3 in the cytoplasm of some spermatogenic cells (arrows), (B) photomicrograph of a section of testis from propolis extract-treated rat showing weak immunostaining for caspase-3 in the cytoplasm of few spermatogenic cells (arrows), (C) photomicrograph of a section of rat testis from doxorubicin control group showing positive immunostaining for caspase-3 in the cytoplasm of spermatogenic cells (arrows) and D. Photomicrograph of a section of a rat testis (group IV) from group treated with doxorubicin and propolis extract showing reduced immunostaining for caspase-3 in the cytoplasm of spermatogenic cells (arrows). Note strong reaction in cytoplasm of myoid cells (arrow heads) (caspase-3 immunostain 400×).
Fig. 10. Effect of propolis extract on the antitumor action of doxorubicin against the growth of solid Ehrlich carcinoma in mice. Data are expressed as mean ± SE (n = 6).

**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**Transparency Document**

The Transparency document associated with this article can be found in the online version.

**References**


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