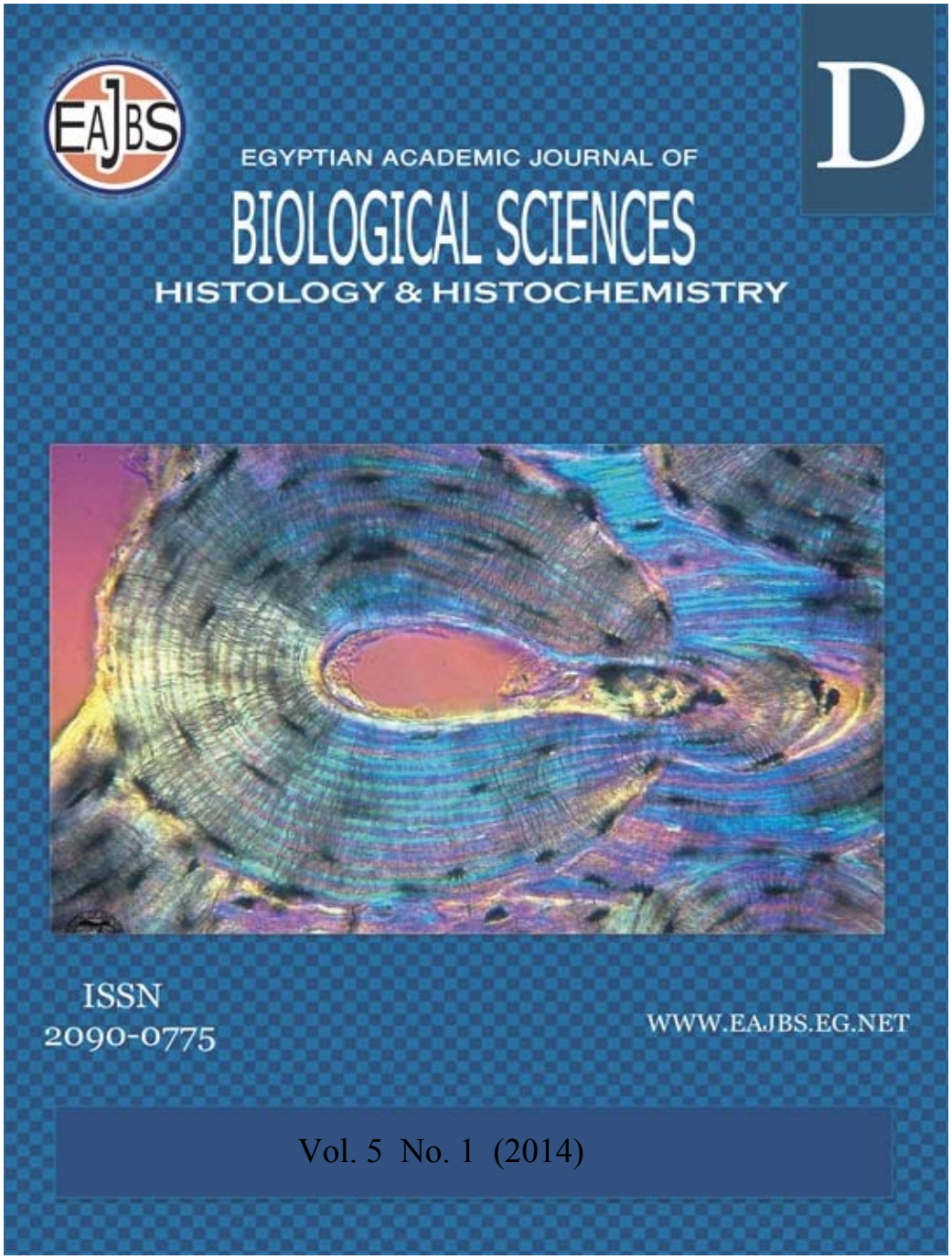

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The Protective Effect of Royal jelly Against Doxorubicin-induced Renal Oxidative Stress, Histopathological and Immunohistochemiacal Alterations in Ehrlich Ascites Tumor Bearing Mice

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ABSTRACT

In the present study, we investigated the potential protective effects of royal jelly against doxorubicin-induced nephrotoxicity in Ehrlich ascites tumor bearing mice. Adult male albino mice were randomly divided into eight groups: the control, royal jelly, doxorubicin, and royal jelly plus doxorubicin groups. Biochemical, oxidative stress, and histopathological and immunohistochemical methods were utilized for evaluation of the reno-toxicity. Blood was collected and analyzed for blood urea nitrogen (BUN), uric acid and creatinine. The renal samples were stored for the measurement of malondialdehyde (MDA), glutathione peroxidase (GSH) and superoxide dismutase (SOD) activities and processed for histopathological examinations. Administration of doxorubicin to mice bearing Ehrlich tumor induced a marked renal dysfunction, characterized with a significant increase in serum BUN, uric acid and creatinine concentrations, and they had higher renal MDA and decreased in GSH and SOD concentrations. In the groups that were feed on RJ in association with DOX, improvement was observed in some oxidative stress parameters and certain other biochemical, histopathological and immunohistochemical parameters. The royal jelly exerted significant protection against renal damage induced by doxorubicin through reduction of the elevated activities of serum renal functions. Moreover, royal jelly blocked doxorubicin-induced lipid peroxidation through decreasing the malondialdehyde formation. In conclusion, royal jelly has a capability to attenuate doxorubicin-induced nephrotoxicity.

INTRODUCTION

Cancer is a major health problem worldwide and is the leading cause of human mortality exceeded only by cardiovascular disease (Varmus, 2006). Most of the currently available anti-cancer drugs fail to differentiate between normal and neoplastic cells or to overcome primary or secondary resistance mechanisms involved in cancer cells.

Thus, there is an urgent need of anti-cancer drugs with high potency, less toxicity to non-cancerous cells and unique target of actions (Chari, 1998).

Doxorubicin (DXR) is an anthracycline glycoside with broad spectrum of therapeutic activity against a variety of human neoplasms (Giri *et al*., 2004). However, the clinical use of DXR has been limited by its undesirable side effects, especially cardiac and renal toxicity (Mohan *et al*., 2010). Oxidative stress has been associated with DOX-induced kidney and renal damage (Akyol *et al*., 2012). Doxorubicin-induced changes in the renal tissue of rats include increased glomerular capillary permeability and tubular atrophy (Saad *et al*., 2001). Although the exact mechanism of DOX-induced nephrotoxicity remains not known, it is believed to be intermediated via formation free radical, iron- dependent oxidative damage of biological macromolecules and membrane lipid peroxidation (Pritsos and Ma, 2000). In view of the truth, widespread the production of free radical is likely to overcome the security of antioxidant, the antioxidants protection of activation of free radical-induced hostile reactions, these antioxidants are expected to reduce the intracellular level of ROS. This increase in depletion of endogenous antioxidants triggers huge immune response and oxidative stress (Rehman *et al*., 2014).

Dietary natural products have shown protection against various degenerative diseases including cancer. They have therapeutic importance because of the virtue of their anti-oxidant and potential of anti-inflammatory activities. The findings of epidemiological also implicate that substantial intake of fruits and vegetables in the diet have protective effects against different types of pathologies (Singletary, 2000). Natural products with anti-oxidant and anti-inflammatory activities are known to exhibit protection against Dox-induced toxicities. Silymarin (Patel *et al*., 2010), Lycopene (Yilmaz *et al*., 2006), *Zingiber officinale* (Ajith *et al*., 2008), *Solanum torvum* (Mohan *et al*., 2010), and Resveratrol (Oktem *et al*., 2012) were found to protect against Dox-induced nephrotoxicity side effects.

Some natural products as royal jelly (RJ) were reported to have a protective role against oxidative damages due to its antioxidant potency and free radical scavenging capacity (Cemek *et al*., 2010). Royal jelly is a secretion produced by the hypopharyngeal and mandibular glands of worker honey bees (*Apis mellifera*). It has many influential compounds with biological activity such as proteins, sugars, minerals and vitamins, free amino acids,and fatty acids (Nakajima *et al*., 2009). Royal jelly has been demonstrated to possess antimicrobial (Chan *et al*., 2009), anti-allergic (Oka *et al*., 2001), anti-inflammatory (Kohno *et al*., 2004), immunomodulatory (Simsek *et al*., 2009) and antioxidant properties (Nakajima *et al*., 2009).

The current study aimed at evaluating the protective effect of royal jelly administration on Dox-induced histopathological, immunohi-stochemistry, antioxidants and biochemical alterations in mice bearing Ehrlich ascites tumor cells.

MATERIALS AND METHODS

Experimental Animals

Sixty- healthy adult male Swiss albino mice, weighing 22.0 ± 2.0 g, 42-64 days old, were received from the National cancer institute (NCI) animal house, Cairo University, Egypt. The animals were kept under standard environmental conditions on 12 hours light/dark cycle under a constant temperature of $(25+1)^0$C, and free access to food and water was allowed at all time. Mice were acclimated to laboratory conditions for two weeks prior to

experiments. Mice were housed five per cage in the sterilized plastic cages with wood shaving bedding.

Chemicals

DOX drug and RJ (1000 mg/Capsule) were obtained from pharco pharmaceuticals Company, Alexandria, Egypt, and were kept at temperature not exceeding 20°C until use and away from moisture. The other laboratory reagents used in this study were analytical of the purest grades.

Experimental Design

Ehrlich ascites carcinoma (EAC) cells were collected from the ascitic fluid of female Swiss albino mouse harboring 8–10 days old ascitic tumor and then diluted with physiological saline to 1×10^7 cell /ml, a total 0.2 ml of $1x10^7$/ml EAC cells were inoculated intramuscularly in right thigh of Swiss albino male mice selected at the start of experiment. The first day, animals were divided with randomization into eight groups as follows: Group I: the control group, animals were injected once saline (0.2 mL, i.p) every 2 days from day 1 to 10), Group II: the royal jelly was given orally once daily at doses of 150 mg/kg for 10 consecutive days from day 1 to 10. Group III: animals were injected intraperitoneally by 15 mg/kg body weight of DOX once every 2 days. Group IV: animals were inoculated intramuscularly in right thigh with 0.2 ml of $1x10^7$/ml EAC cells. Group V: animals were injected intramuscularly in right thigh by 0.2 ml of $1x10^7$/ml EAC ,then the royal jelly was given orally once daily at doses of 150 mg/kg. Group VI: Animals were given orally royal jelly once daily, then 3.5 mg/kg body weight of Doxorubicin was inoculated intraperitoneally once every 2 days . Group VII: Animals were injected intramuscularly in right thigh by 0.2 ml of $1x10^7$/ml EAC, then (15 mg/kg body weight of Doxorubicin was inoculated intraperitoneally once every 2 days and finally. Group VIII: animals were injected intramuscularly in right thigh by 0.2 ml of $1x10^7$/ml EAC, then the royal jelly and DOX were given.

Dissection of Animals and Sample Collection

Before dissection tumor bearing thigh of each animal was shaved the longest and shortest diameters of the tumor were measured with the help of the caliper. At the end of the study, animals were fasted overnight, and were maintained under light ether anesthesia and were, sacrificed by cervical dislocation, and samples were collected from each animal into tubes both with and without anticoagulants. The kidney dissected out, washed and placed in cold isolation buffer containing then fixed in 10% buffered formalin for histopathology.

Sample Collection

Blood was drawn from all animals in each group and centrifuged at 3000 x g for 10 minutes. Plasma was separated and stored at 0°C until analysis. Kidney was removed, and cleared from adhering connective tissue, Part of the kidney was fixed in 10% formalin for histopathological and immunohistochemistry examination. Plasma and Serum samples were stored at 0°C until biochemical analysis in the same day. Piece of the kidney was removed and put in 250 mM sucrose, 10 mM Trizma hydrochloride (Tris–HCl), 1 mM EDTA, pH 7.3.the kidney was minced in isolation buffer before being homogenized.

Kidney tissue was homogenized and the homogenates were centrifuged at 19,000×g at 4°C for 30 min and the supernatant was separated to measure the activity of antioxidant enzymes (GSH and SOD) and oxidative stress marker (lipid peroxidation, MDA).

Renal Functions Biochemistry

Levels of Creatinine, BUN and Uric acid in Serum were determined in accordance with the method provided by

using kits purchased from Diamond Diagnostics Company (Gizza, Egypt).

Histopathological Examination

Light Microscopy:

The right kidney was bisected along its long axis and then fixed in a 10% neutral-buffered formalin solution for overnight. After dehydration via a graded alcohol and xylene series, transverse kidney slices were paraffin embedded, sectioned at five micron thickness and stained for hematoxylin & eosin and iNO immunohistohemistry.

iNO Immunohistochemistry

Sections were treated for iNOS immunohistochemical staining. Briefly, sections were removed from first, the wax, rehydrated and then immersed in 3% hydrogen peroxide in methanol for 30 min to block the endogenous activity of peroxidase. Sections were goat serum incubated (diluted 1:5; Dakopatts, Milan, Italy) for one hour, and serially treated with iNOS (rabbit polyclonal، diluted 1:50; Santa Cruz Biotechnology; Santa Cruz، CA) at 4C overnight. The sections were washed in solution of Tris buffer 0.1M pH 7.4 and subsequent incubated with proper biotinylated secondary antibody and avidin-biotin horseradish peroxidase complex according to the manufacturer's instructions (ABC kit; Dakopatts). The sections were stained with a solution of 0.05% 3,3-diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide. All slides were hematoxylin counterstained، then dehydrated, and mounted. Control reactions were performed in the absence of the primary antibodies.

Statistical Analysis

The mean value of five mice in each group was calculated and data were presented as arithmetic mean + standard deviation calculated. One-way analysis of variance was used for statistical analyses, and Tukey test was used to determine the differences between groups. Statistical analysis for obtained results was carried out with the aid of the SPSS 11 computer software program (one way ANOVA). The statistical significance was defined as p less than 0.05.

RESULTS

In experiments on a DOX, the anti-tumor activities of royal jelly therapy were evaluated on the basis of mean tumor weight after necropsy (Fig. 1). The length and width of tumor were callipered every 4 days for tumor growth, and tumor volume (TV) was estimated using the formula: TV (mm^3) = ($width^2$ × length)/ 2. We also evaluated the toxicological effect of the DOX by measuring mean the biochemical analysis of creatinine, BUN and uric acid (Table 1).

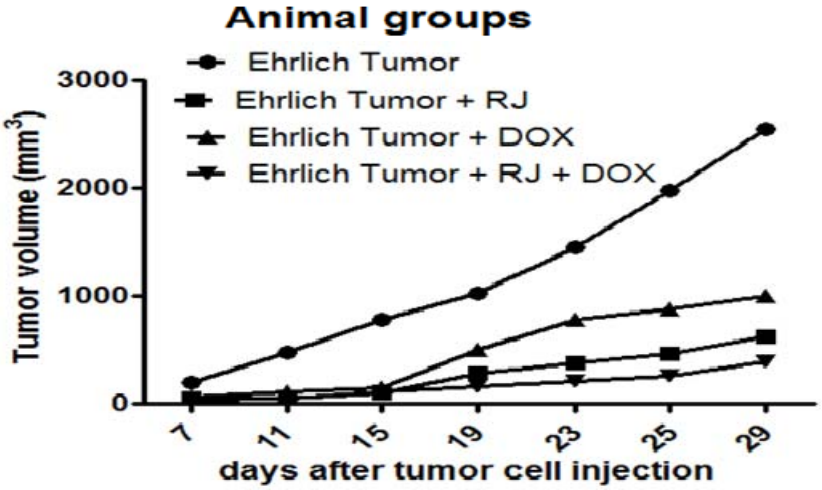

Fig. 1: The Royal jelly and Doxorubicin treatment effects on the tumor volume in different experimental animal groups. Data were expressed as mean + SD.

Table 1: The Creatinine, BUN and uric acid concentrations in different experimental animal groups

	Creatinine (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)
Control	0.48±0.08	36.64±1.9	1.44±0.15
Royal jelly	0.45±0.03	34.34±1.7	1.45±0.13
Doxorubicin (DOX)	2.20±0.09*	82.70±9.9*	3.09±0.28**
RJ + DOX	1.09±0.08*	53.30±3.8*	1.99±0.33
Ehrlich tumor	0.55±0.07	39.12±1.8	1.60±0.07
RJ + Ehrlich tumor	0.49±0.08	37.62±1.4	1.50±0.08
DOX + Ehrlich tumor	2.40±0.09**	86.89±8.8**	2.40±0.21*
DOX+RJ+Ehrlich tumor	1.15±0.06*	59.43±4.8*	1.55±0.31

Data were expressed as mean + SD; significant differences in control group versus treated groups are **P*<0.05, ***P*<0.01.

In Table 2, the significant correlations were presented in all groups which were treated with the doxorubicin when compared with the control and royal jelly groups. The antioxidants GSH and SOD were decreased in the groups DOX, DOX and EAC, but in case of treatment of animals with royal jelly, the levels of the antioxidants were recovered and close of the normal value. The MDA concentration was in high level in all groups injected with the DOX drug, and the normal value was returned in the groups treated with royal jelly.

Table 2: The GSH, SOD and MDA concentrations in different experimental animal groups

Treatment groups	GSH (nmol/gm tissue)	SOD (U/mg tissue)	MDA (nmol/mg tissue)
Control	0.86±0.05	680.07 ± 21.70	0.94 ± 0.11
Royal jelly	0.92±0.03	701.13 ± 22.68	0.89 ± 0.09
Doxorubicin (DOX)	0.29±0.01*	504.19 ± 19.79*	4.35 ± 0.19*
RJ + DOX	0.49±0.04*	630.10 ± 18.66	1.54 ± 0.13*
Ehrlich tumor	0.55±0.08	650.07 ± 14.70	1.04 ± 0.01
RJ + Ehrlich tumor	0.79±0.06*	660.22 ± 21.76	0.93 ± 0.15
DOX + Ehrlich tumor	0.25±0.09**	488.13 ± 28.30**	4.95 ± 0.20**
DOX+RJ+Ehrlich tumor	0.53±0.06*	670.90 ± 15.11	1.81 ± 0.10*

Data were expressed as mean + SD; significant differences in control group versus treated groups are **P*<0.05, ***P*<0.01.

Histopathological of the Kidney

Light microscopic examination indicated a normal structure of the kidney in the controls (Fig. 2A). Exposure to DOX induced degenerative changes in the organ. An enlargement of the renal cells was shown. The chromatin was condensed and cytoplasm was light. Necrosis of single cells, marked by contracted and pyknotic nuclei with condensed chromatin. The vicinity of many renal cells was also infiltrated by mononuclear cells. With the Royal Jelly supply, we noted an absence of nucleus fragmentation and a decrease in the necrosis of renal cells and in the mononuclear cell infiltrations; light cytoplasm was also less met. Co-administration of DOX and RJ practically prevented the changes in the renal structure. In fact we noticed the presence of rare inflammatory sites and some hepatocytes with light cytoplasm. In all control rats, the cortex and medulla of the kidneys had a normal structure. Tubular necrosis and glomerular widening were evident in the kidneys of all DOX-exposed animals. Simultaneous administration of RJ, reduced the DOX toxic signs in the kidney. The kidney morphology of the RJ, Ehrlich treated with RJ, and Ehrlich treated with DOX and RJ groups did not differ from that of the control animals. With the RJ treatment، with the exception of the glomeruli with only few swollen, the current studies noticed a full prevention from the DOX-induced changes in renal structure.

iNOs Immunohistochemistry

In Figure 3, immunohistochemical studies showed that no detectable iNOS was observed both in cortical and medullar structures of the kidney taken from the control, Royal jelly and Ehrlich mice groups. In the renal cortex of DOX treated animals, diffuse, moderate and strong iNOS staining was seen in all the proximal and distal tubules. Low iNOS staining was observed in the glomeruli. It should be noted that the staining was mainly distributed in the basal part of the tubular cells where numerous mitochondria are spread. The vasa recta and Henle loops in outer medulla from DOX-treated mice showed marked iNOS. The mild iNOS was detectable both in cortical and medullar areas after DOX plus RJ treatment.

DISCUSSION

Renal syndromes may develop in many species of mammalian as a result of primary diseases such as change of minimal disease in humans, immune glomerulonephritis in humans or laboratory animals or inflammatory diseases such as membranous nephropathy, or toxic substances exposure such as Doxorubicin (DOX) in rats (Bos *et al*., 2003). Doxorubicin (Adriamycin®) has remained the drug of choice in the management of a wide range of cancers such as breast, prostate, cancers, gall bladder, malignancies, solid lymphomas, soft tissue sarcomas and carcinomas (Petrioli *et al*., 2008; Schelman *et al*., 2009). The anthracyclines are few of the most effective used anti-cancer treatments ever developed, and are effective than any other class of chemotherapeutic agents against more types of cancer (Lelle *et al*., 2014). DOX is a known nephrotoxic substance and creates chronic progressive glomerular disease manifested by increased creatinine of plasma and urea levels associated with extensive lesions of glomerular, dilatation of tubules, renal glomeruli vacuolization, tubular lumen protein deposits and stromal fibrosis (Injac and Strukelj, 2008). The generation of reactive oxygen species (ROS) is the major pathogenic mechanism for DOX toxicity.

A number of synthetic and natural compounds are known to ameliorate DOX-induced toxicity. Their polyphenols possessing significant scavenging activity of free radical are considered important. A number of animal models including dogs, mice, swine, rats, rabbits and hamsters have been used for studying DOX-induced organo-toxicity (Rizk *et al*., 2014).

The RJ used in the current study contained active biologically amino acids including cysteine, aspartic acid, tyrosine, cystine, lysine, glycine, leucine, isoleucine and valine. In brain of young rats, Seminotti *et al*. Seminotti *et al*., (2008) reported lysine induces protein and lipid damage and decreases the reduced glutathione concentrations. In addition, it was reported that the proline inhibitory effect on acetyl colinesterase and cytochrome c oxidase activity is associated with oxidative stress in rats (Delwing *et al*., 2007). Royal jelly (RJ) has been demonstrated to possess several pharmacological activities including hypotensive and vasodilative activities، antitumour activity, disinfectant action, anti-inflammatory, anti-hyperchol-esterolemic activity, and immunomodulatory activities (Šver *et al*., 1996; Shinoda *et al*., 1978) .

In the present study, serum creatinine and urea levels of control and RJ treated groups were comparable and significantly lower than those of DOX and Ehrlich tumor groups. DOX-exposure caused an appreciable increase in serum creatinine coupled with parallel increases in BUN (blood urea nitrogen). Analysis of creatinine to BUN ratio revealed the level of nephrotoxicity. This observation is consistent with many other previous studies (Ajith *et al*., 2008). High

levels of serum creatinine and BUN reflect tissue injury which is dependent upon the production of free radical. Free radicals are produced during biotransformation of a drug which attack lipid membranes and cause the peroxidation of lipid resulting in accumulation of MDA in tissues. The significant increase was observed in serum uric acid levels in mice upon the administration of DOX suggests failure of excretion in other vital organs such as the kidneys. An in mammals, the increasing in the level of urea, and protein catabolism tends to display an increase in renal dysfunctions and increase in the level of uric acid (Van Vleet and Schnellmann, 2003). Ajith *et al*. (Ajith *et al*., 2007) have also reported increases in the blood urea level of experimental rats with intoxication of anti-cancer drug. While the treatment with RJ resulted in a lower serum uric acid level than the only DOX received group.

The plasma MDA levels of animals that received DOX alone was determined to be increased in this study, and this increase was found to be statistically significant compared to the control group ($P<0.05$). The elevations of the MDA levels is an index of peroxidation of the lipid found in kidney tissues and plasma (Robison *et al*., 1989; Bertani *et al*., 1982; Montilla *et al*., 1997). This may explain why kidney tissues and plasma contained higher levels of MDA in the DOX groups than in any other experimental mice groups.

On the other hand, analysis for serum GSH and SOD activities was determined to be decreased. The increase or decrease in the activities of the enzymes can be explained as oxidative stress injury by DOX. The *in vivo* mechanism of DOX-induced nephrotoxicity is complex, and it involves apoptosis, oxidative stress, fibrogenesis, and inflammation. The oxidative stress is actively involved in the pathogenesis of DOX-induced acute kidney dysfunctions. Reactive oxygen species (ROS) directly act on cell components, including proteins, lipids, DNA and destroy their structure. In the presence of DOX, ROS are produced via all these pathways and are implicated in the pathogenesis of acute DOX-induced renal dysfunctions.

In the current study, the histopathological sections of kidney of DOX groups showed inter-tubular hemorrhages, marked congestion with marked degenerative changes, and disrupted epithelium. Bertani *et al*., Bertani *et al*., (1982) reported that doxorubicin (DOX) has the potential to induce renal damage with glomerulosclerosis.

In addition to serum biochemistry, the degree of renal injury was evaluated by H&E, stained kidney sections under a bright field microscope. The renal sections vividly show DOX-induction of nephrotoxicity. Control kidney sections discern intact distal and proximal tubular cells with the normal glomerular architecture. Uniform intensity of the staining reflected intactness of the renal tissue. Unaltered nuclei were found in nearly every cellular unit lining the tubular cells. In contrast, kidney sections derived from animals mice exposed to DOX demonstrated massive dysfunction coupled with distorted cellular morphology. Sections from kidney-RJ treated groups (EAC & DOX groups) showed very mild degenerative changes in tubules, while both the control and RJ groups did not show any significant lesions of pathological importance.

Nitric oxide (NO) is a free radical gas which acts as a cytotoxic agent or a cyto-protective. Nitric oxide (NO) is generated by either inducible nitric oxide synthase (iNOS) or endothelial nitric oxide synthase (eNOS). Possible role of DOX in NOS metabolism occurs via direct or indirect stimulation of NO production, and this might be an

importance of increased free radical generation (Nathan and Xie, 1994). Free radical production and/or NO release induced by DOX is entirely responsible for the DOX-induced toxicity (Radi *et al*., 1991). Mitochondria have been defined as one of the targets in DOX-induced subcellular damage in the tissue. In addition, it has been shown that DOX could stimulate transmembranal arginine transport to provide increased substrate and activate NOS mediated NO production(Cendan *et al*., 1995).

It has been reported that DOX had a toxicological effect on renal tissues and caused renal failure and nephropathy even with a single application at 7 mg/kg dose (Liu *et al*., 2008). In the current study, royal jelly (RJ) application following that of DOX injected was found to be sufficient to prevent formed renal dysfunctions.

The results of this study indicate that royal jelly (RJ) causes satisfactory recovery in DOX-induced nephrotoxicity in bearing tumor mice. Further detailed DOX-induced nephropathy studies may reveal important information about the pathogenesis and treatment by RJ of renal diseases.

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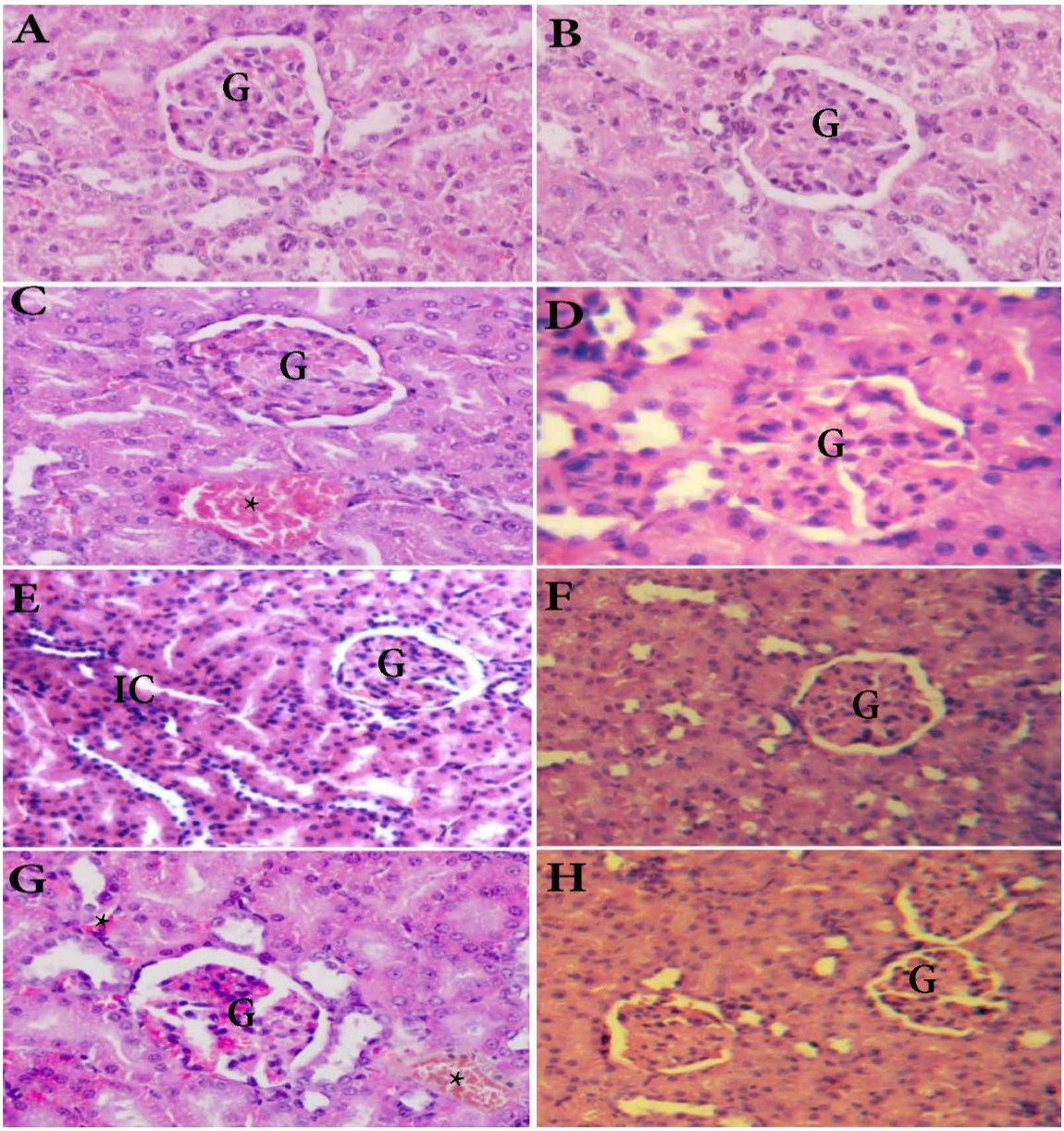

Fig. 2: (A) A normal kidney (GI) and (B) A royal jelly group (GII), The glomerulus is normal in shape (G). (C) The sections of kidney in DOX group, showed marked congestion, inter tubular hemorrhages as the results of doxorubicin effects. (D) The sections of kidney in RJ and DOX treatment group, showed marked glomerulus and renal tubules regeneration as the results of royal jelly treatment against the doxorubicin effects. (E) Section of the kidney of Ehrich tumor cells injected mice showing focal glomerular and tubular damage with mononuclear infiltrate (IC) and diliated renal tubules. (F) Section of the kidney of Ehrich tumor cells bearing mice and treated with royal jelly showing normal glomerular and tubular strcuture with diliated renal tubules. (G) Section of the kidney of Ehrich tumor cells bearing mice and treated with the doxorubicin showing haemorrhage (*), focal glomerular and tubular damage with mononuclear infiltrate and blood vessel congestion, (H) Section of the kidney of Ehrich tumor cells bearing mice treated with royal jelly and doxorubicin, showing normal glomeruli and tubules with some diliated renal tubules, (H&E X 400).

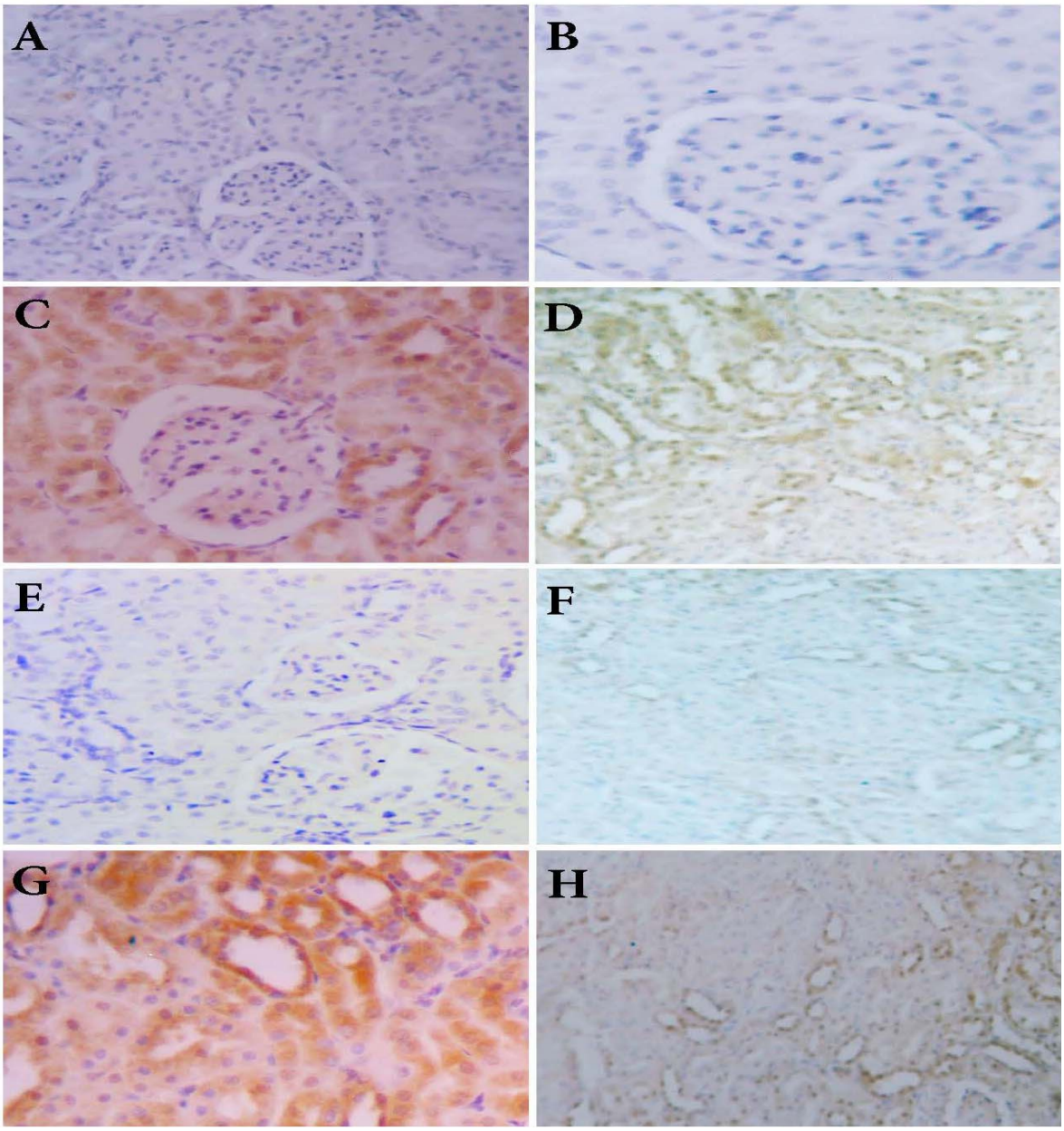

Fig. 3: (A) A normal kidney (GI) and (B) A royal jelly group (GII), showing the very weak iNO-immunoreactive. (C) The picture of iNO-immunoreactive in the doxorubicin treated animals group showing the strong brown positive staining. (D) the picture of iNO-immunoreactive in the doxorubicin and Royal jelly treated animals group showing the weak brown positive staining. (E) The picture of iNO-immunoreactive in the Ehrlich acites tumor bearing mice group showing the very weak brown positive staining. (F) The picture of iNO-immunoreactive in the Ehrlich ascites tumor bearing mice treated with Royal jelly showing the very weak brown positive staining. (G) the picture of iNO-immunoreactive in the Ehrlich ascites tumor bearing mice with Doxorubicin treated group showing the strong brown positive staining. (H) The picture of iNO-immunoreactive in the Ehrlich acites tumor bearingmice treated with Doxorubicin and Royal Jelly group show the weak brown positive staining, (Immunoperoxidas X 400).

ARABIC SUMMARY

التأثيرات الوقائية للغذاء الملكي على الأكسدة والتغيرات النسيجية المرضية والنسيجية المناعية الناجمة عن الدوكسوروبيسين في الفئران الحاملة لاستقاء ورم إيرليش

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تهدف هذه الدراسة إلى تقييم الآثار الوقائية المحتملة لغذاء ملكات النحل على التسمم الكُلوي الناجم عن الدوكسوروبيسين في الفئران الحاملة لورم إيرليش. تم تقسيم الفئران بشكل عشوائي إلى ثماني مجموعات هي (١) المجموعة الضابطة، (٢) فئران تغذت على الغذاء الملكي، (٣) الفئران المحقونة بالدوكسوروبيسين، (٤) الفئران المحقونة بالدوكسوروبيسين والمعالجة بالغذاء الملكي، (٥) الفئران المحقونة تحت الجلد بخلايا إيرليش السرطانية، (٦) الفئران المحقونة بخلايا إيرليش والمعالجة بالغذاء الملكي، (٧) الفئران المحقونة بخلايا إيرليش والدوكسوروبيسين، (٨) الفئران المحقونة بخلايا إيرليش والدوكسوروبيسين والمعالجة بالغذاء الملكي.

تم تعين وظائف الكُلى (كرياتينين، يوريا وحمض اليوريك)، وكلاً من Malondialdehyde، SOD، GSH فى الدم. كما تم تشخيص التغيرات النسيجية بالكُلي عن طريق قطاعات مصبوغة بالهيماتكسلين ايوسين وتعيين اول اكسيد النتروجين المستحث بطريقة كيمياء الأنسجة المناعية.

لوحظ ان الفئران المحقونة بالدوكسوروبيسين والأخري الحاملة للورم إيرليش قد أُصيبت بخلل وظيفي كلوي ملحوظ بزيادة كبيرة في تركيزات الكرياتينين، اليوريا وحمض اليوريك كما زاد تركيز Malondialdehyde وانخفاض في تركيزات GSH و SOD. بينما في المجموعات المحقونة بالدوكسوروبيسين والأخري الحاملة للورم والتي تم معالجتها بالغذاء الملكي ، لوحظ تحسن في وظائف الكُلي البيوكيميائية وزيادة في تركيزات مضادات الأكسدة وانخفاض في عامل الأكسدة (Malondialdehyde) كما ظهر تحسن نسيجي ومناعي لخلايا الكُلي بالفحص الهستوباثولوجي.

من الدراسة الحالية يتضح أهمية الغذاء الملكي لحماية الكُلي ضد التسمم والتلف الناجم عن الحقن بالدوكسوروبيسين من خلال الحد من الأنشطة المرتفعة لوظائف الكلى في الدم علاوة على زيادة تركيزات مضادات الأكسدة وانخفاض في عامل الأكسدة وتحسن في تشخيص الهستوباثولوجي. في الختام، يعتبر غذاء ملكات النحل لديه القدرة على تخفيف وتحسين الأثار السمية والتالفة للكُلي لدى الفئران والناجمة عن الحقن بالدوكسوروبيسين.