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Protective role of propolis on diazinon induced nephrotoxicity in adult male albino rats

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SUMMARY

Diazinon has been reported to produce oxidative stress and adverse effects on many organs. In contrast, propolis components behave as hydrophilic antioxidants. To evaluate the protective effect of propolis on diazinon-induced nephrotoxicity in adult male albino rats, eighty adult male albino rats were divided randomly into four groups: control, diazinon treated, propolis treated and diazinon plus propolis groups. Control group were divided into two subgroups: the first was not given any treatment and the second one received 1.5 ml of sterile distilled water through intra-gastric tube daily for 4 consecutive weeks. The diazinon group was treated with 10 mg/kg through intra-gastric tube, daily for 4 weeks. The propolis group received 50 mg/kg through intra-gastric tube, daily for 4 weeks. The diazinon-plus-propolis group was treated with the same doses as previous groups. Kidneys were removed and processed for haematoxylin and eosin, caspase-3 immunostaining and electron microscopic examination. Renal tissues of diazinon-treated rats showed histopathological and ultrastructural changes such as shrunken glomerulus, hemorrhage, congestion, increased Bowman's space, inflammatory infiltration, degenerated tubules with vacuolated epithelial cell lining, pyknosis and necrotic debris. Rats of the diazinon-plus-propolis group showed a marked reduction in these pathological features. We conclude that propolis can ameliorate the nephrotoxicity induced by diazinon.

Key words: Diazinon – Kidney – Propolis

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INTRODUCTION

Diazinon is an organophosphorus insecticide, primarily used for agricultural purposes and released to the environment through spraying on a wide variety of agricultural crops and agricultural sites for pest control. The main ways of exposure to diazinon (DZN) are ingestion, inhalation and dermal contact (ATSDR, 2008). Toxicities of DZN cause adverse effects on many organs including pancreas (Gokcimen et al., 2007), lung, small intestine (Rady, 2009; Najafi et al., 2014), testis (Sarabia et al., 2009), liver (Nahla et al., 2010), kidney (Sarhan and Al-Sahhaf, 2011) and ovary (Ola-Davies et al., 2015). It also increases the levels of serum glucose, triglycerides and cholesterol, decreases the level of serum total protein and causes severe disturbance of carbohydrates, lipids and proteins metabolism (Al-Attar, 2014).

Toxicity of diazinon was realized to be through altering the normal neurotransmission within the nervous system. Diazinon inhibits the enzyme acetyl cholinesterase, which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions. These results are due to abnormal accumulation of ACh in the nervous system (Timchalk, 2001; Colovic et al., 2015).

Previous studies reported that oxidative stress plays an important role in diazinon toxicity. It was found that diazinon increased the malondialdehyde level and decreased the antioxidant enzymes in rat erythrocytes (Sutcu et al., 2007). Diazinon also enhances renal lipid peroxidation, which is accompanied by a decrease in the activities of renal anti-

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oxidant enzymes and depletion in the level of glutathione (Shah and Iqbal, 2010). Diazoxon (diazinon oxidation product) was found as the most toxic compound. Increasing diazoxon concentrations activates catalase, superoxide dismutase, glutathione peroxidase, and significantly increases malondialdehyde level (Colovic et al., 2015).

Propolis or "bee-glue" is a yellow-green to dark brown resinous and rubbery substance, collected and transformed by honey bees, Apismellifera, from various plant sources to seal holes in their hives and to protect it from intruders (Marcucci, 1995). Propolis has been used since ancient times as a folk medicine for the treatment of many diseases. Numerous biological and pharmacological properties of propolis have been reported in the last decades such as antibacterial, antiviral, antifungal, antiparasitic, antiinflammatory, immunomodulatory, hepatoprotective, antioxidant and antitumor (Banskota et al., 2001; Bankova et al., 2002; Russo et al., 2002; Bankova, 2005; Gómez-Caravaca et al., 2006; Khalil, 2006; Viuda-Martos et al., 2008; Araujo et al., 2012; Pereira-Filho et al., 2014). It is composed of resin (50%), wax (30%), pollen (5%), essential and aromatic oils (10%), flavonoids (quercetin, kaempferol, pinocembrin, apigenin, chrysin, etc.), polyphenolics, beta-steroids, terpenes, minerals, and vitamins (Russo et al., 2002; Gómez-Caravaca et al., 2006; Khalil, 2006; Viuda-Martos et al., 2008). Flavonoids and phenolics compounds have beneficial effects as natural anti-oxidants (Basnet et al., 1997).

So the aim of the study was to evaluate the protective effect of propolis on the nephrotoxicity induced by diazinon in adult male albino rats.

MATERIALS AND METHODS

Chemicals

Diazinon was purchased from ADWIA, emulsifiable concentrate 60%, Cairo, Egypt. Propolis was obtained from honey bee colonies situated at the apiary of the Faculty of Agriculture at Suez Canal University, Egypt.

Preparation of aqueous propolis extract

Propolis freeze until usage, samples were mixed with distilled water, heated gently and filtered through filter paper. It was freshly prepared and given to animals by intra-gastric tube at dose of 50 mg/kg/day daily orally via intra-gastric tube for 4 weeks (Thanaa et al., 2011).

Animals

Eighty healthy male Sprague Dawley albino rats (weighing between 150 and 200 gm and aged 10-12 weeks) were obtained from the animal house of the Faculty of Veterinary Medicine, Suez Canal University, and used throughout the study. They were housed in stainless-steel cages in a fully ventilated room in the Human Anatomy and Embryology Department, Faculty of Medicine, Suez Canal University, and left for 2 weeks before the commencement of the experiment. They received water and food *ad libitum*, and were fed on standard laboratory rat pellets. Rats were weighted daily and observed for behavioral changes. All animal experiments were conducted in accordance with the guidelines of the Institutional Animals Ethics Committee.

Experimental design

Rats were assigned randomly into four groups (20 rats for each):

Group I (control): The animals were divided into 2 subgroups:

Ia: Animals were not given any treatment.

Ib: Animals received 1.5 ml of sterile distilled water through intra-gastric tube daily for 4 consecutive weeks.

Group II (DZN-treated): Animals received DZN (10 mg/kg/ body weight) once daily) orally through intra-gastric tube after dilution in distilled water, for 4 weeks (Kalender et al., 2006).

Group III (DZN plus propolis-treated): Animals received diazinon plus propolis orally for 4 weeks in the same doses as in group II and IV.

Group IV (propolis-treated): Animals received propolis at a dose of 50 mg/kg/day daily orally via intra-gastric tube for 4 weeks (Thanaa et al., 2011).

Histological assessment

All animals were sacrificed 24 hours after the end of treatment by overdose of ether. Kidneys were removed and examined carefully. The right kidneys were prepared and processed for light microscopic examination, while the left kidneys were prepared and processed for electron microscopic examination.

Light microscopic examination

Kidneys were fixed in 10% neutral buffered formalin solution for 24 hours, dehydrated in graded ethanol and embedded in paraffin. Four μm thick serial sections were prepared and stained with Hematoxylin and Eosin (H & E) stain *(Bancroft et al., 1994).* The paraffin embedded sections were also immuno-histochemically stained using Caspase-3 (Rabbit polyclonal Antibody, CPP32, Ab-4, Thermo, UK). Sections were studied blindly using Olympus light microscope, and the histopathological changes were recorded.

Electron microscopic examination

Kidneys were divided into small pieces, fixed in buffered glutaraldehyde 2.5% for two hours and fixed in 1% osmictetroxide. Ultrathin sections were cut using MT 600-XL RMC ultratome and stained with uranyl acetate and lead citrate. They were examined with JEOL-1010 (Japan) transmission

electron microscope, at the regional center for mycology and biotechnology transmitting electron unit, Alazhar University, Cairo and photographed under different magnifications.

Morphometric study

Renal corpuscles were assessed through measuring the diameters of renal corpuscles and glomerulus, thickness of renal space and numbers of glomeruli. Renal tubules were also assessed through measuring the diameters of proximal and distal convoluted tubules and the thickness of the epithelial wall of the proximal and distal convoluted tubules. All morphometric parameters were measured in H&E stained sections by the aid of the micrometer lens in the Olympus light microscope (Power X100). Each parameter was determined in five fields from five serial sections in each rat in all groups.

Statistical analysis

Data of all groups were studied using the statistical program of social science version 12 (SPSS Inc., Chicago, IL, USA). Differences between experimental groups were tested using ANOVA and chi square tests. The statistical significance of the data was determined by P value ($P < 0.05$ was considered significant).

RESULTS

Behavioral changes

There were no behavioral changes in animals in different groups.

Light Microscopy

Haematoxylin and eosin stained sections

Group I (control group):

Both control sub-groups (Ia and Ib) showed the same features. The renal cortex was formed of renal corpuscles and tubules (proximal and distal). Each renal corpuscle consisted of a glomerulus surrounded by double layered Bowman's capsule (simple squamous parietal and visceral layers). A slit-like space was found between the two layers of the capsule, known as the renal space. The glomeruli consisted of tuft of capillaries, mesangial cells and extracellular matrix (mesangium). Juxtaglomerular (JG) cells with basophilic cytoplasm and rounded nuclei were found in the glomerular vascular pole. JG cells were close to the macula densa, which showed crowded nuclei. The proximal convoluted tubules (PCT) were lined with simple cuboidal epithelium, well developed brush borders and narrow lumens. The distal tubules (DCT) were lined with cubical cells with wider lumens (Fig. 1).

Group II (DZN-treated group):

Kidney sections showed glomerular changes including hemorrhage, shrunken glomeruli, increased Bowman's space, congestion of glomerular capillaries, inflammatory infiltration, degeneration in macula densa and JG cells. Some glomeruli showed glomerular hypertrophy, congestion and obliterated Bowman's space. Renal tubules revealed degeneration, vacuolation of epithelial cell lining, pyknotic nuclei and necrotic debris filling their lumens (Fig. 2).

Group III (DZN and propolis-treated group):

Examination of kidney sections revealed capillary congestion with intact renal corpuscles including glomeruli and Bowman's capsule, normal renal space and JG cells. Most of the renal tubules showed normal lumens and epithelial cell wall whereas, few renal tubules showed mild degeneration in their epithelial lining (Fig. 3).

Group IV (Propolis-treated group):

Renal sections of this group showed the same features as in control group.

Immune-stained sections

Group I (control Ia & Ib):

There was a negative Caspase-3 immune-stain in renal corpuscles, proximal and distal convoluted tubules in control subgroups (Fig. 4a).

Group II (DZN-treated group):

Dense positive Caspase-3 immune expression was seen in tubules, glomerular capsule and glomerular cells (Fig. 4b).

Group III: DZN and propolis-treated Group:

Few cells of renal glomerulus and tubules showed faint positive Caspase-3 immune stain (Fig. 4c).

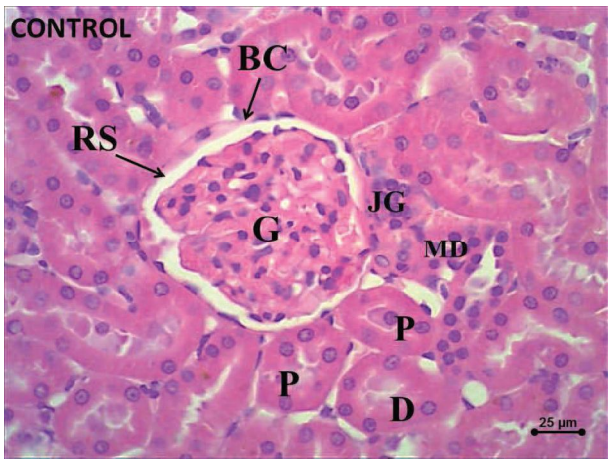

Fig. 1. A photomicrograph of a section in the kidney cortex of control group showing the renal corpuscle consisting of glomerulus (G), Bowman's capsule (BC) and renal space (RS), Juxta glomerular cells (JG), macula densa (MD), proximal convoluted tubules (P) and distal convoluted tubules (D). H&E x400.

Group IV (propolis-treated group):

Renal corpuscles, proximal and distal convoluted tubules showed negative Caspase-3 immunestain similar to control group.

Morphometric results

Morphometric parameters of renal corpuscles revealed a significant decrease in the corpuscular and glomerular diameters in DZN-treated group compared to all other groups. There was a significant increase in the thickness of renal space in DZN-treated group compared to all other groups. The number of glomeruli was significantly decreased in DZN-treated group. DZN and propolis treated group showed a significant improvement in all these parameters compared to DZN-treated group (Table 1). The morphometric parameters of renal tubules revealed a significant increase in the luminal diameters with a significant decrease in the thickness of the tubular walls in DZN-treated group compared to control group. DZN and propolis-treated group also showed a significant improvement in all these parameters compared to DZN-treated group (Table 2).

Electron microscopy

Group I (control group Ia&Ib):

Ultra-structure of the kidney in all animals of control group Ia and Ib revealed glomerular capillaries, lined with endothelial cells forming fenestrated endothelial layer and rested on a basement membrane (Fig. 5). The glomerular capillaries were surrounded by flat podocytes with foot processes. The glomerular basement membrane formed of electron lucid lamina rara externa adjacent to the podocytes, electron dense lamina densa and electron lucid lamina rara interna adjacent to the endothelial cells. Examination of the epithelial cells lining of the proximal convoluted tubule revealed intact brush border, apical located nuclei, lysosome, plenty of mitochondria with intact basement membrane (Fig. 6a). Distal convoluted tubule epithelial cells showed apical located nuclei and plenty of elongated mitochondria, arranged longitudinally in their basal part

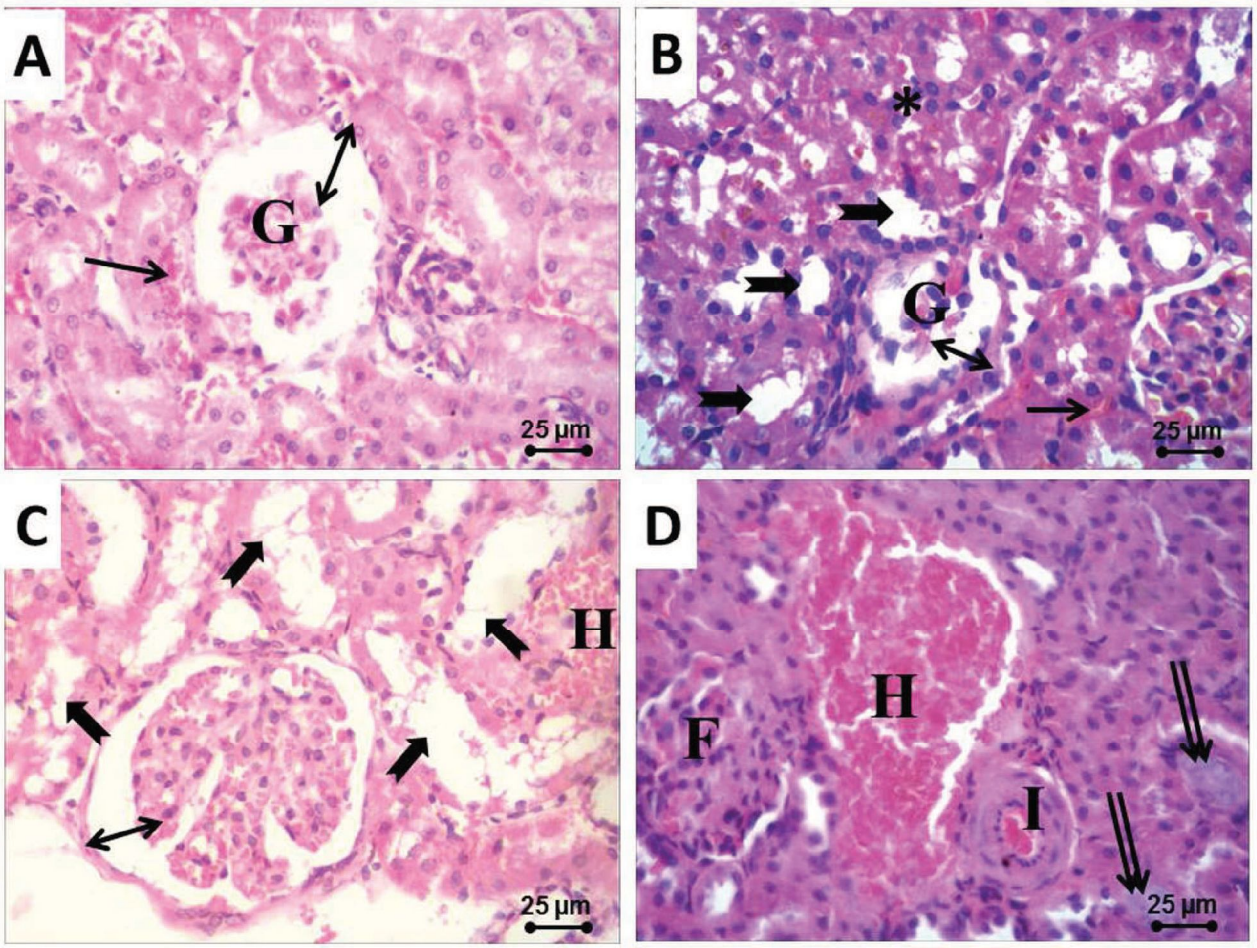

Fig. 2. Photomicrographs of H&E stained sections in renal cortex of DZN-treated group showing: **(A-D)** shrunken glomeruli (G) with degenerated macula densa and JG cells, increased Bowman's space (double end arrow), congested blood capillaries (arrow), degenerated and vacuolated epithelial cells lining of proximal and distal convoluted tubules (thick arrow), hemorrhage (H), pyknotic nuclei (*), congested thick wall blood vessel (I), congested and hypertrophied glomerulus with obliterated Bowman's space and inflammatory infiltration (F) and renal tubules filled with necrotic debris (double arrow). x400.

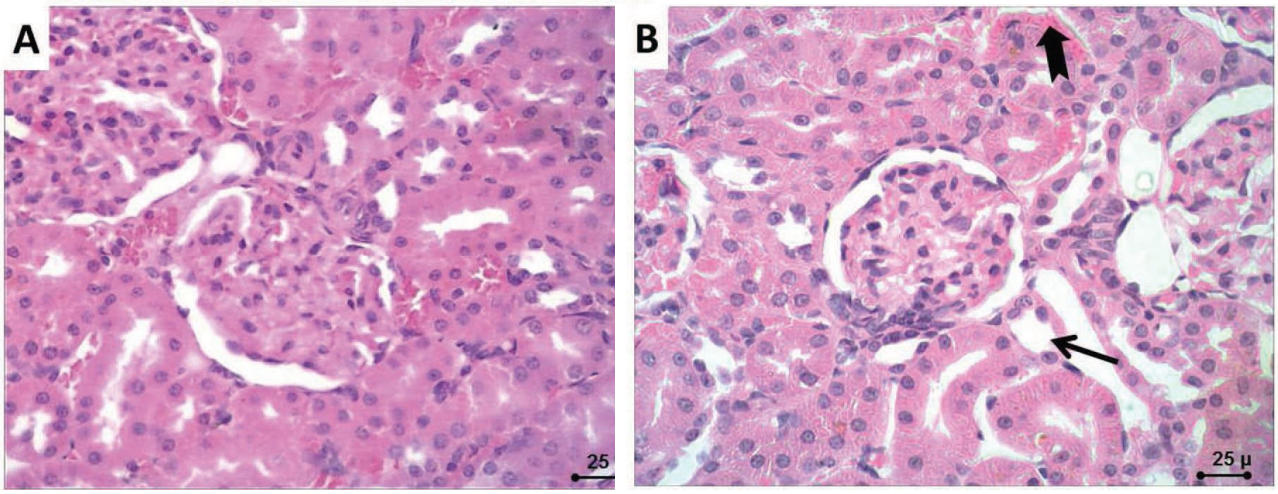

Fig. 3. Photomicrographs of H&E stained sections in renal cortex of DZN plus propolis-treated group showing **(A)** intact renal corpuscles (glomeruli & Bowman's capsule), normal renal space and JG cells, X400; and **(B)** normal lumina, epithelial cell wall and brush border of PCT and appearance of intact DCT lumina and wall. Few renal tubules showed mild degeneration (arrow) and capillary congestion (thick arrow). x400.

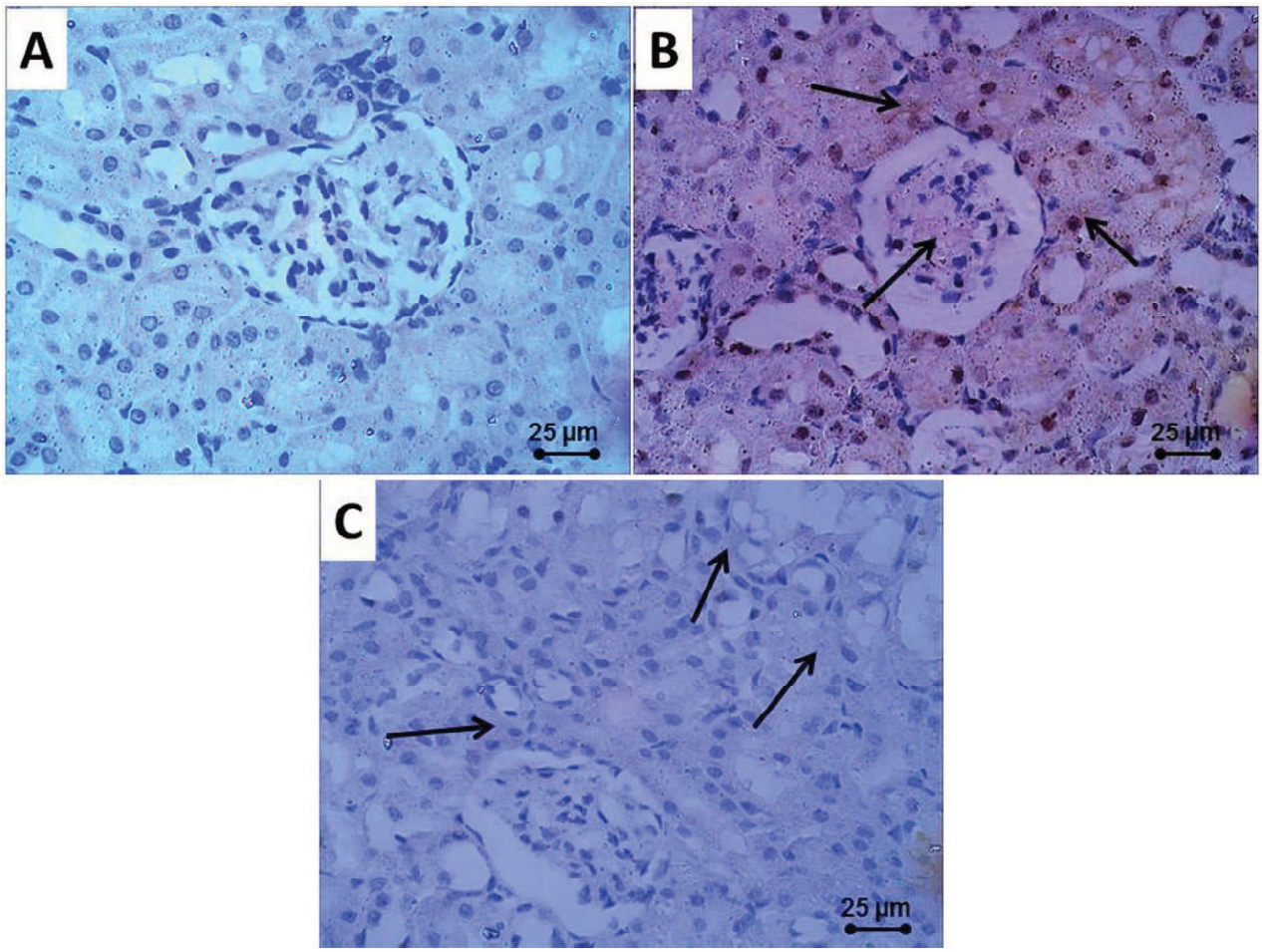

Fig. 4. Photomicrographs of caspase-3 immuno-stained sections in renal cortex of **(A)** control, **(B)** DZN-treated and **(C)** DZN plus propolis-treated groups showing negative caspase-3 reaction in **(A)**, dense positive immune expression (brownish stained cytoplasm) arrows in **(B)** and faint positive immune reaction arrows in **(C)**. x400.

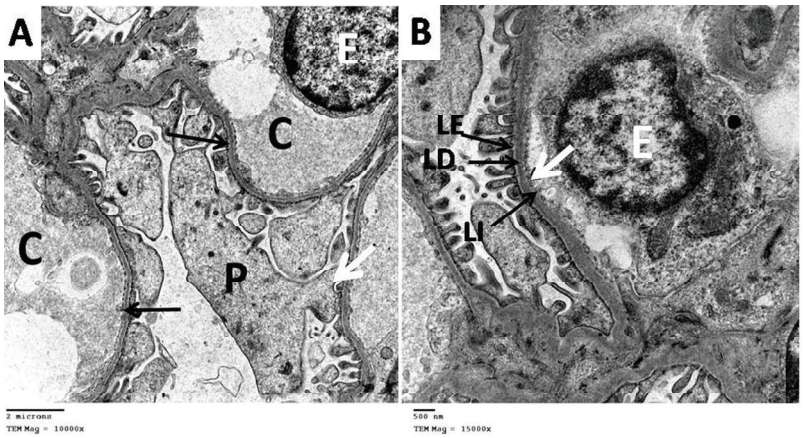

Fig. 5. Electron photomicrographs in rat glomerulus of control group showing **(A)** podocyte (P), glomerular blood capillaries (C), endothelial cell (E), basement membrane (arrow) and foot processes (white arrow). x10,000 and **(B)** endothelial cell (E), the fenestrated endothelial layer (white arrow) and glomerular basement membrane formed from lamina rara externa (LE), lamina densa (LD) and lamina rara interna (LI). x15,000.

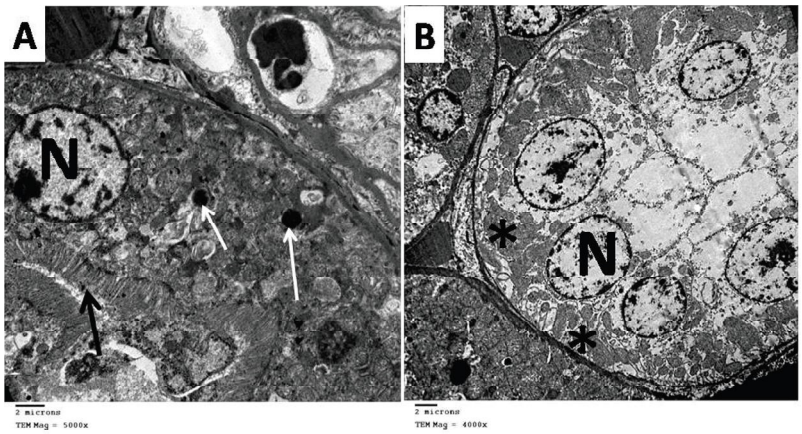

Fig. 6. Electron photomicrographs in rat kidney of control group showing **(A)** proximal convoluted tubule epithelial cells with brush border (black arrow), apical located nuclei (N), lysosome (white arrow), plenty of mitochondria and intact basement membrane. x5000; and **(B)** distal convoluted tubule epithelial cells with apical located nuclei and plenty of elongated mitochondria arranged longitudinally in their basal part (star). x4000.

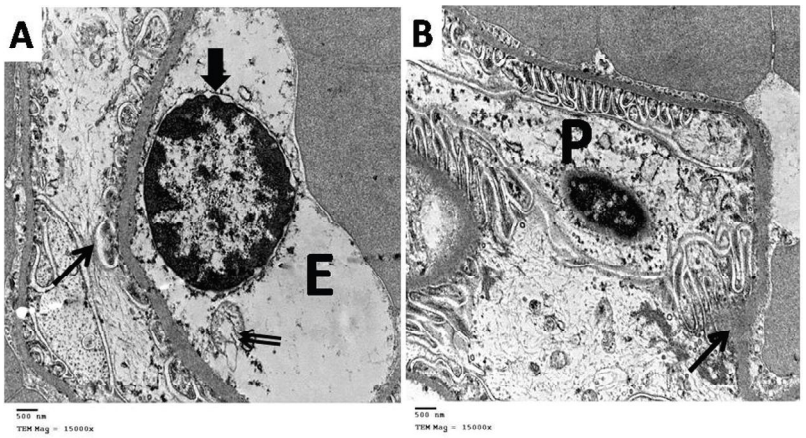

Fig. 7. Electron photomicrographs in rat glomerulus of DZN-treated group showing **(A)** short, flattened and degenerated foot processes of podocyte (arrow) and an endothelial cell (E) with vacuolated cytoplasm, ruptured mitochondria (double arrow), irregular nuclear membrane with chromatin condensation and clumping (thick arrow) and loss of fenestration; and **(B)** a podocyte (P) with vacuolated and edematous cytoplasm, shrunken nucleus, chromatin condensation and thickened basement membrane (arrow). x15,000.

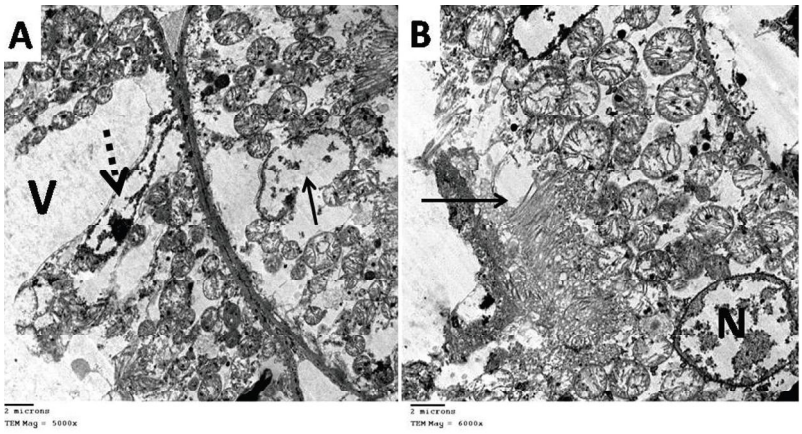

Fig. 8 (right). Rat kidney of DZN-treated group showing **(A)** PCT epithelial cell with compressed flattened destructed nucleus (dashed arrow) surrounded by cytoplasmic vacuolation (V) and another cell showing ruptured destructed nucleus (arrow) and edematous mitochondria. x5000; and **(B)** PCT epithelial cells with areas of lost brush border (arrow), markedly edematous mitochondria and chromatin condensation and clumping (N).

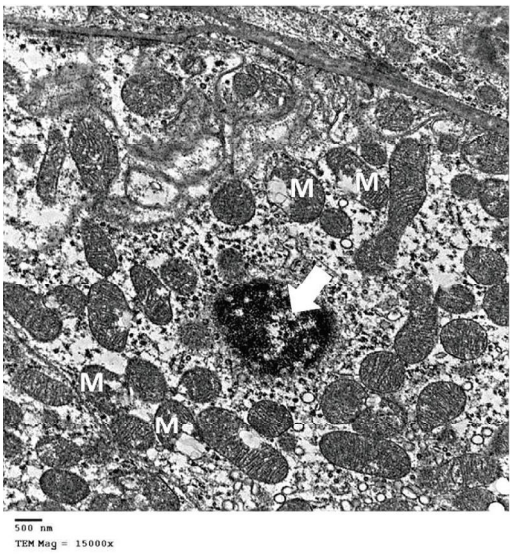

Fig. 9 (above, left). Electron photomicrograph in rat kidney of DZN-treated group showing epithelial cells of distal convoluted tubule with shrunken nucleus, chromatin condensation (thick arrow), ruptured mitochondria (M) and many cytoplasmic vacuoles. x15,000.

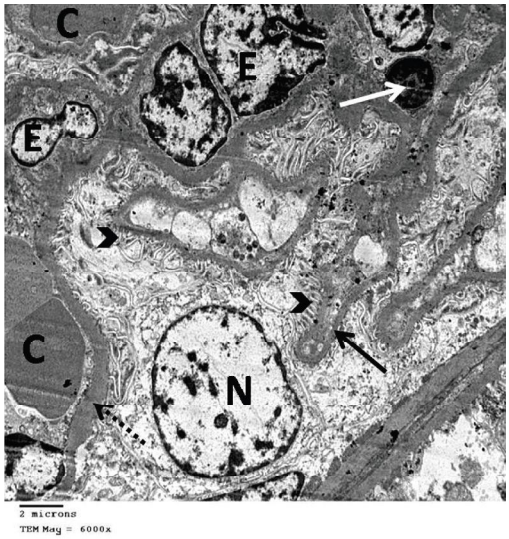

Fig. 10 (above, right). Electron photomicrograph in rat glomerulus of DZN plus propolis-treated group showing blood capillaries (C) lined with endothelial cells (E) showing normal architecture, cytoplasm and nucleus. One endothelial cell showing condensed chromatin of it's nucleus (white arrow). Basement membrane showing normal thickness (black arrow) and increased thickness in other sites (dashed arrow). Podocytes appeared normal with intact foot processes (arrow head) and nucleus (N). x6000.

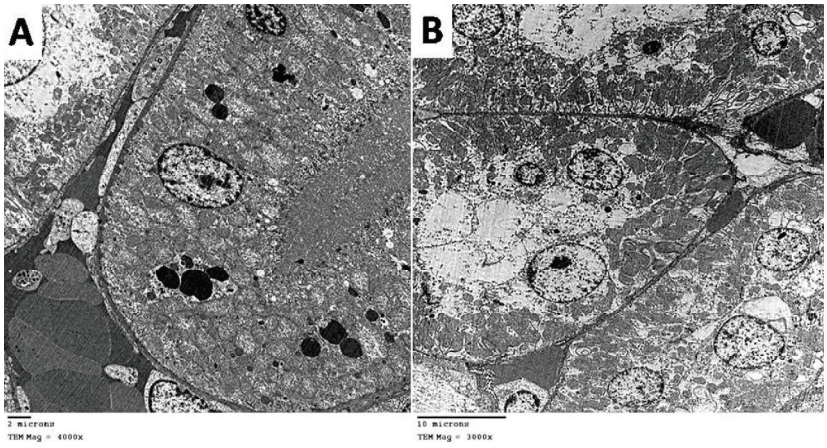

Fig. 11. Electron photomicrograph in rat kidney of DZN plus propolis-treated group showing **(A)** epithelial cells of proximal convoluted tubule with intact brush border, nuclei, lysosomes, plenty of slightly swollen mitochondria with disappearance of cytoplasmic vacuoles and normal basement membrane. x4000; and **(B)** epithelial cells of distal convoluted tubule with nearly normal nuclei, plenty of elongated mitochondria and normal basement membrane. x3000.

Table 1. Morphometric parameters of renal corpuscles.

Group (No. of rats)		Diameter of renal corpuscle[#] µm Mean ± SD	Diameter of glomerulus[#] µm Mean ± SD	Renal space thickness[#] µm Mean ± SD	Number of glomeruli * Mean ± SD
Control (20)	Ia	199.6 ± 5.2	187.1 ± 0.3	12.5± 5.3	42 ± 0.3
	Ib	200.1 ± 7.0	185.8 ± 0.8	11.7± 9.1	45 ± 1.1
DZN-treated (20)		162.2 ± 10.2[a,â,c]	131.1 ± 6.4 [a,â,c]	30.9 ± 8.7 [a,â,c]	10 ± 1.7 [a,â,c]
DZN and propolis –treated (20)		185.9± 6.7[a, â, b, c]	163.8 ± 4.7 [a, â,b,c]	22.1 ± 3.1 [a, â,b,c]	29 ± 0.9 [a, â,b,c]
Propolis-treated (20)		197.9 ± 4.9	183.7 ± 2.6	± 3.9	40 ± 0.6

[#] ANOVA &* Chi square tests: ([a]) P<0.05 compared to control (Ia) group, ([â]) P<0.05 compared to control (Ib) group, ([b]) P<0.05 compared to DZN-treated group, ([c]) P<0.05 compared to propolis-treated group.

Table 2. Morphometric parameters of renal tubules.

Group (No. of rats)		Diameter of PCT µm Mean ± SD	Thickness of PCT epithelilal wall µm Mean ± SD	Diameter of DCT µm Mean ± SD	Thickness of DCT epithelilal wall µm Mean ± SD
Control (20)	Ia	21.8 ± 0.7	40.6 ± 1.4	29.5 ± 1.1	33.8 ± 0.9
	Ib	20.5 ± 2.0	41.3 ± 1.1	28.9 ± 0.9	34.8 ± 1.1
DZN-treated (20)		40.1 ± 6.2 [a, â,c]	30.5 ± 10.2 [a, â,c]	59.4 ± 9.3 [a, â,c]	24.7 ± 5.8 [a, â,c]
DZN and propolis –treated (20)		27.6 ± 1.8 [a, â, b, c]	36.7 ± 2.1 [a, â, b, c]	33.8 ± 1.5 [a, â, b, c]	29.6 ± 2.9 [a, â, b, c]
Propolis-treated (20)		22.2 ± 0.6	39.8 ± 1.8	30.5 ± 0.9	33.1 ± 1.3

ANOVA test: ([a]) P<0.05 compared to control (Ia) group, ([â]) P<0.05 compared to control (Ib) group, ([b]) P<0.05compared to DZN-treated group, ([c]) P<0.05 compared to propolis-treated group. PCT = proximal convoluted tubule, DCT= distal convoluted tubule.

(Fig. 6b).

Group II (DZN-treated group):

Electron microscopic examination of the renal glomerulus revealed podocytes with short, flattened and degenerated foot processes (Fig. 7). Podocytes also showed vacuolated, edematous cytoplasm, shrunken nucleus and chromatin condensation. The basement membrane was thick, and the capillary endothelial cells showed destructed nuclei with irregular nuclear membrane, chromatin condensation and clumping. The endothelial cells also showed loss of fenestration, cytoplasmic vacuolation, markedly edematous and ruptured mitochondria. Examination of the epithelial cells of the proximal convoluted tubules revealed loss of brush border, destructed nuclei, clumping and chromatin condensation with markedly edematous mitochondria (Fig. 8). Cell lining of distal convoluted tubules showed ruptured mitochondria, cytoplasmic vacuoles, shrunken nucleus and chromatin condensation (Fig. 9).

Group III (DZN and propolis-treated group):

Ultra-structure of glomerulus showed capillaries lined by endothelial cells with intact cytoplasm and nuclei (Fig. 10). Few endothelial cells showed chromatin condensation in their nuclei. Most areas of basement membrane showed normal thickness, whereas there were few sites with increased thickness. Podocytes appeared normal with intact foot processes, cytoplasm and nuclei. Examination of epithelial cells lining of the proximal convoluted tubules revealed intact basement membrane, brush border, nuclei and rich cytoplasm with lysosomes and mitochondria with absent cytoplasmic vacuoles (Fig. 11a). Few mitochondria were slightly swollen. The epithelial cells of distal convoluted tubules showed nearly normal nuclei with plenty of elongated mitochondria and normal basement membrane (Fig. 11b).

Group IV (propolis-treated group):

Electron microscopic examination of kidney sections showed the same features as in control group.

DISCUSSION

The DZN-treated group showed many glomerular changes including hemorrhage, shrunken glomeruli, increased Bowman's space, congestion of glomerular capillaries, inflammatory infiltration and degeneration in macula densa and Juxtaglomerular cells. Some glomeruli showed glomerular hypertrophy and obliterated Bowman's space. El-Shenawy et al. (2009) was in agreement with

these results. Moreover, Hala et al. (2011) observed glomerular changes in one-month treated rat with organophosphorus pesticide (Malathion).

Previous studies reported that oxidative stress plays an important role in the renal toxicity of DZN. Diazinon caused lipid peroxidation, free radical generation, decrease in the activities of renal antioxidant enzymes (catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione S-transferase) (Amirkabirian et al., 2007; Abdou and El-Mazoudy, 2010; Shah and Iqbal, 2010). These studies may explain the histopathological changes which appeared in the kidney after exposure to DZN.

Glomerular atrophy may be the result of the toxic effect of the free radicals on the mesangial cells (MCs) that were known to produce growth factors for normal cell turnover, and to resemble vascular smooth muscle cells in phenotype and responsiveness to different stimuli. They were targets of numerous inflammatory mediators, and sometimes assume the role of macrophages (Floege et al., 1993). It was also believed that, mesangial cells play a role in glomerular contraction and modulation of filtration surface area (Rodriguez et al., 2000). As there was no smooth muscle lining in the capillaries, mesangial cell which connect to capillary loops and glomerular basement membrane were believed to be the primary regulators of capillary diameter and to enable overall glomerular contraction (Scharschmidt et al., 1986). Mesangial cells also play a role in the synthesis of the mesangial matrix, a specialized connective tissue which binds the loop of glomerular capillaries and fills the spaces between endothelial surfaces that were not invested by podocytes. They clear the glomerular filter of immune complexes and cellular debris (Standring et al., 2005). So injury of mesangial cells could interrupt their functions, causing glomerular contraction, and decreasing the secretion of mesangial matrix and further glomerular atrophy, as it occurred in the diazinon-treated group in the present study.

On the other hand, Sarhan and Al-Sahhaf (2011) reported that, DZN induced glomerular hypertrophy, which was also in accordance with the results of the current study. The glomerular hypertrophy may be due to inflammatory infiltration and congested glomerular tuft of capillaries which was constantly exposed to comparably high intra-glomerular pressure within glomerular capillaries and mesangium (Iversen et al., 1998). The complex structure of the glomerular tuft consists of glomerular capillaries arranged in a specific folding pattern of the glomerular basement membrane that is primarily sustained by the mesangium. The high intra-glomerular pressures challenge not only the width of the glomerular capillaries, but also the folding pattern of the glomerular tuft (Kriz et al., 1995). So the expansion of the congested glomerular tuft of capillaries may explain the glomerular hypertrophy in the diazinon-treated group.

Renal tubules were also affected in DZN-treated group in the present study, as they showed degenerated and vacuolated epithelial cell lining, pyknotic nuclei and lumina filled with necrotic debris. Enan et al. (1986) mentioned that pesticides caused degeneration of renal tubules with the presence of hyaline casts inside them. These results were in agreement with Hassan et al. (2007), and El-Shenawy et al. (2009).

DZN-treated group also showed positive Caspase-3 immune expression in renal tubules, glomerular capsule and cells. These findings indicate the presence of apoptosis as caspase-3, which was known as the most important effector enzyme in apoptosis that provide a common pathway to both death receptor and mitochondria-dependent apoptotic mechanisms (Krajewska et al., 1997; Rudel, 1999). Moreover, Franco et al. (2009) and Liu et al. (2010) mentioned that the apoptotic changes contribute to high concentrations of reactive oxygen species (ROS).

The morphometric parameters of renal corpuscles and tubules in DZN treated group in the present study confirmed the histopathological and immune- histochemical results.

This group also showed ultra-structural changes in renal glomeruli and tubules. Podocytes, capillary endothelial cells and the epithelial cells of renal tubules showed nuclear destruction and chromatin condensation. These results were in agreement with that of Venees et al. (2011). Podocytes also revealed vacuolation and degenerated foot processes. Moreover, the capillary endothelial cells also showed loss of fenestration, cytoplasmic vacuolation, markedly edematous and ruptured mitochondria with thick basement membrane. These findings were in accordance to Hala et al. (2011) and Mohamed El-Gerbed (2012), as they observed focal segmental thickening and duplication of glomerular basement membrane and podocyte changes after exposure to organophosphorus compounds (malathion and deltametharin).

Datta et al. (1994) reported that the increase in the thickness of the glomerular basal membrane may be due to the fact that all the organophosphorous pesticides were lipophilic and known to have a strong affinity for interaction with membrane phospholipids. Podocyte also was known as the critical component of the selective filtration barrier of the glomerulus, and was susceptible to oxidative damage such as that induced by diabetic nephropathy, as mentioned by Shirong et al. (2008).

Epithelial cells of renal tubule in diazinon-treated group in this study also revealed loss of brush border and markedly edematous mitochondria. Hala et al. (2011) and Mohamed El-Gerbed (2012) also reported that organophosphorus compounds (malathion and deltamethrin respectively) induced ultra-structural changes in the epithelial cells of the

proximal tubules, which included an increase in the number of irregular shaped mitochondria with sparse fragmented cristae, vacuolar degeneration, increased number of lysosomes and loss of apical microvilli. So these findings may explain the previously mentioned histopathological and immune-histochemical results.

Moreover, DZN and propolis-treated group in the current study revealed improvement in the histopathological, immune-histochemical and morphometric parameters of renal corpuscles and tubules compared to DZN-treated group.

Propolis consists of approximately 300 synergistic compounds, with the most important roles being played by phenolic compounds and their anti-oxidizing, anti-rheumatic and disinfectant properties; flavonoids and their anti-inflammatory, anti-microorganism, and anti-neoplastic properties; terpenes with their antibiotic and immune-stimulating properties; and lipid-wax substances which decrease the low density lipoprotein (LDL) fraction of cholesterol in blood. Other important elements present in propolis include calcium, manganese, magnesium, zinc, copper, silicon and iron. Propolis was also rich in pro-vitamin A (beta-carotene), vitamin A (retinol), vitamins B1, B2, B5, B6, C, E and D, proteins and carbohydrates (Kędzia, 2009).

Ichikawa et al. (2002) and Ozguner et al. (2005) reported that propolis prevents lipid peroxidation and enhances reduced glutathione (GSH) in lead-treated groups, and exhibits strong reactive oxygen species scavenging activity in vitro towards both the superoxide anion and nitric oxide radicals. Furthermore, Ozguner et al. (2005) mentioned that caffeic acid phenethyl ester component of propolis exhibit a protective effect on free radical mediated oxidative renal impairment in rats. These findings may explain the improvement in histopathological features of the glomeruli and renal tubules in the group co-treated with propolis and diazinon.

The ultra-structure of glomeruli and renal tubules in the DZN-and-propolis treated group also showed improvement compared to the DZN-treated group that confirmed the histopathological and immune-histochemical results.

Maier and Chan (2002) reported that there is a link between oxidative damage and impaired mitochondrial function. Moreover, among the components of propolis, pinocembrin, (one of flavonoids) was found to have a protective effect on components of mitochondria respiratory chain/oxidative phosphorylation system involving complex I activity, cytochrome oxidase expression and origins of reactive oxygen species (Guang and Du, 2006). Furthermore, Cedikova et al. (2014) found that propolis increases activities of mitochondrial respiratory complexes II and IV without affecting mitochondrial membrane potential. Abdel-Daim.(2014) reported that other antioxidants (ceftriaxone and vitamin C) had a protective role against DZN-induced serum, as well as renal tissue biochemical parameters, and produced synergetic nephroprotective and antioxidant effects. So these results may explain the improvement in the histopathological, morphometric and ultra-structure of renal tissues in the DZN-and-propolis-treated group in the current study.

Conclusion

The current study established the renal histopathological, morphometric and ultra-structural changes induced by diazinon. Propolis has been used empirically in the treatment of multiple pathologies since ancient times. In the present study, it was observed that propolis attenuates the diazinon -induced nephrotoxicity, which confirms it's effectiveness as antioxidant and anti-inflammatory. Further experimental researches are needed, particularly propolis effects on renal functions after long-term oral diazinon exposure.

Ethical approval

All authors declare that all experiments have been performed in accordance with the ethical standards. We further state that we are free of any personal or business association that could represent a conflict of interest regarding the article submitted, and we have respected the ethical principles underpinning the research.

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