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Ethanolic extract of *Carissa edulis* ameliorates dimethoate induced renal damage in male guinea pigs

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Abstract

Carissa edulis (CE), belongs to family Apocyanaceae, is used traditionally to treat many diseases such as headache, cough, rheumatism, epilepsy, syphilis and fever. Our objective aimed to study the ameliorative of *Carissa edulis* (CE) ethanolic extract against dimethoate (DM)-induced renal damage. Healthy adult male guinea pigs were randomly divided into five groups of five animals each. The first group was served as a control group and administered with vehicle orally; the group II administered with DM (14 mg/kg;1/25 LD50) orally. Animals of group III, IV and V were administered with 100 mg/kg of CE extract, 200 mg/kg of CE extract, and 100 mg/kg Liv-52 orally half hour before DM administration respectively. All the previous administrations were repeated daily for 21 days. At the end of experiment, animals were sacrificed and blood was collected from portal vein for biochemical tests. The kidney was removed for histology and lipid peroxidation (LPO)–antioxidant test. Data were analyzed by one-way ANOVA using SPSS. DM caused renal damage as evidenced by elevated urea, creatinine and glucose levels in Group II as compared with Group I. Administration of CE in Group III and group VI caused amelioration and reduction in the rise of blood urea, creatinine and glucose compared with group II. In addition, there was increased LPO and decreased in catalase and GST activities in DM group, while CE significantly reversed the changes toward normal values. Histological examination of DM group showed many pathohistological alterations such as cellular degeneration, vacuolization, hyaline casts, dilatation, necrosis, glomerular atrophy, and infiltration. The ethanolic extract of CE has ameliorative effects against DM-induced renal damage in guinea pigs.

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Introduction

Living organisms are characterized by their ability to produce a large group of metabolic compounds known as secondary metabolites, which is unique to individual species, and have no specific role within cells, but they play a mediating role in interactions with other organisms. This task may be either a defensive role against herbivores and pathogens or as attractive substances for pollinators (Kutchan *et al.,* 2015). These natural products are commonly used to treat several diseases by the oldest folk medicine. The use of these products increases all over the world especially in developing countries (Mahady, 2001). The medicinal plants are the sources of many traditional drugs such as atropine, morphine, ephedrine, digoxin, *etc*. and play an important role in humans and animal health care (WHO, 2000). Approximately 25% of the prescription drugs in folk medicine may contain one or more active ingredients of plant origin (Ahmad *et al.,* 2006).

Many plants have been documented to have antioxidant, antinociceptive and anti-inflammatory activities (Tatli *et al.,* 2009; Demsie *et al.,* 2019) but out of the 250,000 - 500,000 plant species on earth, only 1% to 10% has been identified for their potential medicinal values (Tatli *et al.,* 2009). The genus *Carissa* is a rich source of different natural classes of compounds such as sesquiterpenes, cardiac glycosides, phenolic compound, flavonoids, lignans, and chlorogenic acid derivatives (Pal *et al.,* 1975; Kirira *et al.,* 2006; Wangteeraprasert and Likhitwitayawuid, 2009; Al-Youssef and Hassan, 2010). The chemical constituents of CE in the literature review indicated the isolation of lignans (Pal *et al.,* 1975; Achenbach *et al.,* 1983; Achenbach *et al.,* 1985), flavonoids and phenolic compounds (Al-Youssef and Hassan, 2010; Al-Youssef and Hassan, 2014), and sesquiterpene (Wangteeraprasert and Likhitwitayawuid, 2009). CE is a medicinal plant naturally growing at different geographical areas in South Asia. It is commonly used in folk medicine to treat many diseases such as rheumatism (Giday, 2001), fever, sickle cell anemia, syphilis, helminthoses, and rabies (Nedi *et al.,* 2004; Ibrahim *et al.,* 2007; Ya'u *et al.,* 2008; Youssef and Hassan, 2010). In pharmacological studies, CE showed antiviral activity (Tolo *et al.,* 2006; Tolo *et al.,* 2010), anticonvulsant (Ya'u *et al.,* 2008; Jawaid *et al.,* 2011), antiplasmodial (Koch *et al.,* 2005; Kirira *et al.,* 2006; Kebenei *et al.,* 2011), antimicrobial (Ibrahim *et al.,* 2005), analgesic (Ibrahim *et al.,* 2007), diuretic (Mekaconnen and Urga, 2004), as well as hypoglycaemic activity (El-Fiky *et al.,* 1996). Although, there is no adverse effect reported on CE herbal medicines (Al-Youssef and Hassan, 2010).

The use of chemical pesticides in qat production in Yemen has been increasing dramatically (Date *et al.,* 2004). So, the farmers use these chemicals in agriculture to enhance food production by eradicating unwanted insects and disease vectors (El-Bini *et al.,* 2014). Organophosphorus (OPs) pesticides are widely used for agricultural and domestic pest control especially in developing countries. However, exposure to these pesticides may lead to several problems for the environment, animals, and human health. More recent WHO estimates showed that over 300,000 people die from pesticide poisoning annually (WHO, 2016). The primary effect of OPs insecticides is the inhibition of the enzyme acetylcholinesterase (AChE), an enzyme that hydrolyzes the neurotransmitter acetylcholine at neuromuscular junctions and brain cholinergic synapses (Galloway and Handy, 2003; El-Bini *et al.,* 2014). They irreversibly inactivate this enzyme which leads to nerve dysfunction in insects, humans and many other animals (Čolović *et al.,* 2013). In addition, lipid peroxidation (LPO) caused by free radicals is one of the molecular mechanisms involved in OPs-induced toxicity (Heikal *et al.,* 2011) which exert their toxicity effects through an attack on cellular components of tissues (Heikal *et al.,* 2012) and generation of reactive oxygen species (ROS) (Martínez-Morcillo *et al.,* 2019). While the liver is known to be the primary site of pesticide biotransformation (Hodgson and Goldstein, 2001), the kidney is also considered to be a secondary organ involved in pesticide detoxification due to its high blood flow and its ability to reduce pesticide toxicity (Husak *et al.,* 2014). Moreover, there are many

organs that could be affected by OPs pesticides such as brain (Astiz *et al.,* 2009), pancreas (Kamath *et al.,* 2008), testis (Selmi *et al.,* 2015), ovaries (Kaur *et al.,* 2005), and immune system (Díaz-Resendiz *et al.,* 2015). Dimethoate (DM), as an OPs pesticide, is the most commonly used pesticide in Yemen by farmers to protect crops and qat farms (Al-Youssef and Hassan, 2014). Recently, Al-Awthan *et al.* (2014a) have shown that chronic exposure to DM caused nephrotoxicity and increased LPO and these negative effects were reduced using co-treatment of vitamins (E and C).

The purpose of the present study is to investigate the ameliorative effect of CE ethanol leaves extract administration for 21 days on some biochemical and histopathological parameters in kidney tissues of male guinea pigs intoxicated with sub-chronic doses of DM pesticide.

Materials and methods

Chemicals

Dimethoate (40%) was purchased from local market as a commercial emulsifiable concentrate formulation. Liv-52 was obtained from Himalaya Drug Company, Bangalore, India. Both the DM and Liv-52 were reconstituted appropriately in 0.5% carboxymethylcellulose (CMC) for the final concentration immediately prior to use.

Plant material

The leaves of CE were collected from Jeblah district, Ibb Governorate, Yemen. The plant was authenticated by comparison with reference specimens preserved at the Herbarium of Biology Department, Ibb University-Yemen. Voucher specimens were kept in the Herbarium for future references.

Preparation of the ethanol extract

The powdered material of leaves (2000 g) were macerated with 70% ethanol by continuous stirring at room temperature, and then evaporated to dryness under reduced pressure and finally yield 25%. The dried extracts were dissolved in 0.5% CMC and administrated orally when experiments were performed.

Animals maintenance

Adult male guinea pigs (600 ± 200g) were obtained from the animal house of Biology department, Faculty of Science, Ibb University, Yemen and kept for one week on a commercial diet in environmentally controlled conditions with free access to diet and water *ad libitum*. The experimental procedure was performed in accordance with the national and international guidelines and regulations approved by the ethical committee of Ibb University. In addition, all administrative approvals were taken.

Experimental design

Animals were randomly divided into five groups of five animals each. The control group was given 0.5% CMC suspension by gastric gavage. The animals of groups II were given oral administration of 14 mg/kg DM (1/25 LD50). The animals of groups III were given oral administration of 100 mg/kg CE ethanol leaves extract plus 14 mg/kg DM. Animals of group IV were given oral administration of 200 mg/kg CE plus 14 mg/kg DM. Animals of group V were given oral administration of 100 mg/kg Liv-52 plus 14 mg/kg DM. All the previous administrations were repeated daily for 21 days. At the end of the treatment, the animals of each group were anesthetized with ether and blood was collected directly from the portal vein.

The blood sample of animals in each group was divided in two tubes, one of them mixed with heparin to prevent coagulation and the other was allowed to clot at room temperature for 1 h, and then centrifuged at 3000 rpm and 4°C for 15 min to obtain sera. The separated serum was sampled into clean tubes and kept in a deep-freezer at -24°C for biochemical analysis.

Biochemical analysis

Serum urea concentration was determined using Biodiagnostic kits (Egypt) according to the method of Friedman and Young (1997). Serum creatinine concentration was determined using Biodiagnostic kits (Egypt) according to the method of Bartels *et al.*

(1972). The levels of urea and creatinine were expressed as **(**mg/dl). Glucose concentration was determined using diagnostic kits of Spinreact (Spain) according to the method of Tietz (1995). Lipid peroxidation (LPO) was determined based on that of Ohkawa *et al.* (1979). Catalase (CAT) activity was measured by the method of Aebi (1984). Glutathione-S-transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al.* (1974).

The total protein content of kidney homogenate was determined by the method of Lowry *et al.* (1951). A detailed description of the LPO measurement and other parameters was listed in a previously published article (Al-Awthan *et al.*, 2012).

Histopathological examination

Animals of control and treated groups were put under light ether anesthesia, dissected as quickly as possible, and then pieces kidneys were removed and fixed in 10% neutral formalin for 24 hours, then washed by the running tap water, and stored in 70% ethyl alcohol, until further processing. Small blocks of about 5×5 mm size were dehydrated, cleared and embedded in paraffin wax. Finally, paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and stained with hematoxylin and eosin. Photomicrographs of selected slides were taken using (Sony HD, Japan) built-in digital photo camera.

Statistical analysis

Results of the biochemical estimations were reported as mean ± S.D. To analyze our data, SPSS software version 20 was used. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA) and follow up test (LSD). Differences with a *P*-value of <0.05 were considered as statistically significant.

Results

Biochemical analysis

At the end of the 21 days of our experiment, the control animals' group was compared with all other groups. In addition, the DM-treated group was compared to the CE plus DM-treated group. DM administration (14mg/kg) for 21 days in male guinea pigs caused a statistically significant increase ($P<0.001$) in the serum level of urea (86.09 ± 6.52 U/L), creatinine (1.21 ± 0.13 U/L), and glucose (172.60 ± 11.10 U/L) when compared to control group. These values were 55.74 ± 7.98 U/L, 0.68 ± 0.12 U/L, 124.80 ± 10.98 U/L in normal control guinea pigs, respectively (Table 1).

Table 1. The levels of urea, creatinine, and glucose (Means ± SD), stimulation (S%) and inhibition (I%) in control and different treated group.

Treated groups	Biochemical Parameters		
	Urea (U/L)	Creatinine (U/L)	Glucose (U/L)
Control	55.74 ± 7.98^{a}	0.68 ± 0.12^{a}	124.80 ± 10.98^{a}
DM	$86.09 \pm 6.52^{b***}$	$1.21 \pm 0.13^{b***}$	$172.60 \pm 11.10^{b***}$
S% versus control	54	77	38
100CE+DM	$73.60 \pm 6.34^{c*}$	$0.92 \pm 0.12^{c*}$	$146.80 \pm 10.82^{c*}$
I% versus DM	14	24	15
200CE+DM	$70.40 \pm 5.54^{c**}$	$0.83 \pm 0.14^{c***}$	$125.20 \pm 13.55^{c***}$
I% versus DM	18	31	28
100 Liv-52+DM	$64.91 \pm 5.96^{c**}$	$0.67 \pm 0.13^{c***}$	$131.80 \pm 14.99^{c***}$
I% versus DM	25	45	24

Each value represents the mean ± S.D., n= 5. Values marked with asterisks differ significantly from control animals: $P<0.05$, those marked with the same letter differ insignificantly from control group: $P>0.05$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control, respectively.

The level of these parameters was stimulated by 54%, 77%, and 38% respectively after DM-intoxication when compared to that of the control animals. In addition, ethanolic extract of CE (100 & 200 mg/kg) ameliorate these parameters to around normal levels by the following percentages (14%, 24%, and 15%) and (18%, 31%, and 28%) respectively when compared to DM-treated group (Table 1). Similarly, Liv52 (100mg/kg) also showed a good ameliorative effect by (25%, 45%, and 24%) when compared to DM and CE treated groups (Table 1). On the other hand, levels of LPO were increased (3.03 ± 0.73 nmol mg^{-1} protein) significantly (P<0.001) in the kidney tissue homogenates of DM-treated group as compared to control group alone (1.85 ± 0.32 nmol mg^{-1} protein). However, it was observed that the LPO levels were inhibited (2.21 ± 0.55 nmol mg^{-1} protein), (2.08 ± 0.42 nmol mg^{-1} protein), and (1.99 ± 0.33 nmol mg^{-1} protein) by 31%, 35%, and 37% in the groups that received DM along with 100 mg/kg CE, 200 mg/kg CE, and 100 mg/kg Liv-52 respectively. The inhibition in LPO levels was statistically significant (P<0.01) as shown in Table 2. While, there was a significand decrease in the activity of CAT (2.78 ± 1.11 µmol min mg^{-1} protein) and GST (10.65 ± 1.74 µmol min mg^{-1} protein) (P<0.001) in DM treated group as compared to the control group (5.57 ± 1.50) and (22.28 ± 1.54) respectively. Similarly, the CE plant extract (100 and 200) and Liv52 were found to normalize these parameters to within normal levels by (30%, 57%, and 51%) and (22%, 38%, and 35%) respectively (Table 2).

Table 2. Means ± SD of lipid peroxidation, activities of catalase and glutation-S-transferase, stimulation (S%) and inhibition (I%) in the liver enzymes of control and different treated group.

Treated groups	Antioxidant enzyme		
	LPO (nmol mg^{-1} protein)	CAT (µmol min mg^{-1} protein)	GST (µmol min mg^{-1} protein)
Control	1.85 ± 0.32^{a}	5.57 ± 1.50^{a}	22.28 ± 1.54^{a}
DM	3.19 ± 0.74^{b***}	2.78 ± 1.11^{b**}	10.65 ± 1.74^{b***}
% versus control	72(S)	50(I)	43(I)
100CE+DM	2.21 ± 0.55^{c*}	3.85 ± 1.23	14.72 ± 1.75^{c**}
% versus DM	31(I)	30(S)	22(S)
200CE+DM	2.08 ± 0.42^{c**}	4.37 ± 1.31^{c*}	20.07 ± 2.31^{c***}
% versus DM	35(I)	57(S)	38(S)
100 Liv-52+DM	1.99 ± 0.33^{c**}	4.21 ± 1.20^{c*}	18.95 ± 1.66^{c***}
% versus DM	37(I)	51(S)	35(S)

Each value represents the mean ± S.D., n= 5. Values marked with asterisks differ significantly from control animals: P<0.05, those marked with the same letter differ insignificantly from control group: P>0.05. *P < 0.05, ** P < 0.01 compared with control, respectively.

Histopathological examination

Histopathology of the kidney section of the normal control group (Fig. 1A) showed normal architecture with normal glomerulus and tubules. The kidney sections of the DM-intoxicated guinea pigs (Fig. 1B), showed many histopathological alterations such as cellular degeneration, vacuolization of tubular epithelium, hyaline casts, dilatation of Bowman's capsule, tubular necrosis, glomerular atrophy, and inflammatory cell infiltration with loss of the normal architecture seen in control group. These observations suggested cellular necrosis of the kidney tissue, while the CE (100&200) and Liv52 treated groups (Group III, Fig. 1C, Group IV, Fig. 1D and Group V Fig. 1E) showed lesser damage as compared with DM group as shown in Table 3.

Discussion

In the current study, a marked increase of urea and creatinine confirms an indication of which approved by the histopathological changes in guinea pigs exposed to DM. However, the present increases of

serum urea and creatinine, and histopathological changes are generally in accordance with previous investigations showing increases of these parameters in rats (Hassan *et al.,* 1994; Mahjoubi-Samet *et al.,* 2008; Padma *et al.,* 2012, Hou *et al.,* 2014, Al-Attar, 2015, Abdel-Daim, 2016, Farag *et al.,* 2016, Li *et al.,* 2016), mice (Al-Attar and Abu Zeid, 2013, Selmi *et al.,* 2018), rabbits (Owda, 2003; Sarhan and Al-Sahhaf, 2011) and guinea pigs (Al-Awthan *et al.,* 2014b) exposed to DM and other OPs pesticides. Many mechanisms have been suggested to explain DM induced tissue injury, such as LPO and interaction with cellular components resulting from ROS attack on biological structure (Stohs and Bagchi, 1995). LPO has been extensively used as a marker of oxidative stress.

Table 3. Scoring of the histological features of the guinea pig kidney tissue sections in DM-intoxicated animals and other treatments groups.

Histopathological features	Group I	Group II	Group III	Group IV	Group V
Glomerular atrophy	-	++++	+++	+	++
Dilatation of Bowman's capsule	-	+++	++	+	+
Inflammatory cell infiltration	-	+++	++	+	+
Tubular necrosis	-	+++	++	+	+
Hyaline casts	-	++++	-	-	-

The increase of LPO in the present results were in consistence with previous studies which have shown that acute and sub-chronic exposure to DM generates LPO and alters the antioxidant status of several tissues in rats (Sharma *et al.,* 2004; Sharma *et al.,* 2005; Sayim, 2007; Attia and Nasr, 2009), guinea pigs (Al-Awthan *et al.,* 2014a), and mice (Alarami, 2015). Actually, the involvement of oxidative stress and LPO following exposure to OPs has been reported (Banerjee *et al.,* 2001; Akhgari *et al.,* 2003; Sivapiriya *et al.,* 2005). On the other hand, DM administration in the current study showed a significant decrease in CAT and GST activities.

These enzymes constitute the primary defense system against chemical toxicity associated with free radicals (Pincemail *et al.,* 2002). The observed inhibition in CAT activity in DM group could be due to the adaptive response to the generated ROS indicating the failure of the antioxidant defense mechanism to protect guinea pig tissues from cellular damage induced by DM (Koner *et al.,* 1998). Moreover, the reduced activity of CAT and GST in DM intoxicated group may cause the accumulation of ROS, H_2O_2 or their products of decomposition (Elhalwagy *et al.,* 2008). These findings are consistent with previous investigations which indicated that DM and other pesticides caused oxidative damage which was confirmed by a significant decrease in levels of CAT and GST, and increase in the level of LPO (Padma *et al.,* 2011, Abbassy *et al.,* 2014, Mohamed and Ali, 2014, Beydilli *et al.,* 2015).

Organophosphate insecticides are known to induce various histopathological changes in the kidney tissues of experimental animals (Mohnsen, 2001; Sulak *et al.,* 2005; Kalender *et al.,* 2007; Elhalwagy *et al.,* 2008). In support of our results, a recent study reported that kidney showed glomerular and tubular degeneration, hemorrhage, infiltration, hydropic changes, tubular cast, glomerular shrinkage, and compressed blood vessel following DM intoxication (14 mg/kg) in adult male swiss albino mice (Alarami, 2015).

In addition, previous studies observed glomerular and renal tubular impairments in response to chlorpyrifos (another OPs pesticide) exposure in rats and also found that these changes were mitigating by using antioxidants (Oncu *et al.,* 2002). In addition, DM induced similar histopathological changes in kidney tissues of female rats and their pups (Hassan *et al.,* 1994). Also, previous findings of Sulak *et al.* (2005) and Kalender *et al.* (2007) had found

degenerative changes in kidney of adult rats exposed to methidathion and methyl parathion. Moreover, severe interstitial mononuclear cells' infiltration (Oncu *et al.,* 2002), hyperplasia and hypertrophy of tubular cells (Kalender *et al.,* 2007) had been also observed following pesticide exposure. Interestingly, pretreatment with CE ethanolic extract significantly mitigated DM-induced increases in serum creatinine and urea levels, indicating that CE extract exerted ameliorative effects through maintaining cellular integrity and limiting the leakage of these markers into blood.

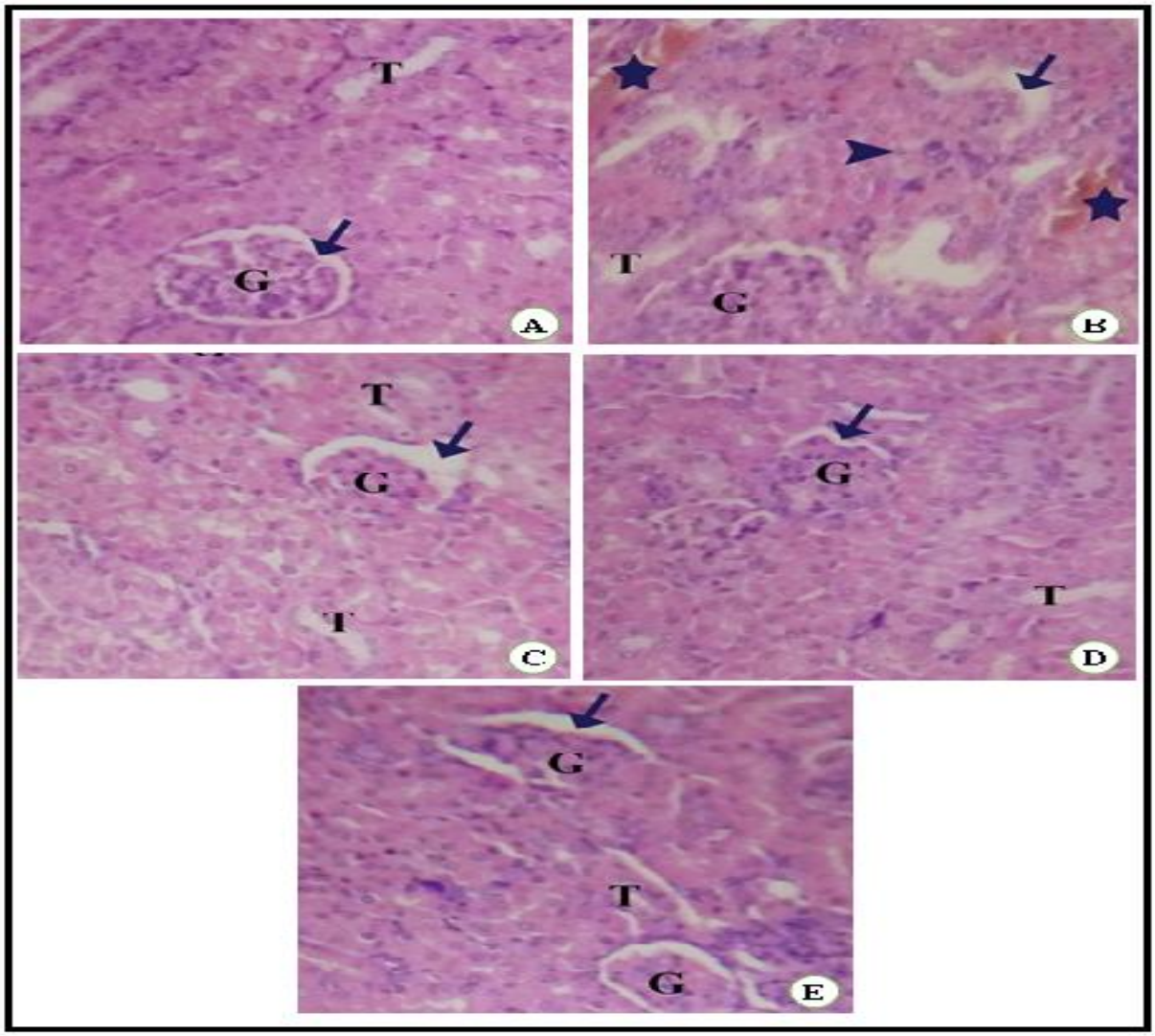

Fig. 1. Photomicrograph of guinea pig kidney tissue sections (H&E ×400): (A) Normal histology of kidney tissue in control animals (Group I) showing normal architecture of the glomerulus (G) and tubules (T). (B) DM-treated animals (Group II) showing mononuclear cell infiltration, glomerular atrophy, hyaline casts (star), dilatation of Bowman's capsule (arrow), and tubular necrosis (arrow head). (C) CE (100 mg kg^{-1} b.wt) + DM, (Group III) showing mild mononuclear cell infiltration with mild glomerular atrophy. (D) CE (200 mg kg^{-1} b.wt) + DM, (Group IV) showing improvement in the architecture resembling the control group. (E) Liv-52 (100 mg kg^{-1} b.wt) + DM, (Group V) showing little mononuclear cell infiltration and mild congestion.

These findings agreed with those in a previous report (Al-Awthan *et al.,* 2014a) which showed that oral administration of vitamins (C&E) protects renal tissues against DM intoxication in male guinea pigs. More recent study also reported that some plant oils showed protective effects against diazinon induced hepatorenal toxicity in male rats (Al-Attar *et al.,* 2017).

Conclusion

Based on the present study, it can be concluded that CE ethanolic extract & Liv52 ameliorate the renal alterations induced by DM intoxication. Additionally, the antioxidant properties of these extracts support the bioactive roles of its protective effects on DM toxicity. To strengthen these findings, further experimental studies are needed to evaluate the effect of other doses of the plant extract as a therapeutic agent against DM toxicity and other toxicants.

Conflict of interest

Authors declare that they have no conflict of interest.

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