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RESEARCH ARTICLE

Comparative immuno-modulatory effects of basil and sesame seed oils against diazinon-induced toxicity in rats; a focus on TNF-α immunolocalization

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Abstract

Diazinon (DZN), a common organophosphorus insecticide (OPI), has hazardous effect to human and animals with its ubiquitous use. Considering the implication of reactive oxygen species (ROS) in the OPIs toxicity, the present study was aimed to evaluate the ameliorative properties of basil (BO) and sesame (SO) seed oils against the toxic effect of DZN. Forty adult male albino rats were divided into four experimental groups (n = 10 rats/group); control, DZN (10 mg/kg b.w/day), DZN + BO (5 ml/kg b.w/day), and DZN + SO (8 ml/kg b.w/day) groups, treated for a period of 4 weeks. DZN-exposed animals showed significant elevation in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (Cr) with a significant decline in testosterone level compared with control. On the other hand, DZN + BO and DZN + SO groups revealed significant decreases in ALT, AST, BUN, and Cr with a significant increase in testosterone level when compared with DZN-exposed animals. Oxidative/antioxidant indices revealed significant increases of malondialdehyde (MDA) levels along with significant decreases of superoxide dismutase (SOD), glutathione peroxidase (Gpx), and catalase (CAT) activities among DZN-treated rats compared with control. Distinctly lower levels of MDA and increased activities of SOD, Gpx, and CAT were evident in both DZN + BO and DZN + SO groups when compared with DZN-exposed animals. Inflammatory and immunomodulatory markers assessment showed a significant increase in TNF-α with a significant decline in IL-10 level in DZN group; meanwhile, both DZN + BO and DZN + SO groups revealed significant declines in levels of TNF-α with significant increases in IL-10. Corresponds immunohistochemistry, the total scores (TS) of TNF-α immunostainings in hepatorenal, testicular, and epididymal tissues of control, DZN + BO and DZN + SO groups were significantly lower than those values of DZN group. Additionally, the examined tissues of DZN + BO group revealed significant lower TS of TNF-α immunostaining compared with DZN + SO group. The overall data suggested that both BO and SO can be efficiently used as preventive herbal compounds against DZN-induced oxidative stress with special reference to their possible antioxidant, anti-inflammatory, and free radical activities. However, BO has more potent protective effect against DZN-induced tissue injury at both immunohistochemical and molecular levels.

Keywords Diazinon · Basil oil · Sesame oil · Immunomodulatory · TNF-α · Oxidative stress

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Introduction

Pesticides have a wide spread utilization in agriculture, veterinary practice, and public health. The misuse of pesticides causes serious environmental pollution and health hazards including acute and chronic poisonings of human (Ellenhorn et al. 1997; Abdollahi et al. 1999). Among pesticides, organophosphorus (OPs), which contain phosphorus (P) derived from phosphoric acid (PA), constitute one of the largest groups of chemical pesticides, and the most toxic to animals as well as humans (De Bleecker et al. 1994; Shah and Iqbal 2010). It is worth noting that pesticides improve both human

and animal nutrition through increasing food availability and storage time, as well as reducing the cost. In spite of the beneficial special effects of using pesticides on food, they are usually toxic to animals and humans, especially the accidental cases of vocational exposure (Kalender et al. 2005).

Diazinon (DZN, O, O-diethyl-O-[6-methyl-2-(1-methylethyl)-4pyrimidinyl] phosphorothioate), one of OPs pesticides, is a common antiparasitic agent used in veterinary field against external parasites like mites and ticks (Oñate et al. 2009). Additionally, it is universally used in agriculture for insects' control (Bailey et al. 2000; Garfitt et al. 2002). DZN can be absorbed via direct contact, ingestion, or inhalation, and it is eliminated mainly by the liver and kidney. Inhibition of acetylcholinesterase is a common mechanism explaining the DZN toxicity where DZN is oxidized within the liver microsomal enzymes producing potent acetylcholinesterase inhibitors: diazoxon, hydroxydiazoxon, and hydroxydiazinon (WHO 1998). Another mechanism of DZN toxicity is the formation of free radicals (FR) and reactive oxygen species (ROS) that induce oxidative stress and lipid peroxidation in different mammalian organs (Altuntas et al. 2004; Ogutcu et al. 2006; Hariri et al. 2010). Abdel-Diam et al. (2018) reported that DZN can induce inflammation in neurohepatic tissue via induction of tumor necrotic factor-α (TNF-α). Moreover, other studies declared that DZN may increase DNA fragmentation of the cells inducing genotoxicity (Aluigi et al. 2010) and apoptosis (Boussabbeh et al. 2016). Additionally, the lipophilic nature of DZN facilitates the interaction with phospholipids bilayer of the cell membranes of most visceral organs (Videira and Antunes-Madeira 2001). The most common target organs for DZN toxicity are the liver and kidney (Nakagawa and Moore 1999; Mansour et al. 2017) and its toxicity extends to impair reproductive organs (Anbarkeh et al. 2019), as well as it causes hematological and biochemical changes (Yassa et al. 2011; Karimani et al. 2018).

For complementary medicine or alternative therapy, many recent literatures reported the use of natural products in attenuating xenobiotics impact (Mohamed et al. 2016; Ismaiel et al. 2017; Abdel-Daim et al. 2020; Awad et al. 2020; Khalil et al. 2020). Accordingly, a great concern has grown to use the natural products and medicinal plants for exploring novel pharmacologically active ingredients (Panda and Naik 2009; Ogbera et al. 2010; Abdellatief et al. 2017). Among these herbs, basil (*Ocimum basilicum*) and sesame (*Sesamum indicum*) are. Basil has an economic importance owing to its phenolic components: lineol, linalool, limonene, estragol, methyl cinamato, eugenol, and geraniol (Sifola and Barbieri 2006). Moreover, the areal part of basil is a very good source of minerals and other phytochemicals, which are responsible for various therapeutic potentials (Tewari et al. 2012). In addition, the basil oil (BO) is a major aromatic agent that is used for food, pharmaceutical, and cosmetic industries (Trevisan et al. 2006). Li et al. (2017) reported that BO shows antioxidant and anti-inflammatory properties via scavenging the DPPH and suppression of TNF-α production, respectively. On the other hand, sesame is a rich source of lignans and phytoestrogens, so it is mixed with human food due to its many benefits for health (Thompson et al. 1991; Mohamed and Awatif 1998).). Sesame lignans are sesamin, sesamol, sesaminol, and sesamolin which have been known to possess anti-inflammatory and antioxidant properties (Wu et al. 2019). A recent study of Hsu and Parthasarathy (2017) reported anti-inflammatory and antioxidant effects of sesame oil (SO) on atherosclerosis. They added that SO decreases lipid peroxidation and nitric oxide production as well as reduces TNF-α production.

For our knowledge, there is limited available information regarding the comparative immunomodulatory efficacy of BO and SO, as natural products, against DZN-mediated metabolic disorders in the liver, kidney, testis, and epididymis. Consequently, the present study was aimed at providing new insights into the possible ameliorative role of both BO and SO as a new herbal therapy against DZN-induced tissue toxicity in rats via assessment of alterations in biochemical parameters, oxidative/antioxidant indices, inflammatory and immuno-modulatory markers, as well as immunohistochemical localization of TNF-α in the examined organs.

Materials and methods

Chemicals, natural products, and reagents

Diazinon-60® was applied as a commercial emulsifiable concentrate containing 60% active ingredient obtained from fertilizers and agricultural pesticides shop (High control company, Cairo, Egypt). It was dissolved in distilled water to obtain the required dose concentration according to Sarhan and Al-Sahhaf (2011). Basil and sesame seed oils were purchased from local market of herbs and medicinal plants (Al-Hedaia, company for natural oils and herbal cosmetics, El-Mahala El-Kobra, El-Gharbia, Egypt). All diagnostic kits were obtained from Biodiagnostic Company (Cairo, Egypt) except kits for testosterone (Cat. number: MBS282195), tumor necrosis factor alpha (TNF-α) (Cat. number: MBS355371), and interleukin 10 (IL-10) (Cat. number: MBS355232) were obtained from MyBioSource Inc. (CA, USA).

Gas chromatography–mass spectrometry (GC-MS) analysis

The phytochemical compositions of both BO and SO were performed using Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 μm

film thickness). The column oven temperature was initially held at 50 °C, then increased by 5 °C/min to 250 °C hold for 2 min, and increased to the final temperature 300 °C by 30 °C/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260 °C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 μl were injected automatically using Autosampler (AS1300) coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times (RT) and mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

Animals model

A total of forty apparently healthy adult male albino rats, *Rattus norvegicus*, (6–8 weeks old), whose mean weight range 150–200 g, were housed in separate well-ventilated stainless-steel cages in fully ventilated room under standard conditions with 12-h light/dark cycle, and the temperature of 25 °C ± 2 °C with relative humidity (50 ± 10). The rats were subjected to free access to drinking water and standard pellet diet given ad libitum until the start of the experiment. Ration was formulated according to National Research Council (1979).

Animal groups and treatment schedule

After 15 days' acclimation, the animals were randomly distributed into four experimental groups ($n = 10$/group) housed in a separate cage: control group, DZN-treated group, DZN plus basil oil-treated group, and DZN plus sesame oil-treated group. Rats of control group were administrated distilled water daily. Rats of DZN-treated group were orally treated with sublethal dose of DZN (10 mg/kg b.w/day) daily for 4 weeks by stomach tube and served as DZN group (Kalender et al. 2006; Karimani et al. 2018). LD50 was 400 mg/kg b.w according to Gokcimen et al. (2007). Rats of DZN plus basil oil-treated group were daily exposed to co-administration of DZN (with dose as DZN group) and basil seed oil (5 ml/kg b.w/day) by stomach tube for a period of 4 weeks and served as DZN + BO group (Farghali et al. 2014). Rats of DZN plus sesame oil-treated group were daily exposed to co-administration of DZN (with dose as DZN group) and sesame seed oil (8 ml/kg b.w/day) by stomach tube also for the aforementioned period of time and served as DZN + SO group (Hsu et al. 2004; Chandrasekaran et al. 2014).

Serum and tissue sampling

At the end of the experimental period, blood samples were individually collected in plain tubes from retro-orbital venous plexus of each rat in all experimental groups. The blood was left for 10 min in a slanted position to coagulate. Sera were separated from non-heparinized blood by centrifugation at 5000 rpm for 10 min followed by storing in deep freeze at − 20 °C until further use for different biochemical analysis.

After blood collection, animals were immediately decapitated under light anesthesia (ketamine-xylazine mixture, 0.15 ml/100 g BW/IP). Hepatic, renal, testicular, and epididymal tissue samples were freshly excised and perfused with cold saline to exclude any blood, then blotted on filter paper, and then quickly fragmented after removing of any adhering blood or fat tissues. Using an electrical homogenizer, tissue specimens were homogenized in potassium phosphate buffers (5 ml buffer of PH 7.4/g tissue). Tissue homogenates were centrifugated at 5000 rpm for 10 min at 4 °C, and then the resultant supernatant was isolated and then quickly preserved at − 20 °C until further use assessment of oxidative and antioxidant parameters (Hamza et al. 2014).

Serum biochemical measurement

Sera were used for assessment of hepatic, renal, and testicular injury biomarkers; ALT, AST, BUN, Cr and testosterone. ALT and AST were assessed according to Bergmeyer et al. (1978). BUN, Cr, and testosterone were measured as described by Chancy and Marbach (1962), Cook (1971), and Tietz (1995), respectively.

Evaluation of oxidative/ antioxidant indices

The stored tissue homogenates were used for measurement of MDA, SOD, CAT, and Gpx. Those parameters were measured as described by the method of Ohkawa et al. (1979); Nishikimi et al. (1972); Aebi (1983); and Paglia and Valentine (1967), respectively.

Assessment of the inflammatory and immune-modulatory markers

Serum TNF-α and IL-10 were assayed using ELISA kits as described by the manufacture instruction.

Immunolocalization of TNF-α

Small tissue specimens from the liver, kidney, testis, and epididymis from each rat in all experimental groups were obtained for immunolocalization of TNF-α using a streptavidin-biotin-peroxidase complex method. The obtained samples were rapidly immersed in 4% paraformaldehyde and then

subjected to the routine histological procedures according to Bancroft et al. (2013). The paraffin embedded samples were cut into 5-μm sections with a microtome. The obtained paraffin sections were deparaffinized in xylene and then rehydrated through descending series of ethanol. Blocking of endogenous peroxidase activity was achieved by incubation of sections into 3% H_2O_2 in methanol for 10 min. Sections were heated at 90 °C with citrate buffer pH 6. The tissue sections were washed with phosphate buffer saline (PBS) and then subjected to blocking buffer (10% bovine serum albumin) for 2 h at room temperature to prevent nonspecific binding of the primary antibody. The sections were then incubated with diluted primary antibody (rabbit polyclonal anti-TNF-α, cat. Number AP20373PU-N, Acris, Germany, at 1:200 dilution) for 1 h. Negative control slides were, in parallel, incubated with 10% normal rabbit serum/PBS. After incubation, sections were washed in PBS and then incubated with conjugated goat anti-rabbit biotinylated immunoglobulin G (IgG) (cat. Number TA130016, Acris, Germany) for 10 mins at room temperature, followed by peroxidase-labeled streptavidin incubation. The reaction was visualized as brown-colored precipitates at the antigen sites, using DAB-nickel revelation. Cellular nuclei were counterstained with Harry's hematoxylin. Stained sections were dehydrated, cleared, mounted, and cover slipped. TNF-α immunoreactivity was detected according to Skondras et al. (2015).

Each slide was individually evaluated and scored in a blinded fashion according to Vermeirsch et al. (2002). At least 10 high-power fields were chosen randomly from each section using a light microscope at a high magnification of × 400. The positive cells were given proportional score (PS) from 0 to 5 for no positive cells to > 65% positive cells, respectively. Moreover, the cells were given intensity score (IS) from 0 to 4 for no staining to very strong staining, respectively (Table 1). The mean ± standard error (SE) total score (TS) was calculated by summation of IS to PS. The total immunostaining was scored at 1–3, 4–6, and 7–9 representing weak, moderate, and strong grades, respectively.

Table 1 Immunohistochemical grading scores

Proportional score (PS)	Intensity score (IS)
Score 0, no positive cells	Score 0, no staining
Score 1, < 1% positive cells	Score 1, weak staining
Score 2, 1–9% positive cells	Score 2, moderate staining
Score 3, 10–32% positive cells	Score 3, strong staining
Score 4, 33–65% positive cells	Score 4, very strong staining
Score 5, > 65% positive cells	

Statistical analysis

The statistical analysis was performed using SPSS (SPSS Inc., Chicago, Illinois, USA). The normally distributed data was analyzed using the parametric test of ANOVA "one-way ANOVA"; meanwhile, the scored data regarding IHC that is not normally distributed was analyzed by Kruskal-Wallis, the nonparametric test of ANOVA, to examine the significance among the studied groups. P values < 0.05 were regarded as significant.

Result

Phytochemical compounds identified in both BO and SO using GC-MS analysis

As shown in Table 2, the GC-MS analysis of BO extract revealed eight distinct peaks at 6.99, 8.92, 11.57, 17.07, 31.29, 32.85, 32.96, and 34.37 m/z. The most intense peak was identified as estragole (53.85%) followed by oleic acid (8.53%), oleic acid, (Z)-, TMS derivative (6.27%), palmitic acid (4.98%), linoelaidic acid (3.45%), methyl eugenol (2.18%), eucalyptol (1.84%), and linalool (1.41%). On the other hand, Table 3 for the GC-MS analysis of the SO extract revealed nine distinct peaks at 12.79, 19.71, 19.80, 23.09, 23.72, 25.10, 28.88, 31.29, and 34.37 m/z. The most intense peaks were identified as bisabolol oxide A (15.12%), and then followed by both carveol and farnesene epoxide, E (8.23%), bisabolol oxide B (6.56%), carvone (3.31%), palmitic acid (2.64%), oleic acid, (Z)-, TMS derivative (2.62%), cyclopropane dodecanoic acid (2.58%), and linolenic acid (1.57%).

ALT and AST activities

Regarding liver enzymes, ALT and AST activities were significantly increased within DZN group compared with control one. On the other hand, there were significant decreases in their activities in both DZN + BO and DZN + SO compared with DZN-treated rats. Meanwhile, DZN + BO group showed a significant decrease in ALT and AST activities compared with DZN + SO group. However, ALT and AST showed significant elevation in their activities in both DZN + BO and DZN + SO compared with control group. (Fig. 1).

BUN and Cr levels

As shown in Fig. 2, significant increases in both BUN and Cr levels were noticed among rats treated with DZN. On the other

Table 2 Gas chromatography-mass spectrometry analysis of BO extract

RT (min)	Compound name	Peak area %	MF	MW	Cas #	Library
6.99	Eucalyptol	1.84	931	154	470–82–6	Replib
8.92	Linalool	1.41	882	154	78–70-6	Replib
11.57	Estragole	53.85	968	148	140–67–0	Mainlib
17.07	Methyl eugenol	2.18	934	178	93–15-2	Replib
31.29	Palmitic Acid	4.98	866	328	55,520–89–3	Replib
32.85	Linoelaidic acid	3.45	929	280	506–21–8	Mainlib
32.96	Oleic Acid	8.53	915	282	112–80–1	Mainlib
34.37	Oleic Acid, (Z)-, TMS derivative	6.27	915	354	21,556–26–3	Replib

RT, retention time; *MF*, matching factor; *MW*, molecular weight

hand, both DZN + BO and DZN + SO ameliorated the level of these parameters close to the control group. Additionally, significant decreases in BUN and Cr levels were noted in DZN + BO compared with DZN + SO.

Testosterone level

DZN-treated rats showed a significant decrease in testosterone level compared with control group. Meanwhile, both DZN + BO and DZN + SO groups revealed significant elevation in the levels of testosterone when compared with DZN group. DZN + BO group revealed a significant increase in testosterone comparing with that of DZN + SO but they do not reach the control level (Fig. 3).

Inflammatory and immunomodulatory markers

Pointing to TNF-α and IL-10 levels, there is a significant increase in TNF-α with a significant decline in IL-10 levels in DZN group compared with control one, while both DZN + BO and DZN + SO groups showed a significant decline in TNF-α with a significant increase in IL-10 when compared with DZN-treated rats. DZN + BO group noted a significant decrease in TNF-α with a significant increase in IL-10 compared with DZN + SO group (Fig. 4).

Oxidative and antioxidant indices

DZN group revealed a significant increase in MDA with significant decreases in SOD, Gpx, and CAT activities compared

Table 3 Gas chromatography-mass spectrometry analysis of SO extract

RT (min)	Compound name	Peak area %	MF	MW	Cas #	Library
12.79	Carvone	3.31	781	150	99–49-0	Replib
19.71	Farnesene epoxide, E	8.23	765	220	83,637–40–5	Mainlib
19.80	Carveol	8.23	717	152	99–48-9	Mainlib
23.09	Bisabolol oxide B	6.56	818	238	NA	Mainlib
23.72	Linolenic acid	1.57	636	262	NA	Mainlib
25.10	Bisabolol oxide A	15.12	843	238	22,567–36–8	Replib
28.88	Cyclopropane dodecanoic acid	2.58	645	366	10,152–65–5	Mainlib
31.29	Palmitic acid	2.64	644	328	55,520–89–3	Replib
34. 37	Oleic acid, (Z)-, TMS derivative	2.62	717	354	21,556–26–3	Replib

RT, retention time; *MF*, matching factor; *MW*, molecular weight

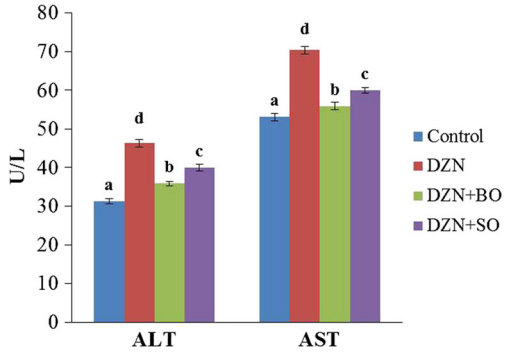

Fig. 1 Mean ± SE of ALT and AST activities in all experimental groups

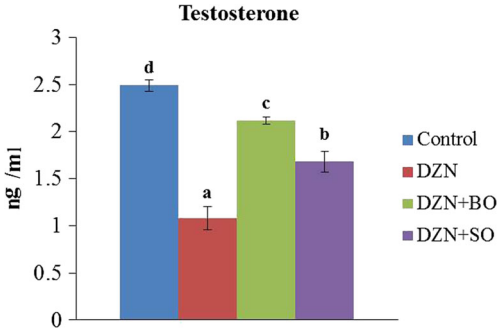

Fig. 3 Mean ± SE levels of testosterone in all experimental groups

with control group. Meanwhile, both DZN + BO and DZN + SO groups showed significant declines in MDA levels with significant elevations in SOD, Gpx, and CAT activities when compared with DZN-treated group. A decline in MDA level and increase in SOD, CAT, and Gpx activities were noted in DZN + BO group compared with DZN + SO group. However, DZN + BO- and DZN + SO-treated groups showed significant elevation in MDA level with significant decline in SOD, Gpx, and CAT activities when compared with control group (Fig. 5).

TNF-α immunolocalization

With regard to immunohistochemical assays, tissues of negative control sections did not reveal any detectable TNF-α immunostaining. Generally, the microscopic appearance of all examined tissues sections obtained from control rats' group demonstrated low TNF-α immunoexpression compared with DZN group that revealed significant increases in TNF-α immunolocalization (Figs. 6, 7, 8, 9).

TNF-α immuno-stained liver of control group showed weak staining with low detected intracytoplasmic TNF-α-positive cells (Fig. 6A). DZN group showed a significant strong intracytoplasmic TNF-α expression in the hepatocytes specially those surrounding the central vein when compared with control rats (Fig. 6B). DZN + BO group showed a significant lower expression of hepatocytic TNF-α in comparison with DZN group (Fig. 6C). Otherwise, DZN + SO group showed moderate expression for TNF-α which appeared significantly higher than DZN + BO group (Fig. 6D).

The renal tissues of control rats showed low glomerular and tubular TNF-α positive staining (Fig. 7A). Meanwhile, in DZN-treated rats, significantly strong TNF-α immunostaining was noticed in both renal glomeruli and tubular epithelial cells (Fig. 7B). However, significant low to moderate TNF-α immunoreactions in the glomeruli and tubular epithelium of DZN + BO group were observed in comparison with DZN group (Fig. 7C). Occasionally, the renal tissue of DZN + SO group exhibited moderate TNF-α immunoreactivity (Fig. 7D).

Testicular tissues of control group revealed no detectable TNF-α immunostaining within the seminiferous tubular cell lining, while a relatively weak interstitial TNF-α immunoreactivity was noted among these groups (Fig. 8A). On the other hand, a significantly strong expression of TNF-α protein was detected in spermatogenic cells of both DZN and DZN + SO groups (Fig. 8B, D). However, the spermatogenic cells of DZN + BO group revealed a significant low expression of TNF-α in comparison with DZN and DZN + SO groups (Fig. 8C).

Current immunohistochemical staining results in epididymal epithelial cells of control group showed no detectable TNF-α reactivity, while a weak TNF-α immunostaining was noticed in the interstitial tissues (Fig. 9A). The expression of

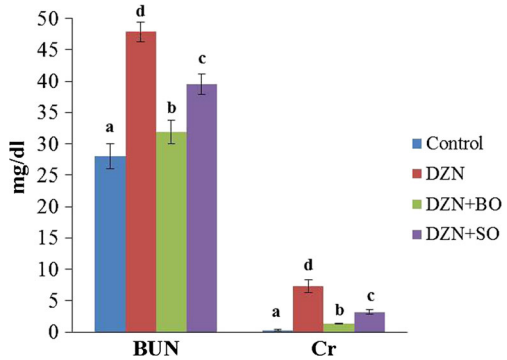

Fig. 2 Mean ± SE of BUN and Cr levels in all experimental groups

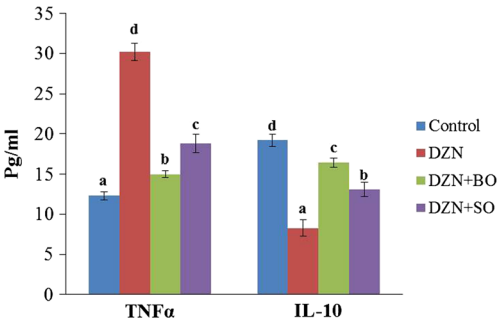

Fig. 4 Mean ± SE levels of TNF-α and IL-10 in all experimental groups

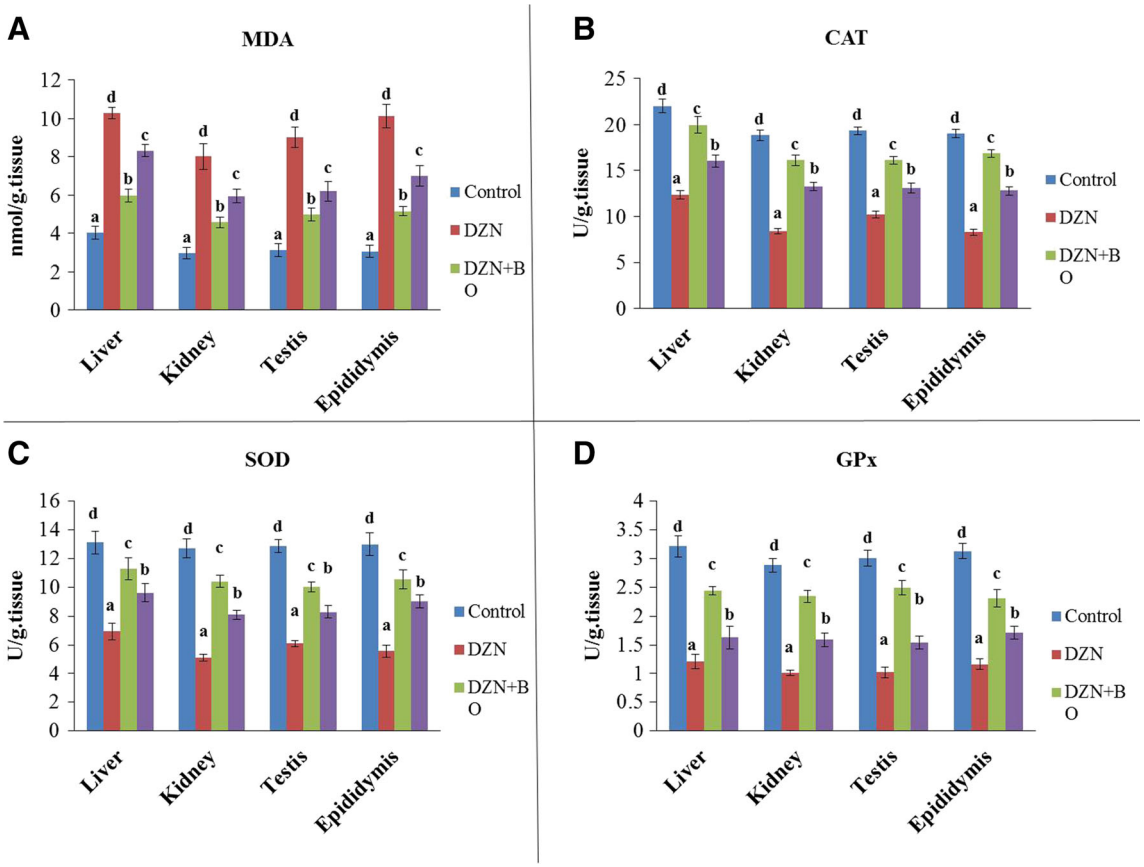

Fig. 5 Mean ± SE of MDA levels (**A**), CAT (**B**), SOD (**C**), and GPx (**D**) activities in different tissues homogenates

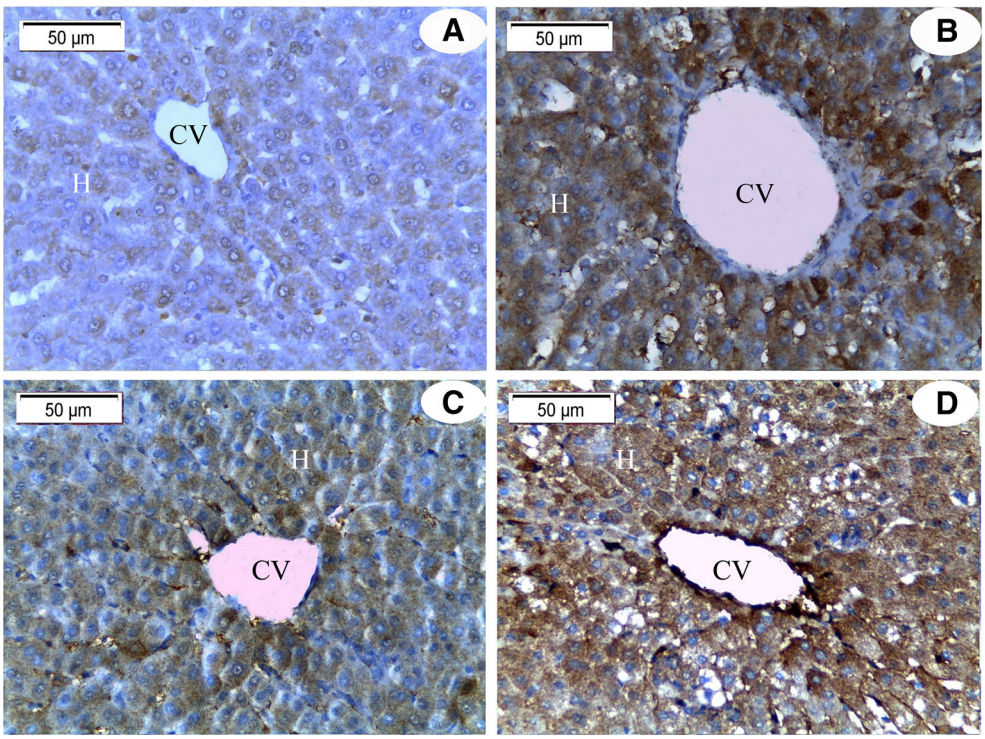

Fig. 6 Immunoperoxidase-stained paraffin hepatic sections for TNF-α. **A** Control group showed weak staining with low detected intracytoplasmic TNF-α-positive cells. **B** DZN group revealed a significant strong intracytoplasmic TNF-α expression in the hepatocytes (H) specially those surrounding the central vein (CV). **C** DZN + BO group showed a significant lower expression of hepatocytic TNF-α compared with DZN group. **D** DZN + SO group showed moderated TNF-α expression

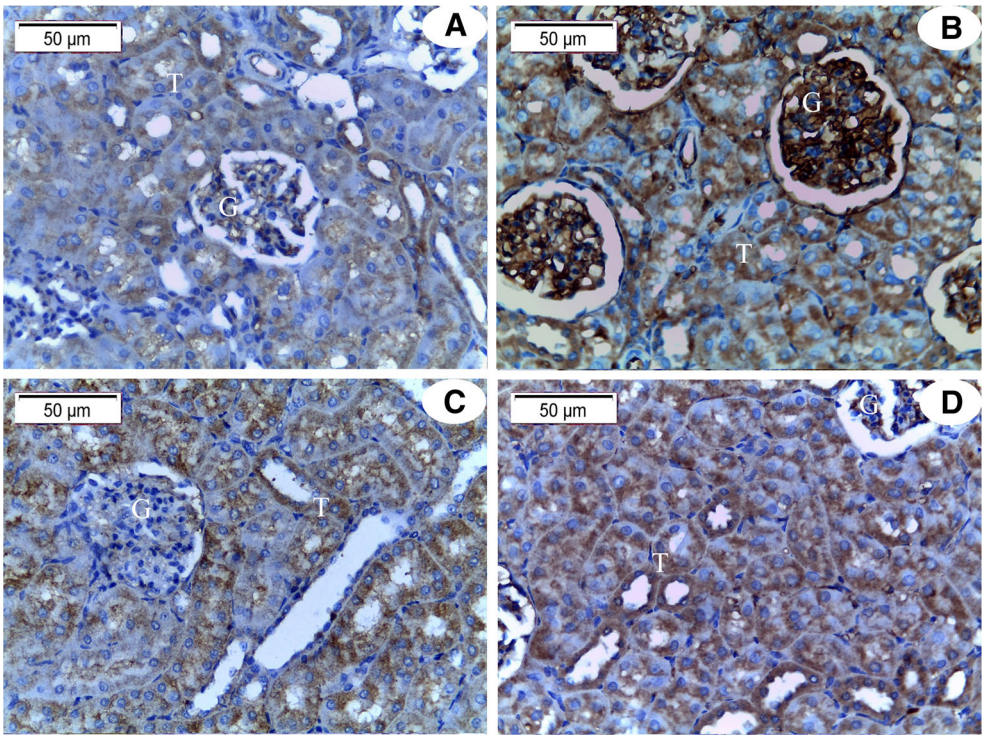

Fig. 7 Immunoperoxidase-stained paraffin renal sections for TNF-α. **A** Control rats showed low glomerular (G) and tubular (T) TNF-α positive staining. **B** DZN-treated rats revealed a significant strong TNF-α immunostaining in both renal glomeruli and tubular epithelial cells. **C** Low to moderate TNF-α immunoreactions was observed in the renal tissue of DZN + BO group. **D** DZN + SO group exhibited moderate TNF-α immunoreactivity

TNF-α was significantly strong in epididymal epithelial cells of DZN-treated rats (Fig. 9B). A distinctly low TNF-α positive immunostaining was recorded in the epididymal epithelial cells of DZN + BO group in comparison with DZN group (Fig. 9C). Additionally, compared with DZN group, DZN + SO displayed a moderate TNF-α immunoreaction (Fig. 9D).

Immunohistochemical investigations showed that the TS of TNF-α immunostainings in the examined tissues obtained from control, DZN + BO, and DZN + SO groups was

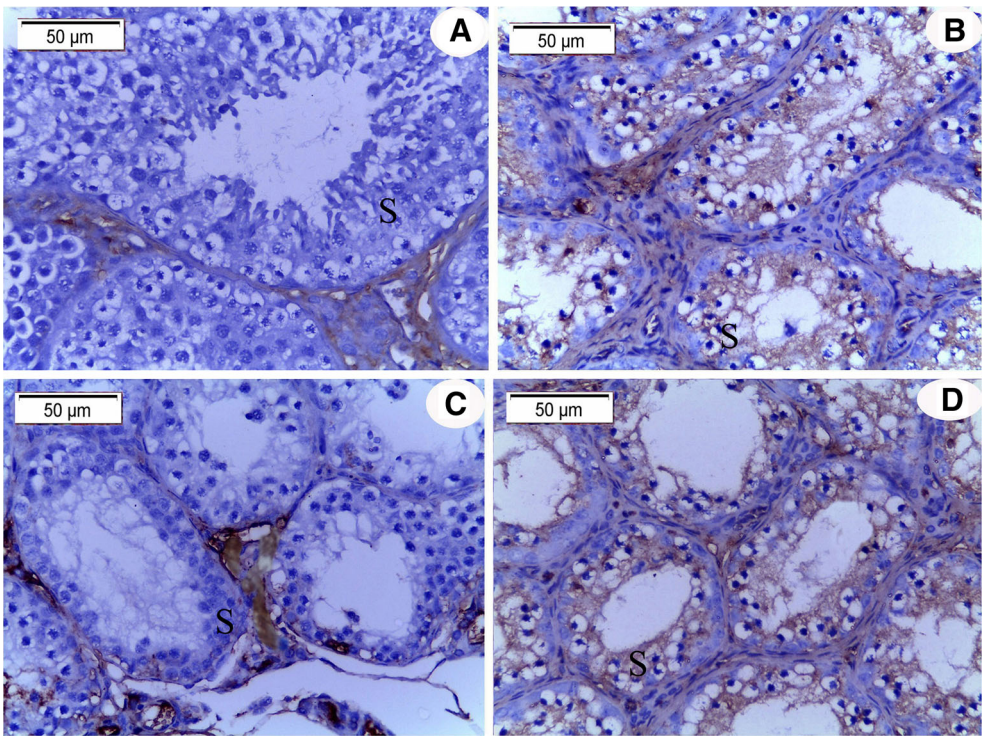

Fig. 8 Immunoperoxidase-stained paraffin testicular sections for TNF-α. **A** Control group revealed no detectable TNF-α immunostaining within the seminiferous tubular cell lining along with a relatively weak interstitial TNF-α immunoreactivity. A significantly strong expression of TNF-α proteins was detected in spermatogenic cells (S) of both DZN **B** and DZN + SO **D** groups. **C** The spermatogenic cells of DZN + BO group revealed a significant low expression of TNF-α in comparison with DZN and DZN + SO groups

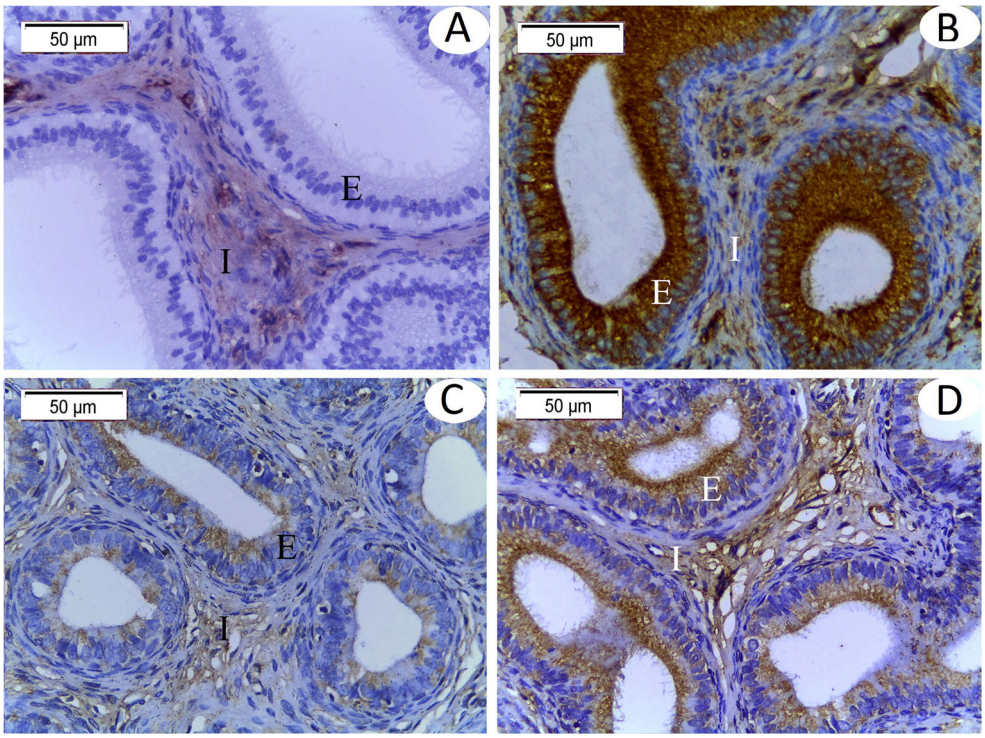

Fig. 9 Immunoperoxidase-stained paraffin epididymal sections for TNF-α. **A** Control group showed no detectable TNF-α reactivity in epididymal epithelial cells **e**, while a weak TNF-α immunostaining was noticed in the interstitial tissues (I). **B** The expression of TNF-α was significantly overexpressed in epididymal epithelial cells of DZN-treated rats. **C** A distinctly low-level TNF-α positive immunostaining was recorded in the epididymal epithelial cells and the interstitial tissues of DZN + BO group compared with DZN group. **D** DZN + SO displayed a moderate TNF-α immunoreaction

significantly ($P < 0.05$) lower than those values in the DZN-treated rats' group (Fig. 10).

Discussion

Within mammalian body, ROS are generated as a consequence of exposure to xenobiotics, exogenous drugs, and/or endogenous metabolic products. The deleterious outcomes of ROS arise due to the imbalance between the formation and inactivation of those species leading to irregularity of the cell structure (Sun 1990). DZN, widely used OPI in veterinary practice and industrial agriculture worldwide, has some serious effects and can probably cause an exposure risk to living organisms and workers in this field. It was puzzled out that DZN-toxicity depends on FR and ROS. The oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance toward a lower oxidative stress status (Abbas 2014; Abdeen et al. 2017).

Although several studies approximately reported the toxicity of DZN, there is limited available information regarding the comparative immunomodulatory efficacy of BO and SO, as natural products, on DZN-mediated metabolic disorders in the livers, kidneys, testis, and epididymis. Hence, the present study was aimed to provide new insights into the possible ameliorative effect of both basil and sesame seed oils as a herbal therapy for prevention of such toxicity and mechanism in their ameliorative action. In the current study, DZN-treated tars revealed significant elevations in the ALT and AST activities with increased levels of BUN and Cr when compared with control group. ALT and AST are very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular membrane is damaged and/or the permeability of hepatocyte increased, the enzymes normally present in the cytosol and released into the blood stream (Kuriakose and Kurup 2010). The obtained results regarding ALT and AST were in agreement with (Al-Attar et al. 2017). BUN and Cr, which are waste products of protein metabolism, are needed to be excreted by the kidney; therefore, the marked increase of these parameters confirms condition of renal functional damage (Panda 1999). The present results were agreed with Al-Attar (2015) who demonstrated that DZN-treated rats display a pronounced impairment in renal function which is confirmed by the increase of serum BUN and Cr levels. These elevations may indicate impairment and hypofunction of the liver and kidneys induced by DZN (Cakici and Akat 2013; Mansour et al. 2017) which resulted from absorption of DZN by the kidneys upon entering the body. It can be oxidized by hepatic microsomal enzymes, producing acetylcholine esterase inhibitors such as hydroxy DZN, diazoxon, and hydroxydiazoxon (Enan et al. 1987) that influence the transportation of materials across mitochondrial membrane and disturbs the activity of p450 cytochrome system in hepatocytes, and releases FR by attacking to intracellular elements (Nakagawa and Moore 1999; Kappers et al. 2001; Sams et al. 2003). Additionally, DZN exposure leads into male reproductive toxicity via decreasing spermatogenesis rate and androgen levels (Damodar et al. 2012). Our results revealed a

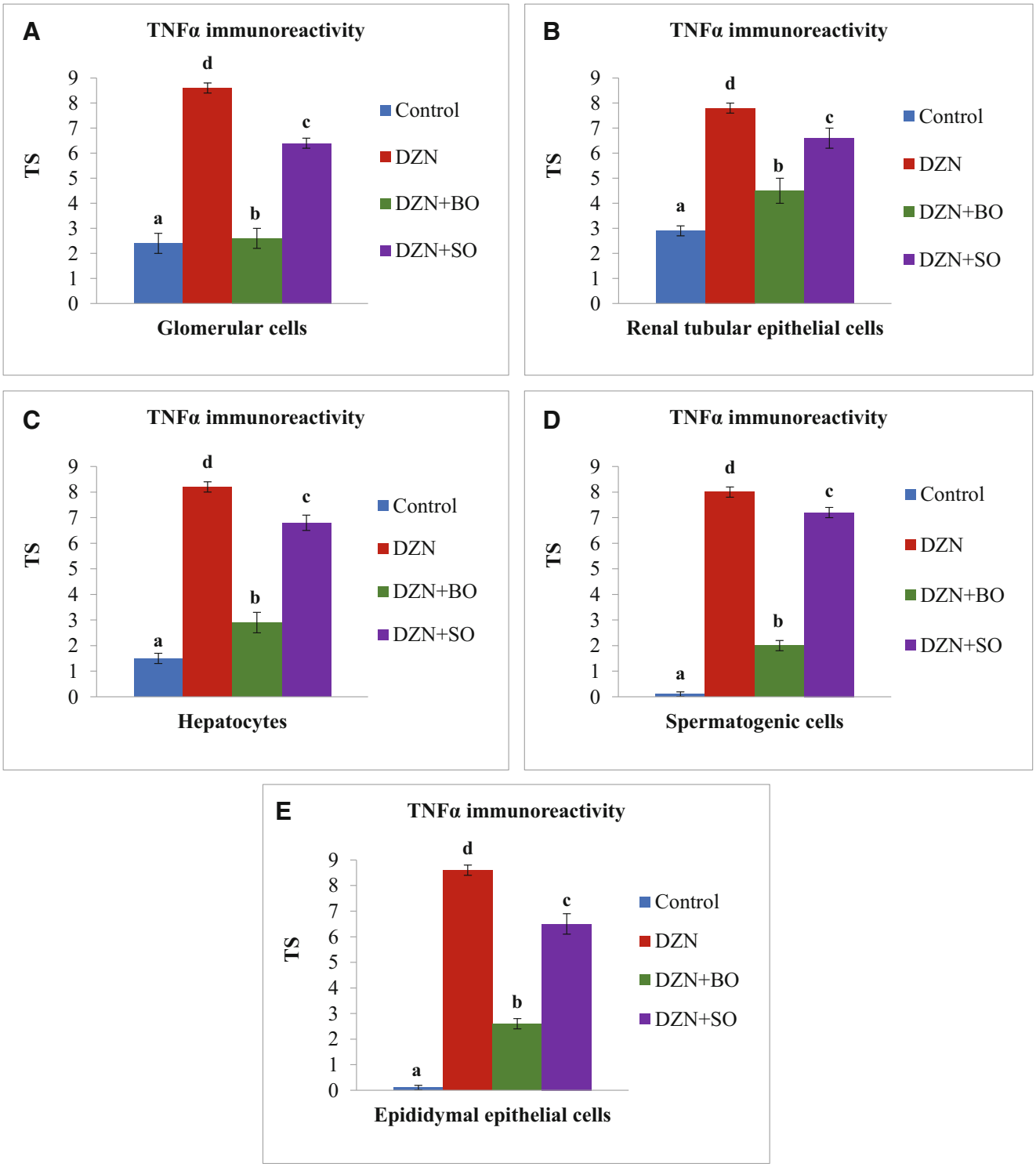

Fig. 10 Mean ± SE total score (TS) of TNF-α immunostainings in, renal (**A** and **B**), hepatic (**C**), testicular (**D**), and epididymal (**E**) tissues. TS of TNF-α immunostainings control, DZN + BO and DZN + SO groups were significantly ($P < 0.05$) lower than those values in the DZN-treated rats' group

significant decline in testosterone level within DZN group, that may be attributing to the adversely effect of DZN on testicular cell, decreased number of spermatogonia available for the production of sperm, as well as the sloughing of immature developing cells (Damodar et al. 2012). Concerning oxidative/antioxidant parameters, there were significant increase in MDA with significant decrease in SOD, Gpx, and CAT activities within DZN-exposed rats. These findings were consistent with previous investigations (Abbassy et al. 2014; Mohamed and Ali 2014; Beydilli et al. 2015; Eraslan et al.

2015). Metabolic oxidation of DZN induces oxidative stress and lipid peroxidation in the different tissues via FR and ROS production as well as depletion of antioxidants scavenging enzymes (Altuntas et al. 2004; Ogutcu et al. 2006; Rahimi and Abdollahi 2007; Hariri et al. 2010; El-Demerdash and Nasr 2014). TNF-α and IL-10 are extremely potent proinflammatory and anti-inflammatory molecules that can mediate acute inflammation (Feghali and Wright 1997). The present finding indicates that DZN treatment causes significant increase in TNF-α with a significant decline in IL-10 levels.

The present study demonstrates that SO and BO individually can counteract the toxic effects of DZN, significantly attenuating the oxidative destruction of tissue damage induction and revealed an enhancement in the biochemical, oxidative/antioxidants and immunological alterations induced by DZN intoxication. The GC-MS analysis of BO extract indicated that the phytochemical compounds of BO are mainly phenols like estragole and methyl eugenol, fatty acids like oleic acid, palmitic acid and linoelaidic acid, as well as aroma compound like eucalyptol. These findings are in the same line with previous reports about BO (Nour et al. 2009; Li et al. 2017). The identified compounds mainly both estragole and methyl eugenol have antioxidant (Chalchat and Özcan 2008) as well as anti-inflammatory properties (Rodrigues et al. 2016). However, the phytochemical compounds identified in SO extract using GC-MS analysis are mainly fatty acids like palmitic acid, oleic acid derivatives, cyclopropane, and linolenic acids. Similar data are reported in previous studies about SO (Elleuch et al. 2007; Carvalho et al. 2012; Gharby et al. 2017; Hou et al. 2019). Additionally, our results identified the presence of volatile terpenoids in SO such as carvone, carveol, farnesene epoxide E, and bisabolol which have antioxidant and anti-inflammatory effects (Kamatou and Viljoen 2010; Çelik et al. 2014). The Chemical structures of the most identified compounds mainly eugenol, bisabolol oxide A and B, and other essential oils in BO and SO have proven the antioxidant properties of the oils under study. These phytochemical compounds have hydroxyl groups (OH) with or without phenol rings, so they act as hydrogen atom donor to neutralize FR and ROS stopping the oxidation chain reaction (Abdeen et al. 2019). Moreover, Sharma et al. (2017) reported that both essential oils and eugenol can protect the cell membrane and mitochondrial membrane against lipid peroxidation via the incorporation into their lipid bilayer.

Significant decrease in ALT and AST activities as well as Cr and BUN levels was noted in DZN + SO and DZN + BO groups, respectively, that were in accordance with Al-Attar et al. (2017), Almalki (2019) and Yacout et al. (2012). Additionally, MDA levels were significantly decline along with significant elevation of SOD, Gpx, and CAT activities that went in harmony with Saleem et al. (2014) who reported that rat administrated SO improved the myocardial antioxidant enzymes, and with Almalki (2019) who also found that SO ameliorates MDA and CAT changes in malathion intoxication rats. This improvement in the biochemical picture in rats administrated SO might be due to its potent antioxidant effect (Yadav et al. 2016), attributing to richness of SO in sesamol and sesamin that possess many actions as antimutagenic, antioxidant, anti-and inflammatory (Sankar et al. 2005), as well as its content of the antioxidant vitamins C and E (Konan et al. 2008; Shittu et al. 2009). Moreover, Makwana and Rathore (2011) reported that basil leaf extract restored both liver and kidney functions in paracetamol-treated rats to its normal values. Lipid peroxidation was suppressed with the significant increases in the activities of SOD, CAT, and Gpx in DZN + BO group suggesting BO role in scavenging ROS and FR induced by DZN. This protective effect of BO owed to the antioxidant activity of its flavonoids (Sakr and Al-Amoudi 2012). Regarding to proinflammatory cytokines, SO diminished the level of TNF-α that agreed with (Yadav et al. 2016), and increased IL-10 level that was in consistent with Assaraj et al. (2018), that might be due to potential anti-inflammatory effect of SO (Shahidi et al. 2006). The relationship between oxidative stress and expression of TNF-α is due to the activation of both nuclear factor kappa B (NFκB) and p38 mitogen-activated protein kinase (MAP kinase) by the ROS, which stimulate the release of TNF-α (Bogoyevitch et al. 1996; Barnes and Karin 1997). This result confirmed that SO decreases the oxidative injury with subsequently inhibit TNF-α expression. BO showed a significant decline in TNF-α with a significant increase in IL-10 that was in consistent with Assaraj et al. (2018). Additionally, BO suppresses TNF-α level in rats express gastric ulcer (Farghali et al. 2014) which contributed to anti-inflammatory properties of BO (Mari et al. 2007). This indicates the effectiveness of BO in prevention of DZN toxicity. In our study, BO gave a significant ($P < 0.05$) better results than SO.

TNF-α is a key mediator of inflammatory tissue damage (Vielhauer and Mayadas 2007). Osawa et al. (2018) suggested that TNF-α is a regulator of hepatocyte apoptosis and proliferation. During liver inflammation, hepatocytes are exposed to high levels of TNF-α as well as oxidative stress causing hepatocyte cell death contributing to liver injuries (Wullaert et al. 2007). Strong expression of TNF-α in hepatocytes was reported in case of acute alcoholic hepatitis (Ohlinger et al. 1993). Moreover, TNF-α has a key role in the pathogenesis of acute and chronic renal disease (Vielhauer and Mayadas 2007). It is differentially expressed in both renal glomerular and tubular cells in response to ischemia and endotoxemia (Donnahoo et al. 2001) and also it is highly expressed in the nephropathic kidney of obese mice (Wang et al. 2017). Mammalian testis normally secretes TNF-α which regulates various processes of spermatogenesis and steroidogenesis (Singhal and Mills 2015). Under normal conditions, weak immunoreaction for TNF-α was found in the canine testes

and epididymis (Payan-Carreira et al. 2012). TNF-α is involved in the pathogenesis of testicular inflammation, triggers germ cell apoptosis (Suescun et al. 2003), and transiently disrupted blood testicular barrier (Li et al. 2006).

For great extent, our immunohistochemical findings in all examined tissues obtained from DZN-treated rats matched with the abovementioned literature indicating the mediator role of TNF-α in tissue damage mediator and inflammation. We demonstrated a strong TNF-α immunopositivity among all tissues of DZN-exposed rats; meanwhile, the protein immune-expressions were declined in both DZN + BO and DZN + SO groups. Therefore, our investigation provides evidence that DZN toxicity may induce inflammation and oxidative stress of tissues in DZN-treated rats. Contrary, basil and sesame seed oil possessed good antioxidant and anti-inflammatory activity against DZN-induced toxicity.

Conclusion

Considering the present finding, it can be concluded that the BO and SO supplementation is beneficial in lowering and improve the biochemical and immunohistochemical alterations induced by DZN intoxication in the liver, kidney, testis, and epididymis. However, the present results suggest the protective, antioxidant, and immunomodulatory effects of both BO and SO on DZN-induced toxicity; the most protective effects were recorded in rats treated with BO. Additionally, the using of BO as feed additive may be a good strategy against oxidative stress induced by DZN. To strengthen these findings, further investigations are needed to explore the mechanism action of BO and/or SO against DZN toxicity.

Author's contribution S.M. Farouk, F.A. Gad, and M.A. Emam contributed to the design and implementation of the research, to the analysis and to discussion of the results.

Data availability Not applicable.

Compliance with ethical standards

All rats used for this experiment were treated in accordance with the guidelines of the NIH (National Institutes of Health) for the Care and Use of Laboratory Animals. The research was considered and approved by Institutional Animal Cares and Use Committee Research Ethics Board at Faculty of Veterinary Medicine, Benha University, Egypt (IACUCREB permit number; BUFVTM 02-04-20).

Conflicts interests The authors declare that they have no conflict of interest..

Consent for publication Not applicable.

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