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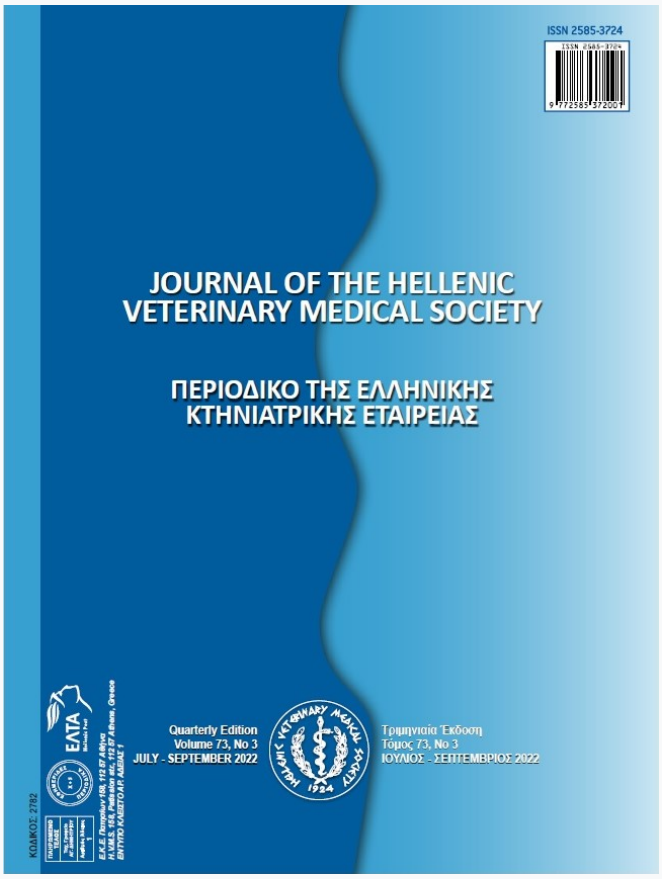

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Emamectin Benzoate-Induced Hepatotoxicity in Rats with Special Reference to Protective Potential of *Nigella sativa* Oil

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ABSTRACT: Extensive use of Emamectin benzoate as insecticide in the agriculture practices leads to continuous animal and human exposure causing adverse health effects. This study was designed to explore the hepatotoxicity of emamectin in male rats and the possible effect of *Nigella sativa* oil (NSO) in ameliorating this. Twenty-eight male rats were used in this study. They were divided into four groups, Control group: rats orally administered distilled water; NSO group: rats administered NSOorally; EMB group: rats administered emamectin benzoate orally; and EMB+NSO group: rats orally co-administered NSO with EMB, with the administrations being performed every other day for 6 weeks. Body weight was measured, **serum** alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were determined, and total protein and albumin levels were recorded. Histopathological examination of the liver was also performed, along with caspase-3 and TNF-α immunostaining of liver tissue. EMB treatment resulted in decreased body weight, while the co-administration of NSO modulated the EMB-induced alterations in body weight. There were also increases in the activities of serum ALT, AST, and ALP and decreases in total protein and albumin levels in the EMB group. Co-treatment with NSO significantly reduced serum ALT, AST, and ALP and improved total protein and albumin levels. Histopathological examination of the liver in the EMB group revealed the presence of different histopathological alterations that were improved by the co-administration of NSO. Immunostaining of caspase-3 and TNF-αin the liver revealed strong expression in the EMB-treated group. Meanwhile, the EMB+NSO group showed weak positivity for immunoreactive cells. NSO can ameliorate the hepatic emamectin toxic effects through improvement Serum liver function markers (ALT, AST and ALP),total protein, albumin levels, histopathological alterations, caspase-3 and TNF-α expression were analyzed.

***Keywords*:** Emamectin benzoate, Hepatotoxicity, Histopathology, Immunohistochemistry, *Nigella sativa* oil

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INTRODUCTION

Pesticide usage in agriculture is widespread and increasing, with major negative health outcomes on animal and humans' populations (Orabi et al., 2013). Pesticides are used to eliminate pests and insects that inflict damage on crops. Pesticides are beneficial to crops, but they have a significant negative influence on the ecosystem. Excessive pesticide use has the potential to destroy biodiversity. The survival of many birds, animals, and aquatic species, is threatened by hazardous chemicals (Mahmoodet al., 2016)

The extensive use of pesticides has contributed to environmental pollution and adverse health effects on human health(El-Ballalet al., 2019;Niuetal., 2020).

EMB works as an activator to GABA-gated chloride channel, increasing permeability of membrane chloride-ion and causing neuronal membrane depolarization, eventually inhibiting nerve impulse conduction, and causing irreversible paralysis, also Emamectin exposure poses a considerable risk to cause liver injury in rats by raising plasma transaminases(Khaldoun-Oularbi et al., 2016). Emamectin induced oxidative stress in many organs, which is caused by an increase in reactive oxygen species (ROS) generation and a decrease in intracellular antioxidants, EMB at a dose of 2.5 mg/kg body weight (BW) induced hepatic damage (El-Sheikh and Galal, 2015)

Emamectin benzoate is a macrocyclic lactone insecticide derived from the avermectin series of natural products. It is a mixture of at least 90% 4-deoxy-4-(methyl amino)-avermectin B1a benzoate (MAB1a or emamectin B1a benzoate) and at most 10% 4-deoxy-4-epimethyl-amino benzoate methyl amino avermectin B1b benzoate (MAB1b or emamectinB1b benzoate) salts.Emamectin is structurally similar to abamectin and ivermectin (Wolterinket al., 2012). Emamectin benzoate is highly effective against a wide range of pests and has been developed for use in various field crops and vegetables (Gacemi and Guenaoui, 2012). EMB is incompletely absorbed in the gastrointestinal tract of mammals. Contamination of human food by insecticides mostly occurs among farmers and agricultural workers (Litchfield, 2005). The European Medicines Agency(EMEA) has set the maximum residue limit for low-dose emamectin benzoate of 10-150 g/kg for food of animal origin, including aquaculture products(Hernando et al., 2007).

Liver is the body's principal metabolic organ. It synthesizes bile, which is important for fat absorption. Liver is also significant in hemoglobin catabolism, while also playing a major role in the metabolism of carbohydrates, fat, and proteins, along with xenobiotics(Campbell, 2006).The central role of the liver in drug metabolism predisposes it to toxic injury and drug toxicity. It has been reported that EMB at 2.5 mg/kg body weight (B.W.) induced liver damage in rat (El-Sheikh and Galal, 2015).

Herbal medicines derived from plant extracts are being increasingly used to treat a wide variety of diseases. Herbal products include chemically defined components that can protect the liver from various injuries (Negi et al., 2007).*Nigella sativa*, an annual plant that is part of the family Ranunculaceae, is abundant in many countries (Khazdair, 2015). The chemical components that make up its black seeds vary.Its major components are alkaloids, as well as fixed and volatile oils. The fixed oils include linoleic acid, oleic acid, and palmitic acid.

Thymoquinone, a volatile oil, is the most active constituent of *N. sativa*. *N. sativa* has many medicinal properties, including neuroprotective (Saleem et al., 2012), hepatoprotective (Pourbakhshet al., 2014), hypotensive and antidiabetic (Khan and Afzal, 2016), bronchodilatory, antibacterial, and anti-tumor (Gholamnezhadet al., 2016), anti-inflammatory (Boskabadyet al., 2011), and antioxidant properties(Gholamnezhadet al., 2015). The antioxidant properties due to thymoquinone can decrease oxidative stress and increase antioxidant defense in the body.

The present study was designed to evaluate the ameliorative effect of *N. sativa* oil (NSO) against EMB-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Ethical approval

This study was approved bythe Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City (VUSC-001-2-20).

Chemicals

Emamectin benzoate (T 20180803) was purchased from Hebei Veyong Biochemical Co., Ltd. (Shijiazhuang, China), and used in the form of anhydrous powder Tomguard 5%.The solution was prepared by dissolving the insecticide in distilled water. Diagnostic kits for assaying serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alka-

line phosphatase (ALP) were obtained from an Egyptian biotechnology company (S.A.E., Cairo, Egypt). Kits for immunohistochemistry for Caspase-3 were purchased from Abcam (Cambridge, UK) and for TNF-α from Celltech, Ltd.(UK).

***Nigella sativa* oil**

NSO was obtained from the plant oil extraction department, National Research Center, Dokki-Giza, Egypt. The extraction of oil was carried out by hydrolytic pressure using the cold press method. The oil was kept in a dark container at 4°C until use.Analysis of the chemical constituents of NSO was conducted in our previously reported study (Madkouret al., 2021) using a trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column (TG-5MS; 30 m x 0.25 mm x 0.25 μm film thickness).

Experimental animals

Twenty-eight adult male Wistar albino rats weighing 110-120 g were used in this experiment. The rats were obtained from Al-Zyade Experimental Animals Production Center (Giza, Egypt). They were housed in standard cages kept in a ventilated room under controlled laboratory conditions of a normal light-dark cycle (12 h light/dark) and temperature (25°C ± 2°C). Standard laboratory chow (Atmida, Egypt) and water were provided ad libitum. The animals were allowed to acclimate for a week before commencement of the study. The experimental protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City (approval number VUSC-001-2-20).

Experimental design

The experimental rats were randomly and equally allocated into four groups (7 rats each): Control group (G1): rats orally administered distilled water by gastric gavage every other day for 6 weeks; NSO group (G2): rats orally administered NSO at a dose of 3 ml/Kg B.W. every other day by gastric gavage for 6 weeks; EMB group (G3): rats orally administered EMB at a dose of 9 mg/Kg B.W. by gastric gavage every other day for 6 weeks; and EMB+NSO group (G4): rats orally co-administered NSO (3 ml/Kg B.W.) together with EMB (9 mg/Kg B.W.) for 6 weeks. NSO was administered 30 min prior to EMB administration. The selected dose of EMB was one-tenth of the oral LD_{50} of EMB (89 mg/Kg B.W.) in rats, in accordance with the work of Wang et al. (2012). The dose of NSO was selected in line with that of Danladi et al.(2013). B.W. was recorded weekly throughout the experimental period (6 weeks).

Sampling

At the end of the experimental period (6 weeks), rats were fasted overnightand euthanized 24 h after the last treatment.Blood samples were collected from the retro-orbital venous plexus in sterile centrifuge tubes, left to clot, and then centrifuged at 3000 rpm for 15 min to separate sera. Serum aliquots were stored at -20°C for further biochemical analysis. The liver from each rat was immediately removed and fixed in neutral buffered formalin for further histopathological and immunohistochemical analyses.

Evaluation of biochemical parameters

Serum alanine aminotransferase (ALT or GPT), aspartate aminotransferase (AST or GOT),and ALPactivities were measured, and total protein and albumin levels were also recorded colorimetrically **(**Reitman and Frankel, 1957**)**using commercially available kits (MDSS GmbH, Hannover, Germany), in accordance with the manufacturer's instructions.

Histopathological examination

Tissue specimens from the liver were collected from the different experimental groups, fixed in 10% neutral buffered formalin, washed, dehydrated, cleared, and embedded in paraffin. The paraffin-embedded blocks were sectioned at 5 μm thickness and stained with hematoxylin and eosin **(**Bancroft and Gamble, 2008**)** for histopathological examination. Stained sections were examined by a light microscope (BX50; Olympus, Japan).

Histopathological lesion scoring

Histopathological alterations were recorded and scored as follows: no change (0), mild (1), moderate (2), and severe (3) changes. The grading was determined based on percentages as follows: <30% change (mild change), <30%-50% (moderate change), and >50% (severe change)**(**Koranyet al., 2019;Azouzand Korany,2021**).**

Immunohistochemistry

Liver tissue sections were deparaffinized in xylene and rehydrated in graded alcohol. Hydrogen Peroxide Block (Thermo Scientific, USA) was added to block the endogenous peroxidase activity. Antigen retrieval was performed with pretreated tissue sec-

tions with 10 mM citrate in a microwave oven for 10 min. Sections were incubated for 2 h with one of the following primary antibodies: rabbit anti-caspase-3 (diluted to 1:1000; Abcam, Ltd.) and TNF-α (dilution 1/100;Celltech Ltd., UK). The sections were rinsed with PBS and then incubated with goat anti-rabbit IgG H&L (HRP) (ab205718; Abcam, Cambridge, UK) for 10 min. The sections were rinsed again with PBS. Finally, the sections were incubated with 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma). The slides were counterstained with hematoxylin and then mounted. Primary antibodies were replaced by PBS for negative controls.

Evaluation of caspase-3 and TNF-α immunostaining

The immunoreactivity of caspase-3 and TNF-α was quantitatively evaluated in the liver sections.In each group, five liver sections were examined. Immunoreactivity was analyzed in 10 microscopic fields per section under a high-power microscope (x400). The percentage of positively stained cells was estimated by color deconvolution Image J 1.52 p software (Wayne Rasband, National Institutes of Health USA).

Statistical analysis

All results are expressed as mean ± standard error of mean (SEM).Statistical analyses were performed using SPSS version 24.0 statistical analysis package (SPSS, Inc., Chicago, IL, USA). The parametric test one-way ANOVA was used for data analysis and comparison was performed using Tukey's posthoc test. In all calculations, a difference of $P < 0.05$ was considered significant according to Snedecor and Cochran (1967).

RESULTS

Evaluation of body weight

Table 1 that showed that EMB intoxication significantly decreased the B.W. from 2nd week to 6th week compared with that in the control rats.The NSO group showed no significant changes in B.W. compared with the control group,The group treated with EMB+NSO also showed no changes in B.W. in comparison with the control group.

Liver function biomarkers

The EMB-treated group (G3) showed significant increases inthe activities of serum ALT, AST, and ALP compared with the control group, while it exhibited significant reductions in total protein and albumin levels. The co-administration of NSO (G4) significantly reduced the elevated levels of serum ALT, AST, and ALP, concurrently with significant increases in serum total protein and albumin levels. On the other hand, NSO alone had no effects on these tested parameters compared with those in the control group (Table 2).

Table 1: Effect of EMB and/or NSO on body weight (g) of the rats in different weeks

Group / Week	Control	NSO	EMB	EMB withNSO
1stweek	101.5 ± 0.86[a]	105.6±2.38 [a]	100±0.412[a]	100.6 ± 1.5[a]
2nd week	152±3.58[a]	141.4 ± 4.24 [ab]	132.5 ± 4.79[b]	145.2 ± 3.06[ab]
3rd week	187± 4.02 [a]	176 ± 5.43 [a]	153 ± 3.85 [b]	183.8±2.48[a]
4th week	223.5 ±4.86[a]	219 ± 4.01 [a]	159.75 ± 7.64[b]	217± 3.83 [a]
5th week	242.25± 3.54[a]	230 ± 6.26 [ab]	145 ± 12.33 [c]	210.2± 4.08 [b]
6th week	240.5± 9.46 [a]	231.2± 10.63[ab]	137.5± 11.27 [c]	210.8 ±2.62 [b]

Values are expressed as means ± SEM. Data with different letters at the same row are significantly different at $p < 0.05$.

Table 2: Effect of EMB and/or NSO on liver function biomarkers in rats

Group / Parameters	Control	NSO	EMB	EMB withNSO
ALT (U/L)	15.2 ± 1.24 [c]	14.8±1.50 [c]	52.2±2.82 [a]	31.6 ± 3.93 [b]
AST (U/L)	22 ± 1.66 [c]	21 ± 1.58 [c]	53.2 ± 3.77[a]	37.67 ± 2.4[b]
ALP (U/L)	61.8 ± 1.28 [c]	64.2 ± 5.08 [c]	113.2 ± 6.04 [a]	87.5 ± 3.48 [b]
Total protein (g/dL)	7.39 ± 0.25[a]	7.43 ± 0.22 [a]	5.64 ± 0.25 [c]	6.19 ± 0.09 [b]
Albumin (g/dL)	3.91± 0.11 [a]	3.9 ± 0.15 [a]	2.44 ± 0.12 [c]	3.09 ± 0.21 [b]

Values are expressed as means ± SEM. Data with different letters at the same row are significantly different at $p < 0.05$.
Alanine aminotransferase (ALT), aspartate aminotransferase (AST),alkaline phosphatase (ALP)

Histopathological findings

Histopathological findings of the liver revealed its normal histological structure in both control (G1) and NSO (G2)-treated groups (Fig. 1a, b). The group that received EMB (G3) showed different histopathological lesions, moderate vacuolar degeneration in most hepatocytes, with sporadic cell necrosis in individual hepatocytes (Fig. 1c), karyopyknosis in some and binucleation in others (Fig. 1d).There were also proliferating Kupffer cells (Fig. 1e), the central veins and blood sinusoids were dilated and engorged with blood, and alsohemorrhage and hemosiderosis were evident in liver parenchyma (Fig. 1f). Portal areas revealed hyperplasia of the bile duct lining epithelium, with multiple newly formed bile ductules, portal blood vessels were dilated and engorged with blood (Fig. 1g), and there was mild fibrosis with the infiltration of a few mononuclear inflammatory cells.Some portal areas revealed proteinaceous edema dispersing portal connective tissue fibers (Fig. 2a).There was also oval cell hyperplasia (Fig. 2b).

The EMB+NSO group (G4) showed mild vacuolar degeneration in a few hepatocytes (Fig. 2 c). Mild central vein and sinusoidal dilatation and congestion were also observed (Fig. 2d).Concerning portal areas, therewere no apparent histopathological changes in portal areas of this group, except for mild hyperplasia in the bile duct lining epithelium (Fig. 2e).

Histopathological lesion score

All recorded lesionsin the liverwere scored according to their severity, as shown in Table 3.

Immunohistochemical findings for Caspase-3 and TNF-α expression

Findings of the percentage areas positive for caspase-3 and TNF-αimmunostaining in the liver of the different treated groups are presented in Table 4. Immunostaining of caspase-3 and TNF-αin the liver revealed no immunoreactive cells in the control and NSO groups (Fig. 3a-d). Tissue from the EMB-treated group revealed strong expression of both caspase-3 and TNF-α (Fig. 3e, f).The group treated with EMB+NSOshowed weak positivity for immunoreactive cells (Fig. 3g, h).

DISCUSSION

Extensively used pesticides and their residues in the environment lead to severe pollution and alsohave adverse health effects. EMB is a relatively new and widely used insecticide that presents a health hazard through the mechanism of oxidative stress **(**El-Sheikh and Galal, 2015).

The present study was an attempt to evaluate the toxicity of EMB on B.W.,liver function parameters,and histopathological findings in male rats and to test the possible ameliorative effects of NSO on this.

Table 3: Scoring of histopathological alterations in liver of all treated groups

Lesions	Control and NSO	EMB	EMB+NSO
Vacuolar degeneration of hepatocytes	0	2	1
Hepatocellular necrosis	0	1	0
Binucleation of hepatocytes	0	1	0
Proliferation of Kupffer cells	0	2	1
Congestion of hepatic sinusoids and central veins	0	3	1
Portal fibrosis	0	1	0
Mononuclear inflammatory cell infiltration	0	1	0
Hyperplasia of bile duct lining epithelium	0	2	1
Formation of bile ductules	0	2	0
Portal blood vessel congestion	0	2	0
Oval cell hyperplasia	0	1	0

The score system was designed as follows: score 0 = absence of lesions in all rats of the group (n= 5), score 1= (<30%), score 2= (<30%-50%), score 3= (>50%).

Table 4: Area % of caspase-3 and TNF-α expression in liver of different experimental groups

Group	Caspase-3	TNF-α
EMB	35.15±0.92[b]	31.54±0.49[b]
EMB+NSO	13.88±0.21[a]	11.56±0.43[a]

Data are expressed as means ± SEM (n = 5).
Different letters at the same column are significantly different ($p \leq 0.05$).

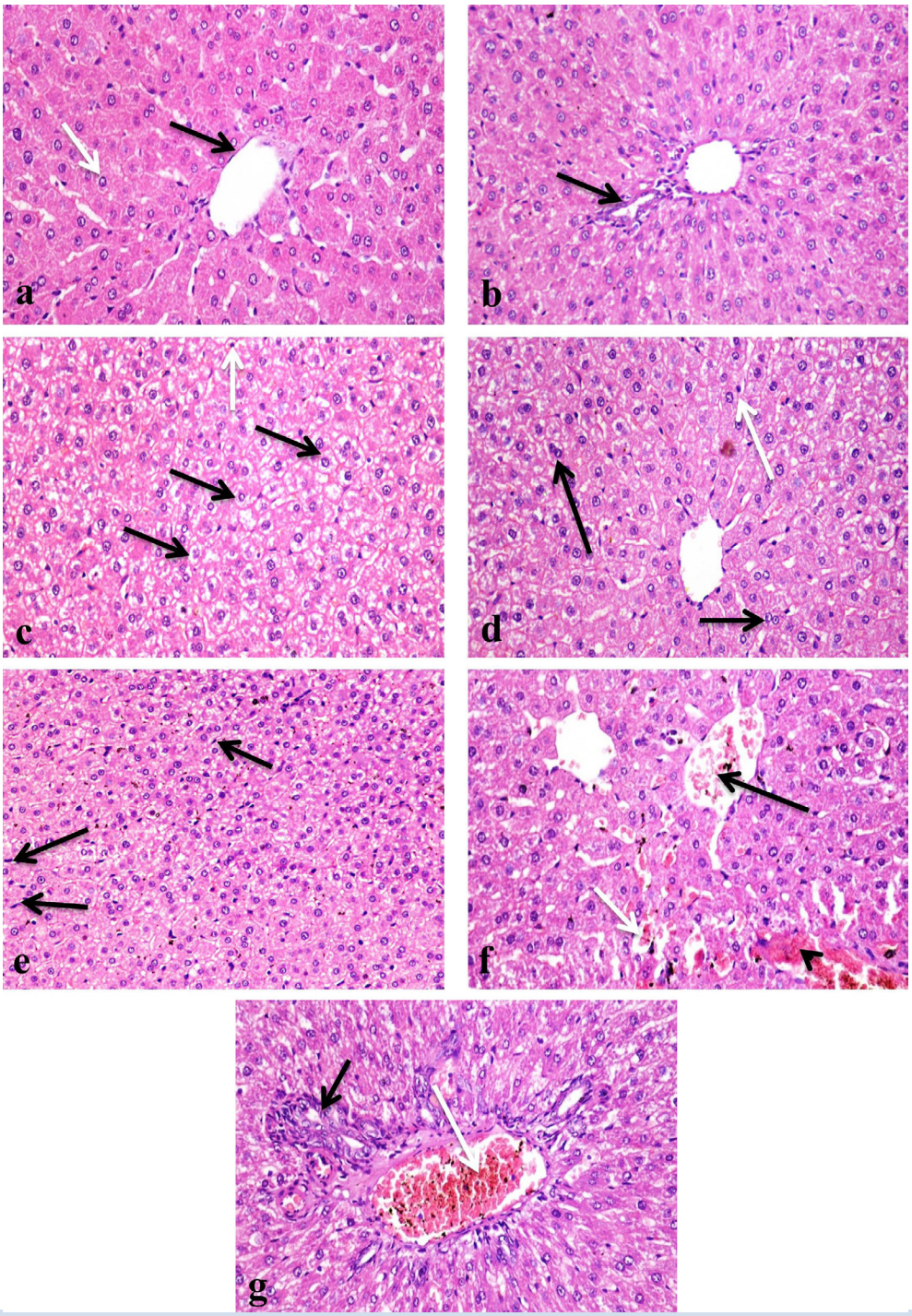

Figure 1. Photomicrographs of the liver of rats in a) control group showing normal histological structure of central vein (black arrow) and hepatocytes (white arrow);b) control group showing normal histological structure of the portal area (arrow); c) group treated with emamectin benzoate showing vacuolar degeneration in a considerable number of hepatocytes (black arrows) with sporadic cell necrosis (white arrow); d) group treated with emamectin benzoate showing hepatocytic binucleation (black arrows) and karyopyknosis of some hepatocytic nuclei (white arrow); e) group treated with emamectin benzoate showing Kupffer cell proliferation (arrows); f) group treated with emamectin benzoate showing congestion of central vein (black arrow) and sinusoids (white arrow) with parenchymal hemorrhage and hemosiderosis (arrow head); and g) group treated with emamectin benzoate showing congestion of portal blood vessels (white arrow) with hyperplasia of bile duct lining epithelium (black arrow) (H&E x400).

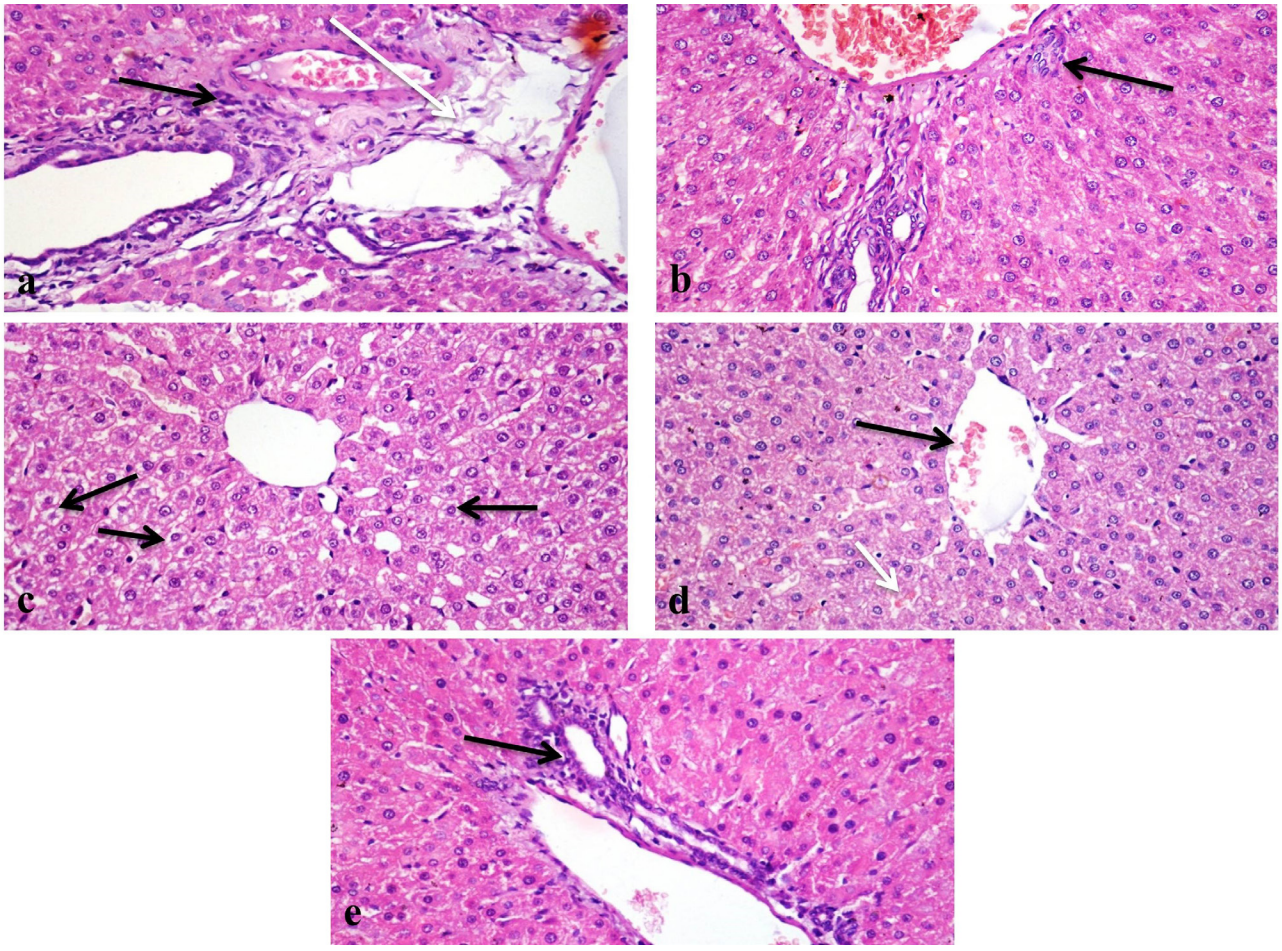

Figure 2. Photomicrographs of the liver of rats in a) group treated with emamectin benzoate showing edema in the portal area (white arrow) with infiltration of a few mononuclear inflammatory cells (black arrow); b) group treated with emamectin benzoate showing oval cell proliferation (arrow); c) group treated with emamectin benzoate and protected by *Nigella sativa* oil (NSO) showing mild vacuolar degeneration in a few hepatocytes (arrows); d) group treated with emamectin benzoate and protected by *NSO* showing mild congestion of central vein (black arrow) and blood sinusoids (white arrow);and e) group treated with emamectin benzoate and protected by *NSO* showing hyperplasia of bile duct lining epithelium (black arrow) (H&E x400).

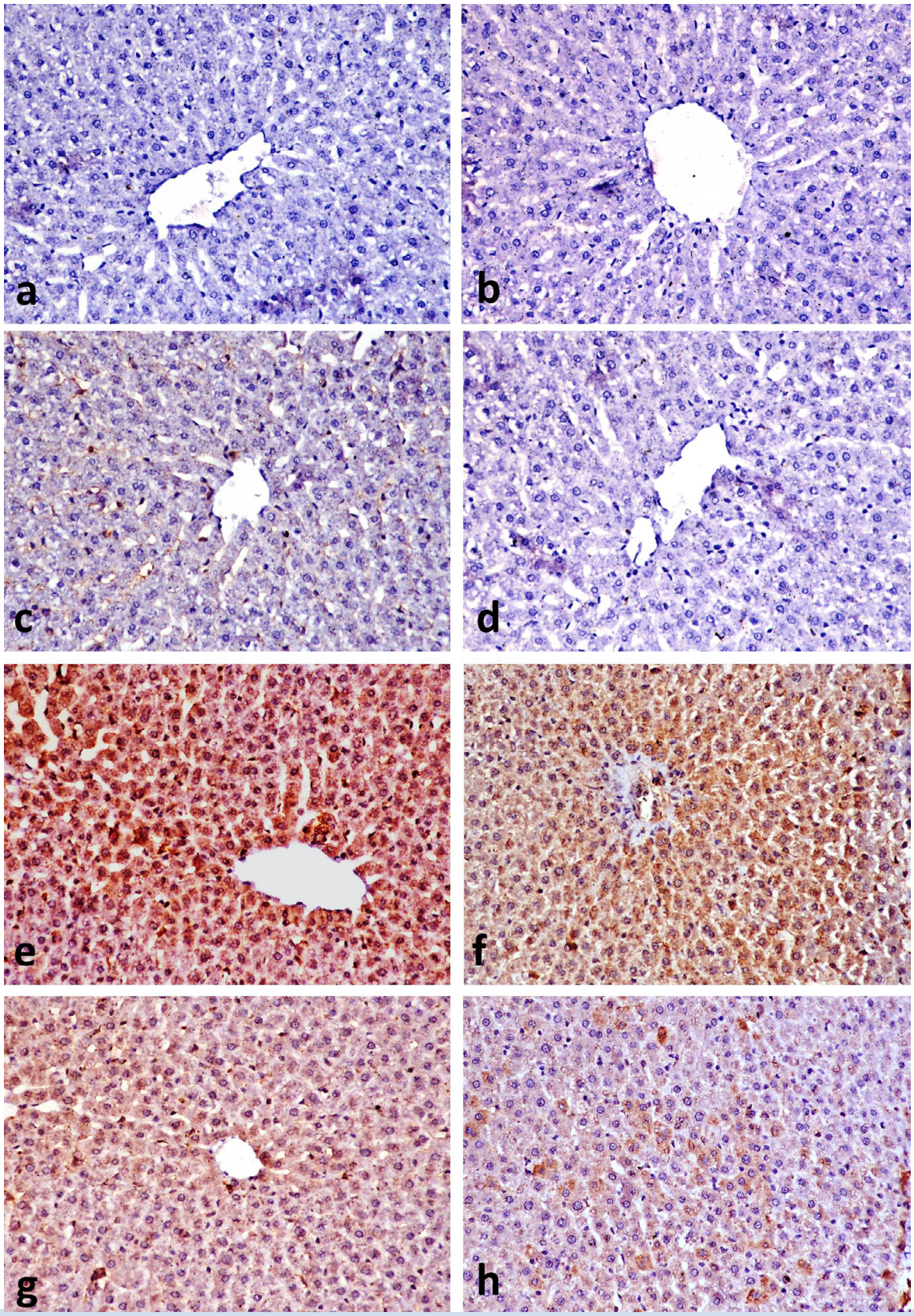

Figure 3. Immunostaining of caspase-3 and TNF-αin liver of a) & b) control rat showing no caspase-3 or TNF-αimmunoreactive cells in liver tissue; c) & d) NSO-treated group showing no caspase-3 or TNF-αimmunoreactive cells in liver tissue; e) & f) EMB-treated group showing strong positive immune expression of caspase-3 and TNF-α; and g) & h) group treated with EMB and protected by NSO showing weakly positive expression of caspase-3 and TNF-α, respectively (Caspase-3 and TNF-α, x400).

Change in B.W. is known to be a sensitive indicator for detecting potentially toxic chemicals (Bailey et al., 2004). The B.W. of EMB-treated rats in the present study was substantially lower than that of the control group. Our results agreed with those obtained by Khaldoun et al. (2014, 2015), Caihong et al. (2000), who found that EMB administration resulted in a decrease in male rat B.W. and Xing et al. (2000), who found that the administration of methyl amino-abamectin (Emamectin) resulted in body weight decrease in male rat. In addition, Al-Amoudi (2018) and Kumar(2018) suggests the body weight reduction in deltamethrin and lambda cyhalothrin induced toxicity models and El-Gendy et al. (2015) who reported the B.W. of abamectin treaded mice was significantly lower than that of the control group ($p< 0.05$). This may have been due to reduced food consumption (food avoidance or anorexia), poor food palatability (Hamed and Abdel-Razik, 2015) or increased lipid and protein degradation due to EMB toxicity (Mansour and Mossa, 2010). It may also have been due to cholinergic overstimulation that triggered an increase in gastric motility and a decrease in food absorption by the gastrointestinal tract and altered efficiency of food conversion. Moreover, Avermectin has been shown to inhibit muscle motility, pharyngeal pumping, and feeding in *Trichostrongylus colubriformis*, a nematode parasite (Sheriff et al*.,* 2002).Furthermore, Yang et al. (2019) mentioned that ivermectin insecticide stimulated farnesoid X receptor, that increase fatty acid oxidation and inhibited genes of lipogenesis that impacted certainly on body weight.

Change in B.W.is known to be a sensitive indicator for detecting potentially toxic chemicals (Bailey et al., 2004). The B.W. of EMB-treated rats in the present study was substantially lower than that of the control group. Our results agreed with those obtained by Caihong et al. (2000), who found that EMB administration resulted in a decrease in male rat B.W. This may have been due to reduced food consumption or increased lipid and protein degradation due to toxicity associated with the treatment (Mansour and Mossa, 2010). It may also have been due to cholinergic overstimulation that triggered an increase in gastric motility and a decrease in food absorption. Avermectin has been shown to inhibit muscle motility, pharyngeal pumping, and feeding in *Trichostrongylus colubriformis*, a nematode parasite(Sheriff et al*.,* 2002). Co-administering NSO to the EMB-treated rats increased B.W. and gained B.W. Our findings are consistent with those obtained by Shahid et al. (2018), who showed that NSO and thymoquinone supplementation stabilized the B.W. of long-term cisplatin-treated rats. The increase in B.W. and weight gain may have been due to the cytoprotective impact of NSO, which kept the B.W. from being decreased.

The present results revealed increments of liver function biomarkers in the EMB-administered group, which could have been due to the EMB-induced oxidative stress that resulted in hepatic tissue damage. The liver is essential for the detoxification of xenobiotics, and the metabolism and biosynthesis of macromolecules for different essential functions (Djordjevic et al*.,* 2011). ALT, AST, and ALP are considered as important indicators of liver tissue damage (El-Maksoud et al*.,* 2020)because they are secreted into the blood upon hepatocellular injury. In addition, total protein and albumin were markedly decreased, which could be attributable to an imbalance between the rates of protein anabolism and catabolism, the low albumin level may be attributable to the toxic effects of EMB on the liver as it is the site where albumin is synthesized, and diseases of the liver can damage hepatocytes and alter their intensity of albumin production. Moreover, the damage could be attributable to the toxic effects of EMB through the generation of ROS, which in turn induce damage to the membrane components of the cell and result in the leakage of cytoplasmic enzymes (Bagchi et al*.,* 1995). Abdel-Hafez and Osman)2013)demonstrated that EMB exposure caused significant liver damage by increasing plasma AST, ALT, and ALP and decreasing total protein in rats. These EMB-induced alterations in ALT, AST, ALP, total protein, and albumin were reversed by NSO treatment. These hepatoprotective effects of NSO could be due to the presence of 5-isopropyl-2-methyl phenol (carvacrol) in NSO, as presented in the gas chromatography-mass spectrophotometry analysis results of NSO in our previous study (Madkouret al., 2021). A previous study by Canbek *et al.* (2008)reported that carvacrol treatment attenuated the increased liver enzymatic activities and enhanced parenchymal cell regeneration in the liver, thus protecting cellular membrane integrity and decreasing enzymatic leakage.

Liver is the largest organ of the human body. It plays a central role in the metabolism and excretion of xenobiotics, which makes it highly susceptible to their adverse and toxic effects (Singh et al*.,* 2011). Therefore, hepatotoxicity is an important endpoint in determining the impact of such xenobiotics. His-

topathological assessment is a widely used tool for identifying organ-specific effects of chemical exposure (Mossa et al., 2012).

In our study, EMB-induced various histopathological changes such as moderate vacuolar degeneration in the liver of treated rats with sporadic cell necrosis, suggesting hepatocellular damage; this result was in agreement with that discussed by El-Ballal et al.(2019). Liver damage could be attributed to the generation of ROS causing damage to various membrane components of the cell and leading to the leakage of cytoplasmic enzymes and cell damage (El-Sheikh and Galal, 2015).

Central vein and blood sinusoids showed dilatation and congestion, which is in accordance with the work of Mahmoud and Mahmoud (2010). There was also proliferation of Kupffer cells. Hemorrhage and hemosiderosis were also observed, as recorded previously by Mossa *et al.*(2017). The portal area showed congestion of portal blood vessels, and moderate fibrosis with the infiltration of few mononuclear inflammatory cells, which agreed with the results of Abou-Zeid *et al.* (2018).

The changes in liver biomarkers and histopathological alterations such as hemorrhage, inflammation, necrosis, degeneration, congestion, and other necrobiotic alterations suggested possible liver tissue damage. These changes could be due to increased free radical and oxidative stress as a result of decreased free radical scavenger formation. However, histopathological alterations in the liver including congestion, hemorrhage, and fibrosis were reported in other studies (Hamed and Abdel-Razik, 2015).

The EMB-treated group revealed strong expression of both caspase-3 and TNF-α. Caspase-3 is considered as a pro-apoptotic protein and an increase in its expression indicates the initiation of toxin-mediated cell damage. TNF-α is considered a pro-inflammatory mediator(Madkouret al., 2021).

The group treated with EMB+NSO showed mild congestion of central vein, blood sinusoids, and portal blood vessels with mild vacuolar degeneration in a few hepatocytes, which agrees with the work of Salman *et al.* (2017), who stated that the oral administration of *N. sativa* induced the restoration of *liver* tissue to the normal state when used to ameliorate the toxic effect induced by liver extract of *Lagocephalus spadiceus* in male albino rats. The recovery of liver tissue may be due to the antioxidant activity of NSO, which possesses radical scavenging and antioxidant potential (Badary et al., 2007). Moreover, the group treated with EMB+NSO showed weak positivity for immunoreactive cells of liver tissue.

The antioxidant properties of *N. sativa* are attributed to the presence of thymoquinone,which has the ability to inhibit iron-dependent lipid peroxidation in a concentration-dependent manner (Nagi and Mansour, 2000). It has potent O_2 scavenger activity (El-Tawil and Moussa, 2006). With these characteristics, thymoquinone can decrease oxidative stress and increase antioxidant defense in the body, so it can help hepatic tissue to avoid oxidative damage by EMB.

CONCLUSION

N. sativa oil has hepato-protective potential against emamectin benzoate-induced hepatotoxicity in albino rats due to its component possessing antioxidant potential.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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