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Protective effect of Nigella sativa oil against hepatotoxicity induced by Emamectine benzoate in rats

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A B S T R A C T

Emamectin benzoate (EMB) is an avermectin insecticide used extensively in pest control on vegetable and field crops. In the current study, we evaluated the hepatotoxicity of EMB on male rats and the possible ameliorative role of Nigella Sativa oil (NSO). Rats (Thirty-five) were randomly divided into 5 equal groups. Control group the rats were treated orally with distilled water, NSO group; the rats received NSO at a dose of 3 ml kg-1 B.W orally day after day for 6 weeks by stomach tube, EMB group; the rats received EMB at a dose of 9 mg kg-1 orally day after day for 6 weeks by stomach tube; EMB+ NSO group; the rats received EMB and NSO day after day for 6 weeks; EMB then NSO group; the rats received EMB orally day after day for 4weeks alone then received NSO day after day for 2 weeks. Intoxication of rats with EMB significantly showed that EMB treatment resulted in a decrease in the body weight in compared with the control group. Regarding to the histopathological examination, EMB treatment induced coagulative necrosis and blood vessels congestion of the liver in treated rats. Furthermore, it resulted in sporadic cell necrosis in individual hepatocytes, central vein and blood sinusoids revealed dilatation and congestion. Portal areas revealed moderate fibrosis with few mononuclear inflammatory cells infiltration and hyperplasia of bile duct lining epithelium. Hemorrhage and hemosiderosis in liver parenchyma. The co-administration of NSO with EMB modulated the EMB induced alterations in body weight and liver structure.

Keywords: Nigella sativa oil, Emamectine benzoate,hepatic toxicity.

1. Introduction

Pesticides are chemical substances that are used to prevent, kill, or minimize harm produced from any pest (Eldridge, 2008). In agriculture are designed to protect crops against unwanted species, such as weeds, insects, and fungus (Bjorling-Poulsen etal. 2008).

The indiscriminate use of chemical insecticides is a frequent popular routine in the management of agricultural pests due to their rapid power in controlling and preventing of the pests (Cooper and Dobson, 2007).

But the extensive use of pesticide has contributed to environmental pollution and adverse health effects to animals and humans (EL- Ballal et al. 2019).

Emamectin benzoate is a macrocyclic lactone insecticide derived from the avermectin series of natural products. It is a mixture of at least 90% (the 4'-deoxy-4'- (methyl amino)-avermectin B1a benzoate (MAB1a or emamectin B1a benzoate) and at most 10% (the 4'-deoxy-4'-epimethyl-amino benzoate methyl amino) avermectin B1b benzoate (MAB1b or emamectin B1b benzoate) salts. Emamectin is structurally similar to abamectin and ivermectin (Wolterink et al. 2012). Emamectin benzoate is highly effective against a wide range of lepidopteron pests and is being developed for use in various field crops and vegetables, such as soybean, cotton, cabbage, and radish (Gacemi and Guenaoui, 2012). The EMB is incompletely absorbed in the gastrointestinal tract of mammals. Oral absorption was estimated at 55% (EFSA, 2012). Contamination of human food by insecticides mostly occurs to farmers and agriculture workers (Litchfield, 2005).

The European Medicines Agency(EMEA) has set the maximum residue limit (MRL) for low dose emamectin benzoate (10 —150 g/kg) for food of animal origin including aqua culture products (Hernando et al. 2007).

The liver is the body 's principal metabolic organ. It synthesizes bile, which is important for fat absorption. Liver is also significant in hemoglobin catabolism. This plays a major role in the metabolism of carbohydrates, fat, and proteins. it metabolizes xenobiotics (drugs, toxin and plant products), usually in two stages. The first stage, phase I, initially inactivates the substance, phase II conjugates the product with a water-soluble molecule that is either excreted in the urine or the bile (Campbell, 2006).

As the central role of the liver in drug metabolism predisposes it to toxic injury and drugs, it has been reported that EMB at 2.5 mg/kg body weight (BW) induced damage in liver (El-Sheikh and Galal, 2015).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. There is no doubt that herbal products chemically defined component that can protect the liver from various injuries (Negi et al. 2007).

Nigella sativa (N. Sativa), which is an annual plant that is a part of the family Ranunculaceae, is widely grown in many countries (Khazdair, 2015). The chemical compounds that make up black seed vary, but 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. S (Al-Saleh et al. 2006). Black seed has many medicinal properties, including neuroprotective (Saleem et al. 2012), hepatoprotective (Pourbakhsh et al. 2014), hypotensive and antidiabetic (Khan and Afzal, 2016), renal protective (Dollah et al. 2013), bronchodilatory, antibacterial and anti-tumor (Gholamnezhad et al. 2016), anti-inflammatory (Boskabady et al. 2011), immunomodulative (Gholamnezhad et al., 2015) and antioxidant properties. The antioxidant properties due to thymoquinone can decrease oxidative stress and increase antioxidant defense in the body. Decrease in malondialdehde and other biomarkers of oxidative stress in parallel with increase in total thiol content and glutathione level are the results of thymoquinone treatment (Seronello et al. 20007)

The present study was designed to evaluate the NSO against EMB induced hepatotoxicity in rats.

2. Materials and Methods

2.1. insecticide. The tested insecticide was emamectin benzoate in the form of anhydrous powder Tomguard 5%. The solution was prepared by dissolving the insecticide in distilled water.

2.2. Animals.

Thirty-five of Wistar male rats, age 6 weeks and weighting (110–120 g), were purchased from Experimental Animals Production Center (Giza, Egypt).

The animals were housed in cages with temperature regulated at 23 ± 2 °C, dark periods of 12-h light/12-h and, 55 ± 5% relative humidity and had free access to a standard Commercial diet of pellets and provided with water ad libitum. The rats have been acclimatized to laboratory conditions for one week before the beginning of the experiment. and had free access to a standard Commercial diet of pellets. The study was ethically approved by the International Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City.

2.3. Nigella sativa oil (NSO).

Nigella sativa oil was obtained from National Research Centre, Dokki-Giza, Egypt. Oil was kept at 4°C in dark container till use.

2.4. Experimental design and animal grouping.
Animals were allocated into 5 groups, 7 animals each. Control group; received D.W. NSO group; the rats were orally administered Nigella Sativa oil (NSO) at a dose of 3 ml kg-1 according to Danladi et al.)2013) day after day orally by gastric tube for 6 weeks. Emamectin group; received emamectin benzoate (EMB)at a dose of 9 mg kg-1 B.W by gastric tube day after day for 6 weeks (equivalent to 1/10th oral LD50 according to Wang et al.) 2012). EMB+ NSO group; The rats were orally administered NSO at a dose of 3 ml kg-1 B.W together with EMB at a dose of 9 mgkg-1 B.W by gastric tube day after day for 6 weeks. NSO administered by half an hour prior to EMB administration. EMB then NSO group; The rats were orally administered EMB a dose of 9 mgkg-1 B.W day after day for 4 weeks then administrated NSO at a dose of 3 ml kg-1 B.W by gastric tube day after day for 2 weeks. Body weight was recorded weekly throughout experimental periods (6 weeks).
2.5. Sample Collection.
At the end of the experiment, rats were fasted overnight, anaesthetized and sacrificed for sample collection. Liver of each rat was collected and was fixed in 10% neutral buffered formalin solution for the histopathological investigation.
2.6. Histopathological examination.
Specimens from liver tissue 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-micron thickness and stained with Hematoxylin and Eosin (Bancroft and Gamble, 2008) for histopathological examination by a light microscope (Olympus BX50, Japan).
2.7. Statistical analysis.
Values are presented as mean ± standard error (SE). Statistical significance of data was determined by one-way ANOVA (Analysis of Variance) followed by Duncan's Multiple range test for post hoc analysis. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) Version 16 released on 2007.

3. Results

3.1. Evaluation of body weights
A significant difference in the body weight gain at the end of the experimental period was observed in treated groups when compared with the control group, Table 1 showed EMB intoxication significantly decrease the body weight gain all over the experimental period compared with the control rats.
Nigella sativa oil group show no significant changes in the body weight compared to the control group,
Group treated with emamectin benzoate and protected with NSO show no any changes in the body weight in comparison with the control group. Meanwhile group treated with emamectin benzoate for 1-month showed decrease in the body weight, but when had been given NSO, body weight gain returned to normal when compared with the control group.
3.3. Histopathological findings of liver tissue
Concerning liver sections of control group and group given NSO, liver showed normal histological structure in both groups (Fig.1 a& b). Group treated with EMB showed moderate vacuolar degeneration in considerable number of hepatocytes (Fig.1 c) with sporadic cell necrosis in individual hepatocytes, central vein and blood sinusoids revealed dilatation and congestion (Fig.1 d). There was proliferation of Kuppfer cells. Hemorrhage and hemosiderosis was evident in liver parenchyma. Portal area showed dilatation and congestion of portal blood vessels, hyperplasia of bile duct lining epithelium with formation of newly formed bile ductules (Fig.1 e). Portal area also showed moderate fibrosis with few mononuclear inflammatory cells infiltration (Fig.1 f). There was oval cells hyperplasia.
Concerning group that treated with EMB and protected by NSO showed mild congestion of central vein, blood sinusoids, and portal blood vessels (Figs.1 g& h) with mild vacuolar degeneration in few number of hepatocytes.
Group that given EMB and then treated with NSO, this group revealed minimal improvement as hepatocytes showed vacuolar degeneration in considerable number of hepatocytes (Fig.1 i), congestion of central vein, blood sinusoid, and portal blood vessels and Kuppfer cells proliferation. Portal areas revealed moderate fibrosis with few mononuclear inflammatory cells infiltration and hyperplasia of bile duct lining epithelium.

4. Discussion

Extensive use of pesticides and their residues in the environment has led to severe pollution and health hazards. Emamectin benzoate is a relatively new and widely used insecticide that cause adverse health effects through the mechanism of oxidative stress (El-Sheikh and Galal, 2015).
The present study was an attempt to evaluate the toxicity of EMB on body weight and histopathological parameters in male rats and to test the possible ameliorative effect of NSO
The changes in body weight are known to be sensitive indicators for the detection of potentially toxic chemicals (Bailey et al. 2004). The body weight of treated EMB rats in the present study was substantially lower than that of the control group. It can be linked to reduced food consumption or increased lipid and protein degradation due to toxicity associated with the treatment (Mansour and Mossa, 2010). It may also be due to cholinergic overstimulation that triggers an increase in gastric motility and a decrease in food absorption. As avermectins have been shown to inhibit muscle motility, pharyngeal pumping and feeding in Trichostrongylus colubriformis, a nematode parasite (Sheriff et al. 2002). Our results agreed with that obtained by Caihong et al. (2000), Who found that EMB administration had resulted in a decrease in male rat body weight. Co-administering NSO to the EMB-treated rats increased body weight and gained bodyweight. Our findings are consistent with those obtained by Shahid et al. (2018), who showed that NSO and thymoquinone supplementation stabilized the body weight of long-term cisplatin-treated rats. This increase in body weight and weight gain due to the cytoprotective impact of NSO (Robert, 1983). So keeping body weight from being decreased.
Liver is the largest organ of the human body. It plays a central role in the metabolism and excretion of xenobiotic 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. Histopathological assessment is a widely used tool for the identification of organ-specific chemical exposure effects (Mossa et al. 2012).

The histopathological changes in the liver as, hemorrhages, inflammation, necrosis, degeneration, congestion, and other necrobiotic alterations in EMB treated rats suggested possible liver tissue damage. These changes could be due to increased free radical and oxidative stress as a result to decrease free radical scavenger formation. Hamed and Abdel-Razik, (2015), Reported that histopathological alterations in the liver include congestion, hemorrhages, and fibrosis.

Group that treated with EMB and protected by NSO, showed mild congestion of central vein, blood sinusoids, and portal blood vessels (figs.1) with mild vacuolar degeneration in few number of hepatocytes, our results agree with Salman et al. (2017), who stated that Oral administration of N. sativa and vitamin E induced restoration to the liver tissue to the healthy when used to ameliorate the toxicity induced by liver extract of Lagocephalus Spadiceus in male albino rats. The tissues recovery of the liver suggested due to the antioxidant activity of NSO which possessed radical scavenging and antioxidant (Badary et al. 2007).
The antioxidant properties of N. sativa came back to the presence of thymoquinone that has the ability to inhibit iron-dependent lipid peroxidation in concentration-dependent manner (Nagi and Mansour, 2000). It is a potent O-2 scavenger activity (El-Tawil and Moussa, 2006). With this characteristic, thymoquinone can decrease oxidative stress and increase antioxidant defense in the body. So keeping hepatic tissue from oxidative damage by EMB.

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

Groups Parameters	Control	NSO	EMB	EMB with NSO	EMB then NSO
1st week	101.5 ± 0.86^{ab}	105.6±2.38 ^{a}	100±0.412 ^{bc}	100.6 ± 1.5 ^{abc}	96 ± 1.47 ^{c}
2nd week	152 ±3.58^{a}	141.4 ± 4.24 ^{ab}	132.5 ± 4.79^{b}	145.2 ± 3.06^{ab}	137.25 ± 7.15^{ab}
3rd week	187± 4.02 ^{a}	176 ± 5.43 ^{a}	153 ± 3.85 ^{b}	183.8±2.48^{a}	156.5 ± 8.53 ^{b}
4th week	223.5 ±4.86^{a}	219 ± 4.01 ^{a}	159.75 ± 7.64^{b}	217± 3.83 ^{a}	160± 13.07 ^{b}
5th week	242.25± 3.54^{a}	230 ± 6.26 ^{ab}	145 ± 12.33 ^{c}	210.2± 4.08 ^{b}	218.18± 8.61^{b}
6th week	240.5± 9.46 ^{a}	231.2± 10.63^{ab}	137.5± 11.27 ^{c}	210.8 ± 2.62 ^{b}	219.25 ± 38.79^{ab}

Values are expressed as means ± SE (standard errors).
Data with different letters in the same row are significantly different at P< 0.05.
G1: (control group), G2: (Nigella sativa oil group), G3: (Emamectin benzoate group), G4: (Emamectin benzoate with Nigella sativa oil group), G5: (Emamectin benzoate then Nigella sativa oil group).