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Assessment of the potential ameliorative role of *Origanum vulgare* on permethrin-induced toxicity

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Abstract

Origanum vulgare (O. vulgare), is a medicinal plant which is traditionally used to treat different diseases. In this study, we evaluated the potential protective effects of *O. vulgare* against the toxic effects of permethrin (PM) in rats. Thirty adult rats were orally treated with normal saline as a control group, PM (150 mg/kg) as the second group, and (PM (150 mg/kg) + *O. vulgare* (300 mg/kg)) as the third group, once a day for 21 consecutive days. After completion of the study, clinical signs and body weight were assessed. Tissues and blood samples were collected for histopathological examination, biochemical alterations, and hematological analysis. The findings of this study demonstrated that PM induced some clinical signs and significantly declined the body weight of the treated rats. The tissue sections of animals in the second group showed some tissue complications in the liver, heart, lung, and kidney. In addition, hematological parameters including WBC, RBC, Hb, HCT, MCV, MCH, PLT, and biochemical parameters including ALT, AST, ALP, BUN, and creatinine were significantly changed. On the other hand, in the third group, *O. vulgare* reduced hematological, biochemical, and histopathological abnormalities, significantly. Furthermore, this plant could improve clinical signs and body weight changes. In conclusion, our results demonstrated that *O. vulgare* could be a potential herbal medicine for reducing the toxicity of PM.

Keywords *Origanum vulgare*, permethrin, toxicity, rats.

1. Introduction

Pesticides are an important group of environmental pollutants. Environmental contamination of pesticides is a threat to life because pesticide residues are found in the food chain, soil, and water. The pyrethroids are popular insecticides with extensive applications. They consist of two groups according to their chemical structures. Type I pyrethroids are without α-cyano moiety at the α-position such as permethrin (PM), while; type II pyrethroids have α-cyano moiety such as cypermethrin [1-2]. PM is one of the most widely-used synthetic pyrethroids with a wide spectrum insecticidal activity, which is used to control different pests in agriculture, veterinary, and domestic in the world [2]. This insecticide is applied in sprays, pet flea shampoos, lice shampoos, and home mosquito abatement products. It is also applicable on agricultural crops, particularly fruits and vegetables. Humans can be exposed to PM with ingestion of contaminated foods or inhalation of polluted air. Occupational exposure can happen via both inhalation and dermal contact at workplaces where PM is manufactured or used [2, 3]. Although scientists believe that pyrethroids are safer than organochlorines,

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organophosphates, and carbamate pesticides, the European Union (EU) classifies permethrin as hazardous to the environment [4], and an increasing number of investigations have demonstrated that PM can induce a variety of toxicities in animals and humans, such as carcinogenicity, neurotoxicity, immunotoxicity, cardiotoxicity, hepatotoxicity, reproductive toxicity, digestive system toxicity and cytotoxicity [5-11]. As we know, there is no special antidote for pyrethroids poisoning. As a result, treatment is supportive and symptomatic. In this regard, the identification of new ways for treatment of pyrethroids poisoning will be valuable. Traditionally, the use of plants for the treatment of different poisonings was popular because many of the plants have no adverse effects. One of the most precious plants is *O. vulgare* which grows in Iran extensively. This plant is used to treat hyperglycemia, leukemia, and other diseases. The major constituents of this plant are rosmarinic acid, eriocitrin, luteolin-7-oglucoside, origanol A and B, and ursolic acid. This plant demonstrated a potent antioxidant and free radical scavenging feature [12]. On the other hand, one of the most important mechanisms for pyrethroids poisoning is stress oxidative. Therefore, *O. vulgare* may be effective for treating pyrethroids poisoning which is related to stress oxidative. As far as we know, there is no study in the field of the protective effects of *O. vulgare* against pyrethroids poisoning in experimental animals. Therefore, in the present investigation, we assessed the potential ameliorative effects of *O. vulgare* against the toxic effects of PM in rats.

Materials and methods

Animals and test materials

Thirty adult Wistar rats were bought from the Pasteur Institute of Iran. All rats were housed in separate cages and allowed to be adapted with lab environment before the experiment. After a period of one week adaptation to laboratory environment, rats were randomly allocated into 3 groups. They were kept under hygienic and standard conditions (temperature of (22 ± 2)°C, humidity (55 ± 5) %, and a 12:12 light/dark cycle) with adequate standard laboratory food and tap water. All animals were kept according to the recommendation of the animal care committee of the Tehran University based on the 'Guide for Care and Use of Laboratory Animals' (NIH US publication 86-23, revised 1985). Permethrin (Pale yellow liquid; CAS No. 52645-53-1; purity = 95%) was obtained from Shanghai Bosman Industrial Co., Ltd. (China).

Preparation of extract

O. vulgare plant was purchased from (Zarin giah company, Iran) and the plant samples were identified by a botanist. Then, 50 grams of plant powder were mixed in double distilled water and ethanol for 72 hours. Then, the hydroethanolic extract was filtered, and the filtrate was concentrated through evaporation under a vacuum at 40°C. Then, the extract was kept at -20°C until experiments.

Treatment

Thirty rats were randomly divided into three groups, ten in each group. The first group was the control which received saline. The second and third groups were considered as the experimental groups. A single dose of PM was administered orally by gavage to animals at the dose of 150 mg/kg (second group) for 21 days. In the third group of animals, a single dose of PM (150 mg/kg) and a single dose of *O. vulgare* (300 mg/kg) were administered orally by gavage to animals simultaneously. At the end of the study, the animals were sacrificed by intraperitoneal injection of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg).

Hematological assessment

Hematological parameters including RBC, HB, HCT, MCV, MCH, MCHC, WBC, and PLT were analyzed using a hemocytometer (ADVIA, Hematology system). The results represent the mean ± standard deviation (SD) per percentage of cell suspension.

Biomarker assessment

Blood samples were collected into the test tubes containing EDTA, kept for 30 min, and centrifuged at 3000 rpm for 20 min. Finally, we separated the serum samples and measured the levels of blood alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), the levels of blood urea nitrogen (BUN), and creatinine by (Model BT3000 auto analyzer Italy and commercial biosystems kits, Spain) using the manufacturer's guideline.

Histological analysis

Tissues such as heart, lungs, kidney, and liver were isolated and immersed in 10% buffered formalin for 48 h at room temperature and then sectioned transversely in 3–4 µm slices. Samples were dehydrated in a graded series of alcohol and xylene and embedded in paraffin. For histological processing, the sectioned tissues were stained with hematoxylin-eosin and examined for morphological and histological parameters by light microscope (Labomed Lx 400, USA).

Statistical Analysis

All data are expressed as mean ± SD. The mean of all parameters between the two groups was compared using the Student's t-test. Data was analyzed using the SPSS software (version 19) and a $p<0.05$ is considered statistically significant.

Table 1. The mean of hematological parameters following oral administration of PM and (PM + *O. vulgare*) in rats.

parameters	First	Second	Third
WBC	8.63±1.32	4.61±0.74*	7.15±1.67**
RBC	6.70±0.97	8.2±1.45*	7.4±1.55**
HB	12.05±1.51	14.2±1.22*	12.51±1.23**
HCT	33.6±5.80	40.25±6.28*	36.7±6.48**
MCV	50.56±1.50	58.05±2.68*	55.07±1.69**
MCH	18.1± 0.97	18.52±1.64	18.47± 1.5
MCHC	34.18±1.16	34.76±3.80	34.91±1.52
PLT	578±187	181±37*	362±49**

Mean values (standard deviation) are shown for ten animals in each group. *significant difference between the second and the control groups ($p<0.05$), ** significant difference between the third and the second groups ($p<0.05$)

Results

Hematological assessment

The mean of hematological parameters in the groups could be seen in table 1. The mean of parameters including RBC, HB, HCT, and MCV in the second group was significantly higher than those in the control group. While, the mean of WBC and PLT in this group was significantly lower than those in the control group. In this group, the mean of MCH and MCHC did not show significant different in comparison with the control group. In the third group, *O. vulgare* ameliorated the mean of RBC, HB, HCT, MCV, WBC, and PLT parameters in comparison with the second group, significantly.

Biomarker alterations

Serum ALT, AST, ALP, BUN, and creatinine concentrations were significantly boosted in the second group of animals compared to the control group. In the third group of animals, *O. vulgare* improved biochemical parameter abnormalities, significantly (Figures 1 and 2).

Histopathological evaluation

Oral administration of PM in the second group induced different histopathological complications such as hepatocyte vacuolation, sinusoidal enlargement, inflammation, and congestion in the liver of animals. Interlobular congestion, distal and proximal cell vacuolation, and degeneration of proximal renal tubules were observed in the kidney of animals. Also, this pesticide induced important pathological abnormalities including interstitial inflammation, hemorrhage and congestion in lungs and congestion in the heart of animals. On the other hand, *O. vulgare* improved histopathological abnormalities in the third group in comparison to the second group (Figure 1).

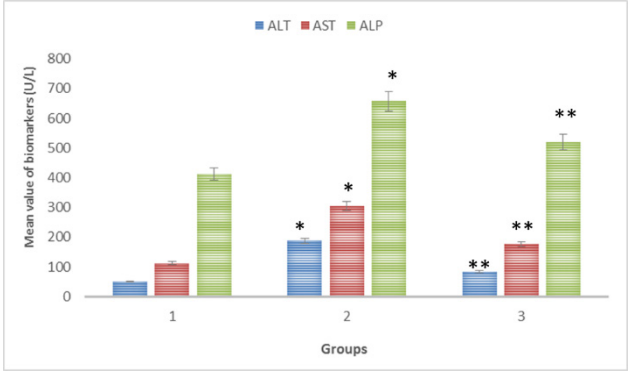

Fig 1. Effect of PM and (PM + *O. vulgare*) on ALT, AST, and ALP blood levels in rats; Values were given as means ± SD for ten animals in each group. *Significantly different from the control group ($P < 0.05$). **Significantly different from the second group ($P < 0.05$).

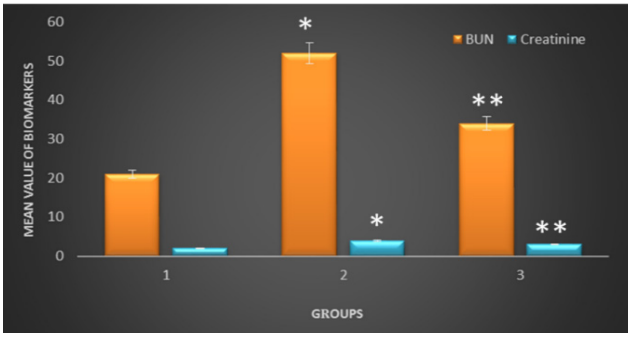

Fig 2. Effect of PM and (PM + *O. vulgare*) on the BUN and creatinine blood level in rats; Values were given as means±SD for ten animals in each group. *Significantly different from the control group ($P < 0.05$). **Significantly different from the second group ($P < 0.05$).

Clinical symptoms and body weight

Mortality was not observed in any of the experimental groups. PM induced some clinical signs such as anorexia, depression, muscular tremors, and ataxia. In addition, this pesticide decreased significantly the body weight of treated rats in the second group of animals. While, oral administration of *O. vulgare* and PM simultaneously improved clinical symptoms and body weight in the third group of rats in comparison to the second group. The body weight of all experimental rats is summarized in Table 2.

Discussion

In this study, we analyzed the ameliorative effects of *O. vulgare* against the toxic effects of PM in rats. The mechanism of action of PM is to interfere with sodium channels, receptor-ionophore complexes, and neurotransmitters. Previous studies have reported that PM can induce a variety of toxicities in animals and humans [9-11]. Our data revealed that oral administration of PM induced histopathological alterations such as hepatocyte vacuolation, sinusoidal enlargement, inflammation, and con-

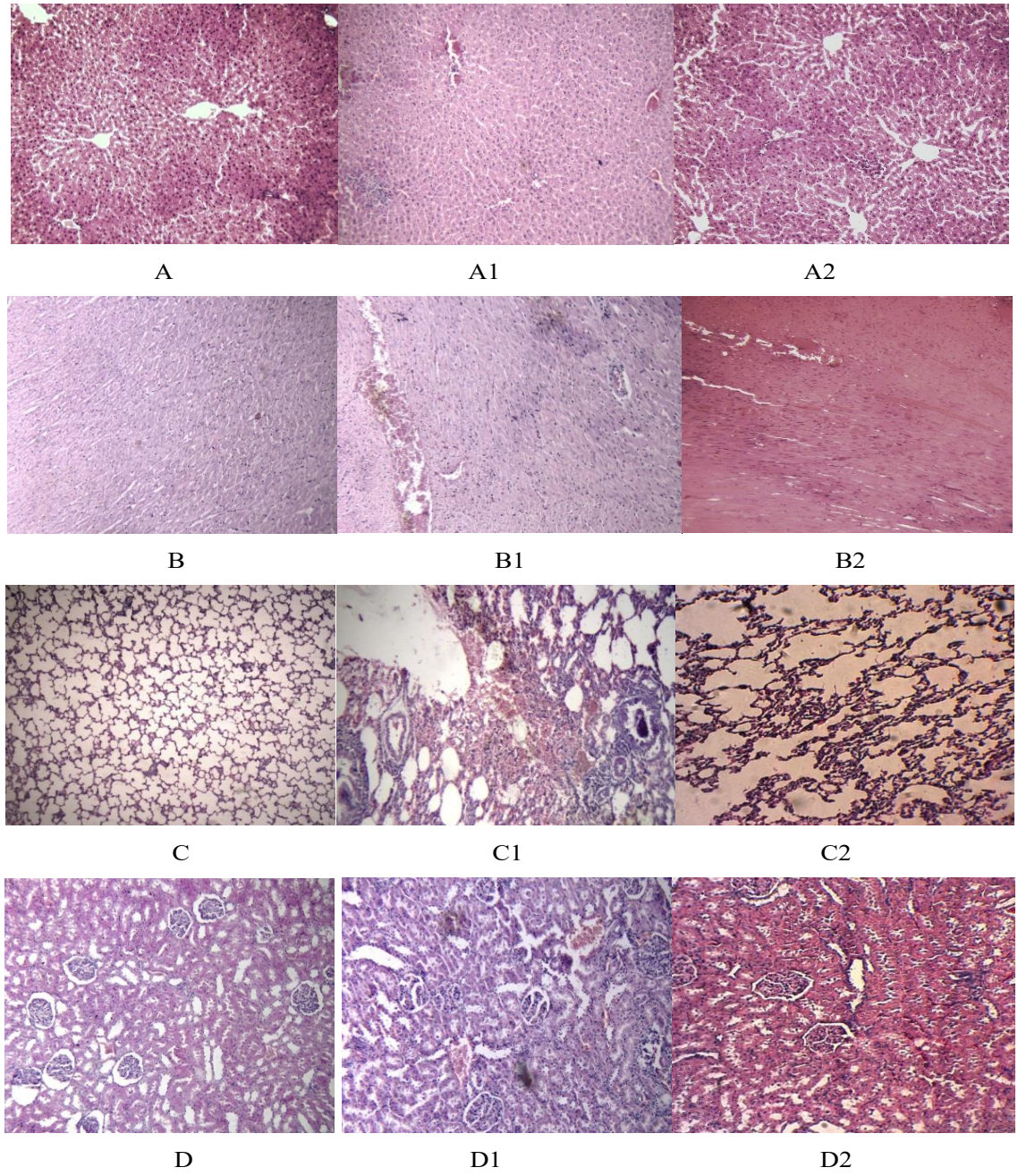

Fig 3. Photomicrographs of liver, heart, lung, and kidney sections were obtained from rats exposed to PM, and (PM + *O. vulgare*). Panels A: normal liver; A1: inflammation, congestion in the liver; A2: mild inflammation in the liver; B: normal heart; B1: congestion in the heart; B2: mild congestion in the heart; Panel C: normal lung; C1: interstitial inflammation, hemorrhage and congestion in the lung; C2: mild congestion in the lung; D: normal kidney; D1: interlobular congestion, distal and proximal cell vacuolation and degeneration of proximal renal tubules in the kidney; D2: mild congestion in the kidney (Staining with hematoxylin and eosin).

gestion in the liver, interlobular congestion, distal and proximal cell vacuolation, degeneration of proximal renal tubules in the kidney, interstitial inflammation, hemorrhage, and congestion in the lung and congestion in the heart of the experimented rats. There are some reports about pathological abnormalities in animals which were induced by pyrethroids. Kotila et al [13] demonstrated negative effects of permethrin on follicular and corpus luteum cell morphology in a dose dependent manner. Furthermore, some studies reported inflammation, congestion, edema, and hemorrhage in some organs following administration of some other sorts of pesticides such

Table 1. Body weight following oral administration of PM and (PM + *O. vulgare*) in rats.

Groups	Bodyweight (g) on the first day	Bodyweight (g) on the last day
First	201 ± 20	311 ± 24
Second	201 ± 22	266 ± 19*
Third	205± 23	301 ± 23**

Values were given as means±SD for ten animals in each group. *Significantly different from the control group ($P < 0.05$). **Significantly different from the second group ($P < 0.05$).

as fipronil and chlorpyrifos in animals. Congestion or hyperemia represents the increase of blood in an organ, due to dilatation of small vessels. However, mechanism of congestion induced by this pesticide is not clear and needs further investigations. Some studies concluded that oxidative stress may be a potential mechanism of toxicity for PM. In addition to stress oxidative, PM can decrease the antioxidant defense system resulting in damage to cellular macromolecules, including DNA, lipids, and proteins [3]. The hematological results in the experimented group demonstrated that parameters including WBC and PLT were significantly lower than those in the control group. Platelets are the blood cells involved in coagulation. The platelets should be in sufficient size, number and function for blood coagulation [14].Our results showed a platelet count reduction, following PM exposure. This result agrees with a study that reported a decrease in platelet count in rats exposed to pyrethroids [14]. The mean values of RBC, Hb, HCT and MCV in the group received PM were significantly higher than the control group. Our results suggest PM treatment may induce abnormalities in bone marrow and hematopoietic progenitor cells. Moreover, the activity of ALT, AST, ALP, BUN and creatinine was significantly raised following PM treatment in rats. The AST and ALT increase is one of the most important indexes in the diagnosis of liver abnormalities. Furthermore, BUN, and creatinine level rise is an essential factor for the identification of renal dysfunction. Our results are compatible with a previous study which indicated that pyrethroids such as cypermethrin could induce liver and kidney failure [15]. In the present investigation, we realized that *O. vulgare* could improve hematological, biochemical, and histopathological abnormalities induced by PM. Furthermore, this plant could ameliorate clinical signs and body weight changes induced by PM. Some plants have special components such as flavonoids and phenolics which have antioxidant and free radical scavenging characteristics, and they could be used as a medicine for the treatment of different kinds of diseases [16, 17]. In a study, Arami et al reported that *O. vulgare* could protect blood lymphocytes from DNA damage and reduced genotoxicity which induced by irradiation [18]. Carvacrol and thymol are the two main phenolic compounds in *O. vulgare*. These components are strong antioxidants. Therefore, the antioxidant activity of *O. vulgare* could be attributed to these phenolic compounds [19]. In another study, Shokrzadeh et al reported that *O. vulgare* could be used to relieve the adverse effects of cyclophosphamide [20]. It has been proved that PM induces toxicity by oxidative stress mechanism. Therefore, *O. vulgare* probably declines toxicity by oxidative stress inhibition.

Conclusion

To sum up, our findings indicate that *O. vulgare* can be a potential supportive herbal medicine for treatment of PM poisoning.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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