*Journal of Reports in Pharmaceutical Sciences* 2018, 7(1), 108-115

# The Effect of *Satureja avromanica* Maroofi Extract on Oxidative Stress Induced by Malathion in Rats

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**ARTICLE INFO**

**Article Type:**
Research Article

***Article History:***
Received: 2017-11-22
Revised: 2017-12-20
Accepted: 2017-12-25
ePublished: 2018-02-13

***Keywords*:**
Inflammation
Malathion
Oxidative toxic stress
*Satureja avromanica*

**A B S T R A C T**

Malathion is an organophosphate (OP) pesticide that has been shown to induce oxidative stress in various organisms through the generation of free radicals and alteration of the cellular antioxidant defense system independent of its anticholinesterase effects. *Satureja avromanica* Maroofi species has been extensively investigated as a source of natural products with potential antioxidant and antimicrobial activity. The aim of this study was to investigate the possible protective role of hydroalcoholic extract effects of *S.avromanica* on interlokin17 (IL17), tumor necrosis factor (TNFα), total antioxidant capacity (TAC) and lipid peroxidation (LPO) in male rats poisoned with malathion. Effective doses of malathion (150 mg/kg/day) and *S.avromanica* extract (1000 mg/kg/day) were administered alone or in combination for 7 days by intraperitoneal injection. At the end of the experiment, the plasma of the animals was separated. In the blood plasma, the (IL17), (TNFα), (TAC) and LPO were measured. The results showed that the *S.avromanica* reduced the level of IL-17 compared to the control group, but this difference was significant only compared to the malathion group. Also, receiving *S.avromanica* caused increased serum TAC levels in rats, which this difference was significant compared to both control and malathion groups. In addition, it was observed that *S.avromanica* group showed significantly decreased in LPO level, compared to the group treated with malathion. As the results display, *S.avromanica* plant improves the oxidative stress status and related immune system factors.

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## Introduction

The organophosphate (OP) pesticide malathion, like other OP compounds, is known to inhibit acetylcholinesterase (AChE) activity, an effect that is thought to underlie the neurotoxicity elicited by these compounds [1]. In this regard, studies have reported neurotoxic effects of malathion exposure in both human beings and experimental animals [2]. Reactive oxygen species such as singlet hydroxyl radicals, oxygen, peroxyl, superoxide, and peroxynitrite can damage the body by cellular or oxidative stress. This leads to the development of diseases like diabetes, cancer, cardiovascular, and cirrhosis. Free radicals generated in the body can be removed by its own natural antioxidant defense systems that include glutathione peroxidase, catalase, superoxide dismutase, etc. Endogenous antioxidant defense are not completely efficient, therefore natural antioxidants and dietary are required to reduce the effect of oxidative stress due to excessive free radicals occurring in our system [3-5].

The genus *Satureja* L., (Lamiaceae) contains about 200 species of herbs, shrubs and mainly aromatic plants with wide distribution in the Mediterranean area, Asia and boreal America [6]. From ages past, *Satureja* species have been used as culinary herbs, spices and flavorings and in Iranian folk medicine the aerial parts of several species of this genus are used to treat various diseases such as urinary tract infections, upper respiratory tract infections, gastroenteritis, wounds and diarrhea [7]. *Satureja* species has been extensively investigated as a source of natural products with potential antimicrobial, antioxidant, analgesic, antiseptic, antiviral, antiproliferative, antiprotozoal, antidiarrheal, anti-inflammatory and antinociceptive activities [8-11]. The *S. khuzestanica* interferes with malathion-induced stimulation of hepatic cells glycogenolysis and gluconeogenesis through its antioxidant potential and increasing acetylcholinesterase activity [12]. *S. avromanica*, one of the species in the *Satureja* genus, is an indigenous plant that has only been found in the Avraman-Kurdistan region (West of Iran) which is frequently used as a spice. In our previous study *S. avromanica* has been extensively investigated as a source of natural products with potential antioxidant and antimicrobial activity [13]. The aim of this study was to investigate the possible protective role of hydroalcoholic extract effects of *S. avromanica* on interlokin17 (IL17), tumor necrosis factor (TNFa), total antioxidant capacity (TAC) and lipid peroxidation (LPO) in male rats poisoned with malathion.

## Materials and Methods

### *Plant material*

The aerial parts of *S. avromanica* were collected in September 2014 from Avraman Mountains (Kurdistan Province, Iran). Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands Researches by Hossein Maroofi, Sanandaj, Iran, under voucher no. (8504).

### *Preparation of the extract*

The air dried plant (200 g) was extracted with 500 mL of methanol/water (70/30) by using a maceration method for 72 h. The resulting extract was filtered using Whatman filter paper (no. 1) and then concentrated in vacuum at 40 °C using a rotary evaporator [14]. The residues obtained were stored at 4 °C until tested and analyzed.

### *Animals*

20 adult male Wistar rats, weighting 220-250 g were used in study. All animals were housed under standard laboratory conditions and allowed free access to normal laboratory rat chaw and water. All experiments were performed according to the animal welfare Act [12] (Act P.L. 99–198 approved by Ethics Committee of Hamadan University of Medical Sciences (No: 940215719).

### *Animal treatment*

Animals were randomly divided into four groups comprising of five animals and all treated for 28 days. Treatment groups were as follows: group 1 received only normal saline daily, group 2 received effective dose of malathion at 150

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mg/kg/day [15] , group 3 received hydroalcoholic extract of *S. avromanica* (1000 mg/kg/day) [16] and group 4 received combination of hydroalcoholic extract of *S. avromanica* and malathion in water.

### *Sampling*

At the end of the specified treatment, the animals were anesthetized by intraperitoneal administration of pentobarbital sodium (60 mg/kg), and then the liver was removed by transverse abdominal incision, perfused with cold 0.9% saline and kept frozen at -70 °C until homogenized. Blood samples were collected under anaesthesia by cardiac puncture was taken after 7 days treatment into vials containing heparin. The plasma was separated and kept at -20 °C for further analyses.

### *TAC assay*

Antioxidant power of plasma was determined by measuring their ability to reduce $Fe^{3+}$ to $Fe^{2+}$ established as named FRAP test that described previously [17] . Briefly, in this test, the medium is exposed to $Fe^{3+}$ and the antioxidants present in medium start to produce $Fe^{2+}$ as an antioxidant activity. The FRAP reagent prepared freshly, contained 25 ml of 300 mM acetate buffer (pH 3.6) plus 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 2.5 ml of 20 mM ferric chloride ($FeCl_3.6H_2O$). The complex between $Fe^{2+}$ and TPTZ gives a blue color with absorbance at 593 nm.

### LPO Assay

The method based on the reaction of LPO as the end product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of LPO extent. In this test the reaction of LPO with thiobarbituric acid (TBA) creates a complex which is determined spectrophotometrically while LPO in samples are assessed [18].

### *Interleukin 17 assay*

To measure the amount of IL-17 in serum samples of rats, an ELISA kit now Bioasay Technology Laboratory rats were used for E0115Ra code.

## Results

The potential effect of *S. avromanica* on the serum level of IL-17, as a key pro-inflammatory cytokine released following microglia activation, was investigated. The results indicated that malathion increased the IL-17 level about 115% of control group. Elevation of IL-17 level was prevented by treatment with *S. avromanica* but not significantly, when compared with malathion group (Fig. 1). IL-17 levels in the group receiving the *S.avromanica* extract alone represent a significant ($P<0.01$) reduction compared to the group treated with malathion. However the serum level of IL-17 in the group receiving the *S.avromanica* extract alone reduced when compared to the control group, but not in a significant manner.

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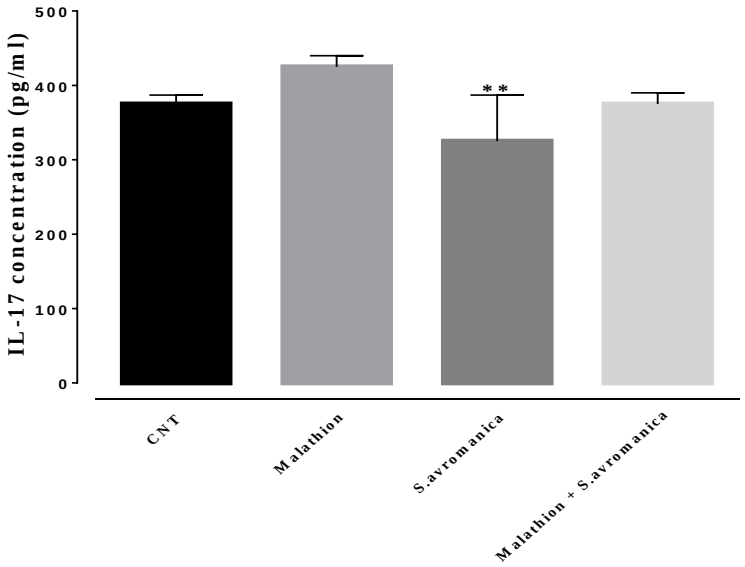


**Fig. 1.** Interlukine 17 (IL17) in plasma of rats. *Significantly different from control group at $p < 0.05$. **Significantly different from malathion group at $p < 0.05$. (CNT = Control)

To investigate the possible involvement of LPO as a marker of the oxidative stress in malathion poisoning we appraised the level of LPO in the serum. In the present study malathion injection given to the rats resulted in a significant ($p < 0.01$ vs. control) increase in LPO in serum, as measured by an increase in the level of LPO (Fig. 2). The MDA level in the serum was found to reduce from 4.4±1.5 µmol/ml in malathion poisoned group to 3±0.51 µmol/ml, in malathion plus *S. avromanica* treated groups, but not in a significant manner, (Fig. 2). Treatment of the healthy rats with *S.avromanica* extract alone had no effect on LPO levels in compared to the control group.

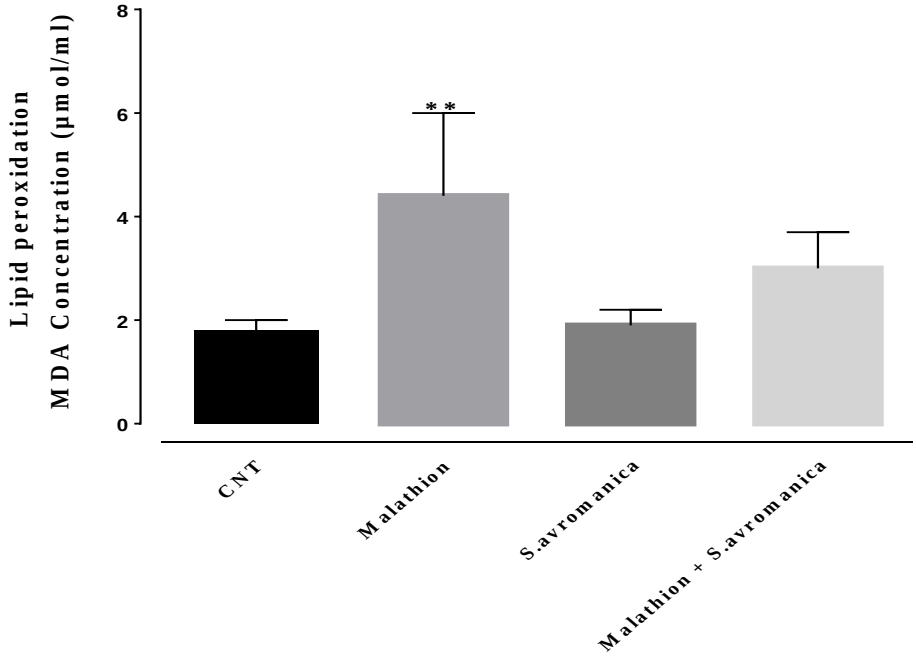


**Fig. 2.** Lipid peroxidation (LPO) in plasma of rats. *Significantly different from control group at $p < 0.05$. **Significantly different from malathion group at $p < 0.05$. (CNT = Control)

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As shown in the figure 3 we evaluated the effects of malathion and *S. avromanica* on the serum level of total antioxidant capacity (TAC). In the present study malathion decreased TAC level in serum of rats when compared with control group. Treatment of poisoned animals with *S. avromanica* extract increased total antioxidant capacity level in compared to malathion poisoned group, but not in a significant manner (Fig.3). However, in the groups receiving *S. avromanica* extract alone, the level of TAC was increased significantly ($p < 0.01$) compared to control animals.

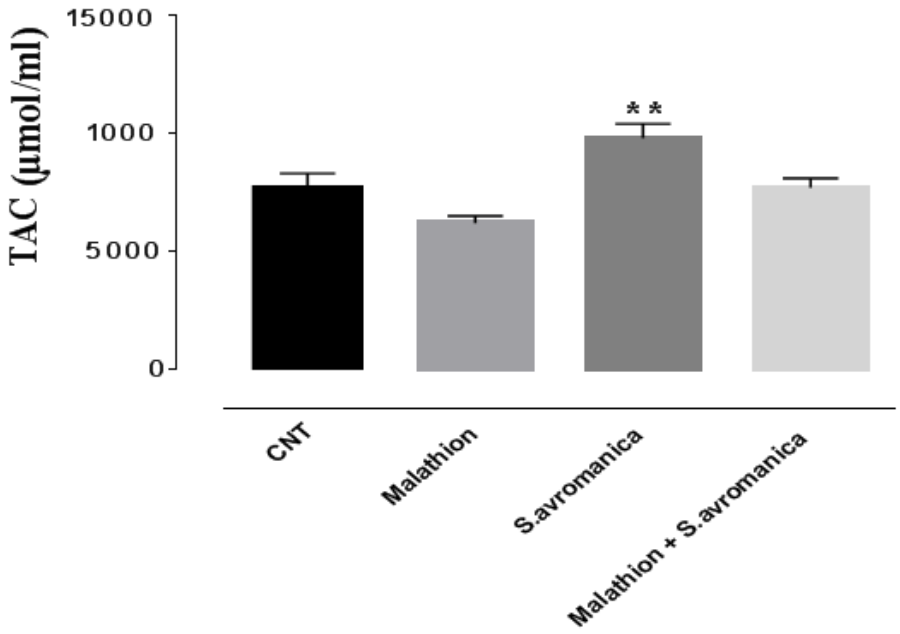


**Fig. 3.** Total antioxidant capacity (TAC) in plasma of rats. *Significantly different from control group at $p < 0.05$. **Significantly different from malathion group at $p < 0.05$. (CNT = Control)

## Discussion

Malathion is a widely used pesticide that affects various organs throughout oxidative toxic stress. This study showed that malathion significantly increase the levels of ILl17 and LPO and decreases TAC concentration. On the other hand, administration of *S. avromanica* significantly decreased both ILl17 and LPO changes in blood and increased TAC level alone or combination with malathion (Fig,3). In this study, the amount of LPO as the products of cell membrane were also increased in blood of rats received malathion. LPO is involved in the pathogenesis of various tissue damage such as brain, liver, kidney caused by malathion by decreasing cell membranes integrity [19-21]. Taking collectively, these data indicate that toxicity of malathion is mediated through oxidative toxic stress and *S. avromanica* induces its positive effects via improving of oxidant/antioxi-dant balance. Treatment by *S. avromanica* prevented LPO which can be attributed to presence of antioxidant ingredients in hydroalcholic extract of this plant and its ROS scavenging activity in malathion-induced oxidative toxic stress (Fig,3). Tlymphocytes produce an array of small proteins that are involved in cell growth, inflammation, immunity, differentiation, and repair. These protein mediators referred to as cytokines are not produced constitutively by T cells, but rather are induced after receptor-mediated T cell activation [22]. Recently, Th17 phenotype cytokines (i.e. IL-17) have been implicated in the development of various disorders [23]. Our findings demonstrated that IL-17 level induced by malathion and synergistic activities cause ROS generation, which was attenuated by treatment

with *S. avromanica* (Fig. 1). Pro-inflammatory cytokines are reported to transduce cellular signaling through ROS (as second messenger) under acute and chronic OP poisoning conditions [24, 25]. In current study, malathion administration led to a significant increase in the content of IL17 in blood, which can be a compensatory response to reduce toxicity of malathion. Also, treatment with *S. avromanica* decreased levels of IL17, probably due to the increase of inflammation in OP exposure especially malation. Therefore, in this study we showed antiinflammation and antioxidative effects of *S. avromanica* extract on malathion toxicity.

We can conclude that despite the different mechanisms of malathion toxicity, the main cause of oxidative effects of malathion is induction of oxidative stress. The antioxidant compounds such as *S. avromanica* in the treatment of poisoning caused by pesticides, especially OP are important. Consequently, identification and purification of these antioxidants will be helpful in planning for future therapeutic strategies. Antioxidative effects of *S. avromanica* may be the result of its ROS scavenging activity due to its bioactive antioxidants and its capacity in improving the enzymatic antioxidants defense system. The genus *Satureja* is known to be a rich source of biologically active compounds, such as phenols and flavonoids. Rosmarinic acid, an economically important metabolite is a biologically active phenolic acid from *Satureja* species. This compound showed various biological properties including antioxidative, anti-inflammatory, antibacterial, antimutagen, and antiviral activities. It has been confirmed that plants nonenzymatic antioxidant compounds such as phenols, flavonoids, and tannins are free radicals scavengers and these compounds can delay or inhibit oxidative damages [26, 27].

So, further studies are needed to find out, extract and purify compounds with the antioxidant activity in *S. avromanica* hydroalcholic extract. Further studies are required to elucidate the mechanism of action of *S. avromanica* on cellular and molecular mechanisms in various tissues.

## Conclusion

Knowledge of wild foods and endemic plant species with their biological properties could be of great value to further understand and make better use of these foods. As the results display, *S. avromanica* improves the oxidative stress status and related immune system factors. The data of this study can be considered in foods and industries.

## Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

## Acknowledgments

This study was supported by a grant from Vice Chancellor of Research of Hamadan University of Medical Sciences for student pharm thesis. (grant number:940215719).

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