Original Paper:

Evaluation of the Protective Effects of Hydroalcoholic Extract of *Satureja avromanica* Against Malathion-induced Oxidative Stress in the Liver: An Experimental Study

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ABSTRACT

Background: Studies have shown that organophosphorus pesticides such as malathion induces oxidative stress injury and tissue damage.

Objectives: This research aimed to determine the effects of the hydroalcoholic extracts of Satureja Avromanica (SA) on the liver function of malathion-poisoned animals.

Methods: Twenty-eight rats were divided into four groups of the control, SA (20 mg/kg), malathion+SA, and malathion. Animals received malathion 150 mg/kg and SA 20 mg/kg for one week through intraperitoneal injection. Then, their liver and blood samples were extracted, and alanine aminotransferase, aspartate aminotransferase concentrations in serum as well as biomarkers of oxidative stress such as Lipid Peroxidation (LPO), Total Antioxidant Capacity (TAC) and Total Thiol Groups (TTG) in the liver tissue were measured.

Results: The results showed that the SA administration reduced the level of liver LPO compared with that in the malathion group. Also, receiving SA increased liver TAC and TTG levels in rats, which this difference was significant compared with the malathion group. Besides, the SA group showed a significant decrease in liver enzyme levels, compared with the malathion-treated group.

Conclusion: According to the results, SA exerted protective effects against malathion poisoning, through reduction of oxidative stress. Therefore, SA may be an antioxidant to counteract the harmful effects of malathion poising in liver tissue.

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Introduction

Pesticides are chemical materials used in agriculture to protect crops from insects, weeds, bacterial, or fungal diseases during growth and can also preserve foods during storage [1]. Most of the commonly-used pesticides are Organophosphorus (OP) insecticides [2]. Unfortunately, the broad application of OP insecticides is hazardous for humans, animals, plants, and the environment and produce severe acute and chronic toxicity [3-5]. The poisoning mechanism of OP insecticides causes irreversible inhibition of Acetylcholinesterase (AChE), an increase of acetylcholine (ACh), and then stimulation of muscarinic, cholinergic, and nicotinic receptors [6].

Malathion, O,O-dimethyl-S-(1,2- dicarcethoxyethyl) phosphorodithioate, is an OP pesticide, which inhibits AChE. It has been extensively used to control not only pests [7] but also reduces animal ectoparasites, human head and body lice, and household insects [8]. Malathion damages various tissues, such as the liver, pancreas, and the reproductive system by irreversible inhibition of AChE activity, which disrupts mitochondrial membrane transport, i.e., cytochrome P450 system [9].

Oxidative toxic stress refers to the imbalance between the production and the neutralization or elimination of the Reactive Oxygen Species (ROS) and reduces the production of antioxidants in the body [10]. It has been shown that malathion increases the production of free radicals. ROS attack on the cellular constituents increases Lipid Peroxidation (LPO) and phospholipids degradation, causes hepato- and neuro-toxicity, and plays an essential role in the pathogenesis of malathion toxicity [11, 12].

Satureja is a member of the Lamiaceae family that encompasses more than 30 species. The genus *Satureja L.* (*Lamiaceae*) is widely distributed in the Mediterranean Region, Asia, and the boreal forests in North America [13]. *Satureja* species have been extensively examined as a source of natural products such as thymol, carvacrol, terpinene, p-cymene, and β-caryophyllene. Many studies have reported the antimicrobial, antioxidant, analgesic, antiseptic, antiviral, antiproliferative, antiprotozoal, antidiarrheal, anti-inflammatory, antinociceptive, and vasodilatory activities of genus *Satureja L.* [14-16]. Accordingly, the objectives of this research are to determine the ameliorative property of *Satureja avromanica* (SA) on liver oxidative stress in an experimental study of a malathion-poisoned model.

Materials and methods

Plant materials

The aerial parts of SA were collected from Hawraman Mountains (Kurdistan Province, Iran) and dried in shadow. Hossein Maroufi deposited voucher specimens at the Herbarium of the Research Institute of Forests and Rangelands Research, Sanandaj, Iran (voucher No. 8504) [17].

Methanolic extraction from SA

The air-dried plant (100 g) was extracted successively with 500 mL of methanol by applying a soxhlet extractor (ISOPAD, Heidelberg, Germany) for 24 h in a hot water bath at a temperature not exceeding the boiling point of the solvent [18]. The resulting extract was filtered with Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany). The collected residues were stored at 4°C until further analyses.

Chemicals and drugs

Malathion, Tris base, 1,1,3,3′-tetraethoxypropane, 2-thiobarbituric acid (TBA), dithionitrobenzoic acid (DTNB), trichloroacetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ) and n-butanol were purchased from Merck Chemical Company (Germany). All the other chemicals used were of analytical grade.

Animals and experimental design

Male Wistar rats weighing 220-250 g were obtained from Animal Care Center, Hamadan University of Medical Sciences. Animals were kept in standard conditions (12:12 h dark/light cycle at 22±2°C). The Medical Ethics Review Board of Hamadan University of Medical Sciences approved the study (No. 930222666).

After a period of adaptation, the animals were randomly divided into four groups (7 in each group): control, rats received SA (20 mg/kg), rats received malathion (150 mg/kg), rats received a combination of hydroalcoholic extract of SA (20 mg/kg) and malathion (150 mg/kg) in distilled water. Animals received malathion and SA for one week through intraperitoneal injection. In the next step and 24 hours after the last injection, the fasted rats were anaesthetized with ketamine (50 mg/kg). Blood samples for biochemical analyses were collected and stored at -20°C. Also, the liver tissue was extracted from all rats and perfused with cold 0.9% saline and fro-

zen in liquid nitrogen immediately after separation and stored at -70°C until further analysis.

Estimation of oxidative stress parameters

Assay of malondialdehyde

The amount of LPO was estimated as the concentration of thiobarbituric acid reactive output of Malondialdehyde (MDA) according to Yagi's method. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA+MDA adducts in the samples [19].

Assay of total antioxidant power

TAC measurement was done manually by the ferric reducing ability of plasma method. The method is based on the capacity of the sample to reduce Fe3+ to Fe2+ in the presence of TPTZ (Tripyridyl-s-triazine). The interaction of Fe2+ and TPTZ produces a blue color complex. Maximum optical density was measured at 593 nm [20].

Assay of total thiol groups

To evaluate the plasma total thiol groups, DTNB was used as a reagent. DTNB reacts with thiol molecules and creates a yellow complex which has an absorbance measurement at 412 nm [21].

Assay of liver enzyme

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were assayed by Pars Azemoon kit, Tehran, Iran.

Statistical analysis

The statistical analysis was conducted in SPSS v. 16.0 (SPSS Inc., Chicago, IL, U.S.A) and GraphPad Prism v. 6.0 (GraphPad Software, San Diego, CA, USA). One-Way Analysis Of Variance (ANOVA) followed by the post hoc Tukey test was used to detect the statistical significance between groups. The obtained data were expressed as Mean±SD, and a P-value of less than 0.05 was considered statistically significant.

Results

Lipid peroxidation

To investigate the possible involvement of LPO as a marker of the oxidative stress in malathion poisoning, we measured the level of LPO in the liver tissue. In the present study, malathion injection to the rats resulted in a significant (P<0.01 vs. control and SA groups and P<0.05 vs. malathion+SA group) increase in LPO in the liver, as estimated by the rise in the level of LPO (Figure 1).

Total antioxidant capacity

As shown in Figure 2, we evaluated the effects of malathion on the liver tissue of TAC. In the present study,

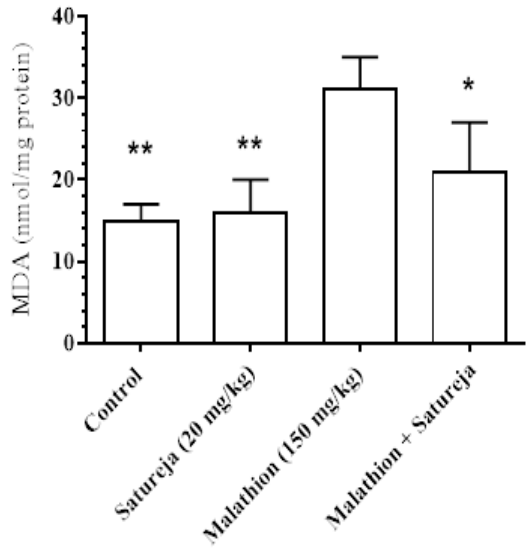

Figure 1. The effect of Satureja avromanica on Lipid Peroxidation (LPO) in the liver tissue of rats

Results were expressed as Mean±SD. Significantly different from the malathion group at *P<0.01 and **P<0.01.

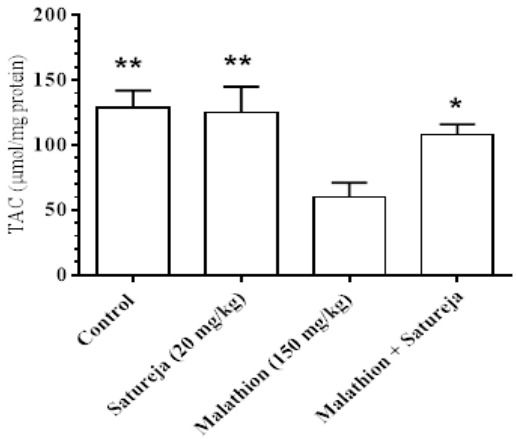

Figure 2. The effect of Satureja avromanica on Total Antioxidant Capacity (TAC) in the liver tissue of rats

Results were expressed as Mean±SD. Significantly different from the malathion group at *P<0.01 and **P<0.01.

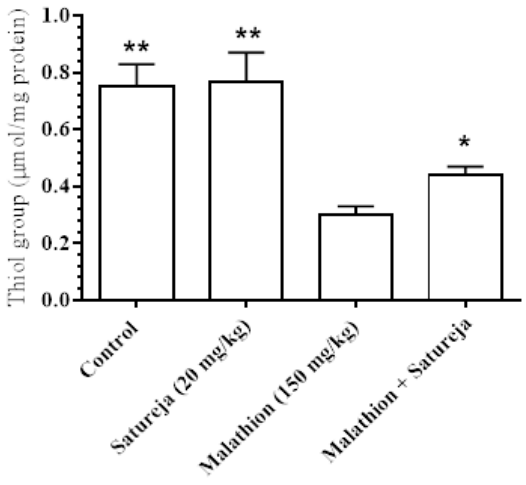

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Figure 3. The effect of Satureja avromanica on total thiol groups (TTG) in the liver tissue of rats

Results were expressed as Mean±SD.

Significantly different from the malathion group at *P<0.01 and **P<0.01.

malathion significantly decreased the TAC level in the liver of rats compared with the control and SA groups. In the poisoned animals with malathion, the TAC level significantly decreased versus malathion+SA group.

Total thiol groups

The potential effect of *Satureja* extract on the liver TTG was investigated. In our study, malathion injection given to the rats resulted in a significant (P<0.01 vs. control and SA groups and P<0.05 vs. malathion+SA group) increase in TTG in the liver, as measured by the rise in the level of TTG (Figure 3).

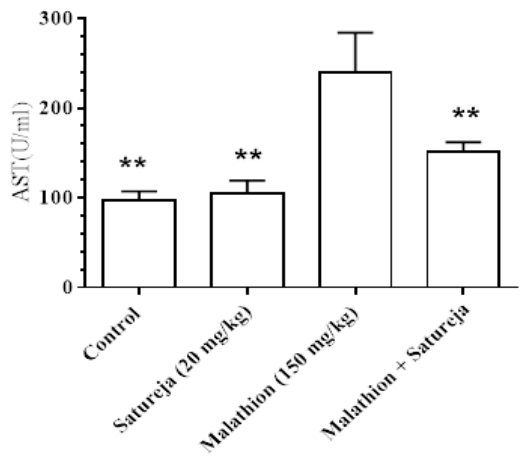

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Figure 5. The effect of Satureja avromanica on aspartate aminotransferase (AST) in the serum of rats

Results were expressed as Mean±SD.

Significantly different from the malathion group at *P<0.01 and **P<0.01.

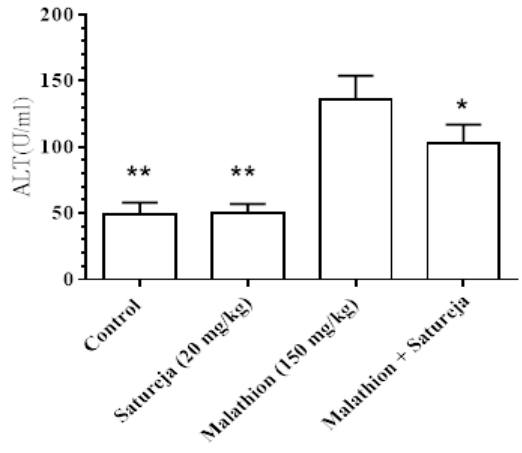

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Figure 4. The effect of Satureja avromanica on alanine aminotransferase (ALT) in the serum of rats

Results were expressed as Mean±SD.

Significantly different from the malathion group at *P<0.01 and **P<0.01.

Alanine aminotransferase

The potential effect of SA extract on the ALT as a biochemical indicator of liver function and malathion toxicity was investigated. In the present study, malathion-induced rats resulted in a significant (P<0.01 vs. control and SA groups and P<0.05 vs. malathion+SA group) increase in ALT level in serum, as measured by the rise in the level of ALT (Figure 4).

Aspartate aminotransferase

The potential effect of SA extract on the AST as a biochemical indicator of liver function and malathion toxicity was investigated. In the present study, malathion-induced rats showed a significant (P<0.01 vs. control and SA groups and P<0.01 vs. malathion+SA group) increase in AST level in serum, as measured by an increase in the level of AST (Figure 5).

Discussion

The genus *Satureja* is known to be a rich source of biologically active compounds, such as phenols and flavonoids. Plants have several compounds such as phenols, flavonoids, and tannins with antioxidant properties, as

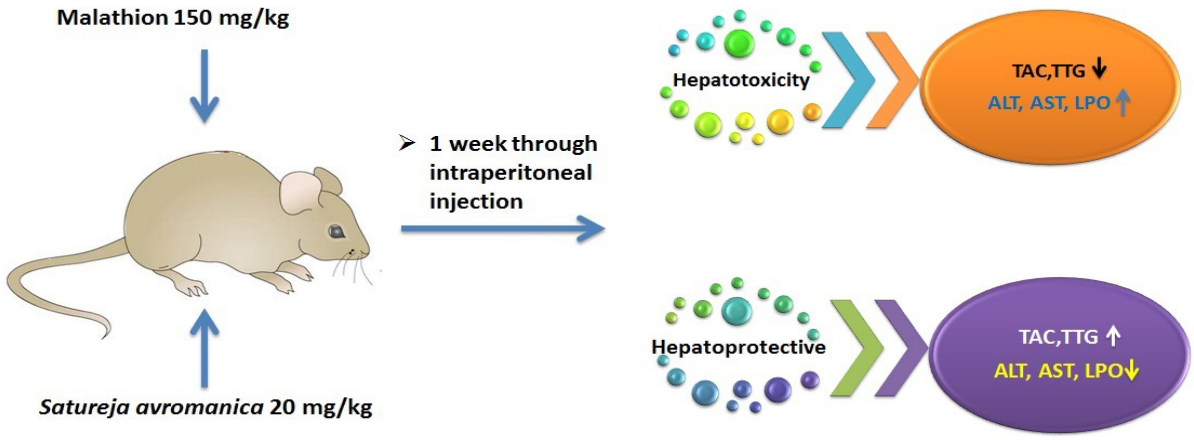

Figure 6. The effects of *Satureja avromanica* against the malathion-induced oxidative toxic stress in rat liver PBR
Malathion decreases the levels of Total Thiol Groups (TTG), Total Antioxidant Capacity (TAC), and increases the levels of Lipid Peroxidation (LPO), also leads to an increase in the levels of Aspartate Transaminase (AST) and Alanine Transaminase (ALT). Satureja avromanica administration improved all examined parameters induced by malathion.

well as free radical scavengers that can delay or inhibit oxidative toxic stress [22]. In the present study, the protective effect of SA was investigated against malathion-induced hepatic oxidative damage. Malathion is a widely-used pesticide that affects various organs throughout oxidative toxic stress. This study showed that malathion significantly increased the levels of ALT, AST, and LPO and decreased TAC and TTG levels. On the other hand, the administration of SA significantly diminished liver aminotransferase and LPO in the liver and increased the TAC level (Figure 6).

Oxidative toxic stress occurs in the models of subacute, acute, and chronic exposure to OP agents. In this study, oxidative stress status was investigated, and the result indicated that the level of LPO increased in malathion-poisoned animals. Malathion damages various tissues such as the brain, liver, kidney through LPO generation, and decreasing cell membranes integrity [23]. Also, SA significantly improves LPO in the liver of rats which received malathion. The antioxidant effects of SA is a strong justification for its liver damage protective. Studies revealed that thymol, carvacrol, p-cymene, γ-terpinene, linalool, and camphor are the main components in the essential oil of *Satureja* species [16].

It is well documented that carvacrol, C6H3 (CH3) (OH) C3H7, 1,8-cineol, and C10H18O in the hydroalcoholic extracts of SA have antioxidative effects. The findings of Ahmadvand et al. showed that *Satureja* khuzestanica essential oil has a beneficial impact on the antioxidant enzymes activities in alloxan-induced Type 1 diabetic rats. *Satureja* khuzestanica significantly increased the serum level of glutathione and the serum activity of glutathione peroxidase, superoxide dismutase, and catalase in the treated group compared with the untreated diabetic group [24]. The antioxidant activity of SA is related to its rosmarininc acid contents. SA suppresses oxidative stress and inflammation in the serum of animals poisoned by malathion [25].

In addition, treatment by SA significantly increased TAC and TTG, which can be attributed to the presence of antioxidant ingredients in the hydroalcoholic extract of this plant. The study about the effect of *Satureja* khuzestanica essential oil on male rat fertility has shown that *Satureja* khuzestanica can improve male rat fertility by its antioxidative effect [26]. It has been widely accepted and proven that the phenolic content of a plant extract is associated with its antioxidant activity. The total phenolic of methanolic extract of SA was reported using the Folin-Ciocalteu assay (95.3 mg GAE/g sample) [17]. The other study investigated the protective effect of *Satureja* montana extract against cyclophosphamide-induced testicular injury in rats. *Satureja* montana also suppressed LPO and significantly enhanced the lowered TAC [27].

Liver tissue is the largest glandular organ in the body and performs many vital functions to keep the body pure of toxins and harmful substances. Without a healthy liver, a person cannot survive. High levels of aminotransferases (ALT, AST) are the most sensitive and widely used markers for the detection of liver injury [9]. Farrokhi et al. animal study about the effect of malathion insecticide on liver tissue and enzymes showed that ALT increased significantly 23 days after exposure to malathi-

on [28]. Assaei et al. reported that *Satureja* khuzestanica essential oil lowered AST and ALT levels [29]. Similar to other studies, we demonstrated that SA decreased liver enzyme activity in the serum of rats. SA seems to preserve the structural integrity of the hepatocellular membrane, as evidenced by a significant reduction in the activities of the liver aminotransferases.

Conclusion

The main mechanism of malathion hepatotoxicity is the production of ROS. According to our study, the use of *Satureja* species is very helpful in the treatment of malathion-induced liver injury in the poisoned rats. Further studies are needed to find out, extract, and purify compounds with the antioxidant activity in SA hydroalcoholic extract to elucidate the cellular and molecular mechanisms of SA action in various tissues.

Ethical Considerations

Compliance with ethical guidelines

The study followed the principles of the declaration of Helsinki and was approved by the Medical Ethics Review Board of Hamadan University of Medical Sciences (IR.UMSHA.REC.1395.56).

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Authors' contributions

Conceptualization: Nejat Kheiripour, Akram Ranjbar; Methodology: Nejat Kheiripour, Negar Mehri, Hassan Ghasemi; Writing-original draft: Dara Dastan, Farzaneh Kazemi Najafabadi, Narges Dehkhodaei; Funding acquisition: Akram Ranjbar; Supervision: Nejat Kheiripour; Writing-review & editing, investigation: All author.

Conflict of interest

The authors declared no conflict of interest.

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