

The Inhibitory Effect of *Satureja khozestanica* Essential Oil and Carvacrol on Nitric Oxide Production in Macrophage Cell Line

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Article information	Abstract
<p>Article history: Received: 2 Sep 2012 Accepted: 10 Jan 2013 Available online: 26 May 2013 ZJRMS 2014; 16(3): 31-34</p> <p>Keywords: Satureja Khozestanica Carvacrol J774A.1 macrophage cell line Nitric oxide Inflammation</p> <p>*Corresponding author at: Department of Immunology, Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran. E-mail: ghasemmosayebi@arakmu.ac.ir</p>	<p>Background: <i>Satureja khozestanica</i> is among the native Iranian plants which grow mostly in Lorestan and Khozestan. A number of reports show that <i>Satureja</i> plant possesses anti-inflammatory effects. The aim of this study is to determine the effects of <i>Satureja khozestanica</i> essential oil on nitric oxide in the presence or absence of lipopolysaccharide in J774A.1 macrophage-like cell line.</p> <p>Materials and Methods: J774A.1 cells are cultured at a concentration of 10⁶ cells per ml in RPMI 1640 medium with 10% fetal Bovin serum. Then, the cells were treated in the presence of lipopolysaccharide (1µg/ml) containing 0.004%, 0.008%, and 0.016% doses of <i>Satureja</i> essential oil and 0.004, 0.008, and 0.016 macromolar of carvacrol over 12, 24, and 48 hours. Next, the level of nitric oxide in the cell culture supernatant was measured using Griess's technique.</p> <p>Results: The results showed that <i>Satureja khozestanica</i> extract significantly decreased the production of nitric oxide in J774A.1 macrophage cell line, dependent on dose and time. In addition, in the presence of lipopolysaccharide, <i>Satureja khozestanica</i> extract inhibited the production of nitric oxide more effectively than carvacrol.</p> <p>Conclusion: The results demonstrate that <i>Satureja khozestanica</i> essential oil is effective in lowering inflammation through inhibition of nitric oxide production. Therefore, this extract may be effective in treatment of inflammatory diseases.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Satureja khozestanica Jamzad is an herbaceous, aromatic, and endemic plant in Southwestern Iran, with distribution in Lorestan and Khozestan. This new species was introduced in 1994 by Jamzad [1]. In the recent years, several studies have been conducted on the extract from this plant to find out about its different treatment benefits including: antimicrobial [2], anti-inflammatory and analgesic [3], fertility improvement [4], antioxidant and anti-diabetic, and lowering plasma triglycerides. In addition, no sign of toxicity was observed in the pregnant rats treated by this plant [5].

The major components of the *Satureja khozestanica* essential oil are carvacrol (86.29%), paracemenu (3.35%), terpinol (2.11%), Mersin (1.16%), and other known compounds that constitute a small percentage of the essential oil [6]. Carvacrol as one of the main compounds of *Satureja khozestanica* is a monoterpenoid phenol. Several reports on different properties of carvacrol such as antioxidant, antimicrobial, antibacterial, anti-parasitic, anti-inflammatory and analgesic, antispasmodic, and anti-cell proliferative have been provided [7]. Nitric oxide (NO) is a product of amino acid arginine metabolism which is found in many tissues. It is among the most important inflammatory mediators. Nitric oxide relaxes the muscles within the walls of blood vessels, resulting in dilation of them and so blood pressure reduction;

therefore, it is known as endothelium-derived relaxant factor [8]. However, NO is biologically essential, but overproduction of it in the patients with acute infections can be harmful due to over-reduction of blood pressure and induction of septic shock [9]. Given that some of the previous reports maintain anti-inflammatory properties of *satureja* plant and that the effect of this plant on nitric acid production, as an important anti-inflammatory factor, has not been investigated, this study examine the effect of *satureja* essential oil on J774A.1 cell line in vitro condition.

Materials and Methods

Cell line: In this study, murine macrophage-like cell line J774A.1 was used provided by National Cell Bank of Iran (code NCBI-C483). Preparation of *Satureja khozestanica* essential oil. After collecting *Satureja khozestanica* (both wild and agricultural types) within Khorramabad, Lorestan, it was identified by the Rangeland, Forestry, and Medical Plants Research Institute of Iran. Dried powder of the aerial part of the plant were mixed and boiled in a pot for five hours by distillation in distilled water using Clevenger apparatus. Water and essential oil mixture was evaporated and the vapor mixture entered into the cooler through an interface tube. Due to the

density difference between water and the oil, the obtained yellow oil was isolated. Then, the suspended water particles were observed by sodium sulfate and stored at 4°C [10]. The effect of carvacrol and *Satureja khozestanica* essential oil on NO production first, J774A.1 macrophage cells were cultured in RPMI 1640 with fetal Bovin serum 10%, containing 100 µg/ml streptomycin and 100 µg/ml penicillin, at the concentration of 10⁶ cell/ml. Then, the cultured cells were treated with Carvacrol and *Satureja khozestanica* oil (0.004%, 0.008%, and 0.016 %) for 12, 24, and 48 hours, in the presence and absence of lipopolysaccharide (1 µg/ml). Cell culture supernatants were collected to measure nitric oxide and then frozen at -20°C.

Nitric Oxide Measurement: Nitric oxide measurement was done using Griess's technique [11], in that by preparation of ternary serial dilutions of 100 nm sodium nitrite, the standard curve was plotted. An amount of 100µl of each serum sample was, dually, poured into a 96-well plate. In addition, 100 µl of sulfanilamide solution (1 g sulfanilamide in 100 cc phosphoric acid 5%) was added to the wells, containing the sample and standard. The plate was incubated for 5-10 min in the dark at room temperature. Then, 100 µl of NED solution "N-(1-N-naphthyl) ethylenediamine dihydrochloride" was added to the wells and the plate was again incubated for 5-10 min in the dark at room temperature. The reading for light absorption was taken after half an hour employing spectrophotometer at the wavelength of 530 µm, and the samples nitrate was quantified using the standard curve.

Results

Data analysis was carried out using one-way ANOVA and post hoc multiple comparisons employing Turkey test. The significance level was set at less than 0.05.

In this study, *Satureja khozestanica* essential oil (0.004, 0.08, and 0.016 doses) was incubated, in the presence and absence of lipopolysaccharide, with J774A.1 macrophage cells over 12, 24, and 48 hours. Then, the production of nitric oxide was measured at the mentioned periods. The obtained results showed that incubation with *satureja* and carvacrol essential oil for 12 hours with 0.018 and 0.008 doses caused inhibition of nitric oxide production, and with 0.004 dose led to reduction of nitric oxide production. Compare with carvacrol, *satureja* has more inhibitory impact on the production of nitric oxide (Fig. 1). Incubation with carvacrol and *satureja* essential oils for 24 hours with 0.016 dose, in the absence of lipopolysaccharide, caused inhibition of nitric oxide production, while in treatment with 0.008 dose, *satureja* essential oil had more declining impact on the production of nitric oxide (Fig. 2). Twenty-four hours after incubation with 0.016 dose of *satureja* essential oil, in the presence of lipopolysaccharide, a reduction in the nitric oxide production was observed. Similarly, carvacrol decreased nitric oxide production. Compared with carvacrol, treatment with 0.008 and 0.004 doses of *satureja* essential oil causes further reduction of nitric oxide production (Fig. 3). Forty-eight hours incubation

with 0.016, 0.08, and 0.004 doses of *Satureja Khozestanica* and carvacrol, in the absence of lipopolysaccharide, results in further reduction of nitric oxide by carvacrol than *Satureja khozastanica* (Fig. 4). Therefore, in the similar condition and in the presence of lipopolysaccharide, the declining effect of *satureja* essential oil on macrophage-like cell-produced nitric oxide is higher than that of carvacrol (Fig. 5). The findings demonstrate that the effect of different doses of *Satureja Khozestanica* and carvacrol on inhibition of NO production over 12, 24, and 48 hours, in the presence and absence of lipopolysaccharide, is dose dependent with significant differences. This significant difference is 0.014 ($p=0.014$) for *satureja* essential oil and 0.02 ($p=0.02$) for carvacrol. Inhibition of NO production under the treatment of J774A.1 macrophage-like cells with 0.004, 0.08, and 0.016 doses of carvacrol and *satureja khozestanica* essential oil, in the presence of lipopolysaccharide, over 12, 24, and 48 hours shows significant changes ($p=0.042$), indicating dose-dependent changes. Therefore, this reduction of NO production, in the absence of lipopolysaccharide, was not significant. The results demonstrate that the effect of *Satureja Khozestanica* essential oil and carvacrol under the treatment with 0.004 and 0.008 doses in incubation over 12, 24, and 48 hours significantly and time-dependently decrease NO production. This effect significantly changes by increasing the time ($p=0.03$). Hence, these changes under the treatment with 0.016% dose were not significant, indicating that NO production changes depended on incubation time in low and medium concentrations of essential oil. The comparison between the effect of *Satureja khozestanica* and carvacrol on the inhibition of NO production, in the absence of lipopolysaccharide, revealed no significant difference. Therefore, a significant difference is seen in the presence of lipopolysaccharide in form of greater impact of *Satureja khozestanica* essential oil on the inhibition of NO production in comparison with carvacrol at the same concentration.

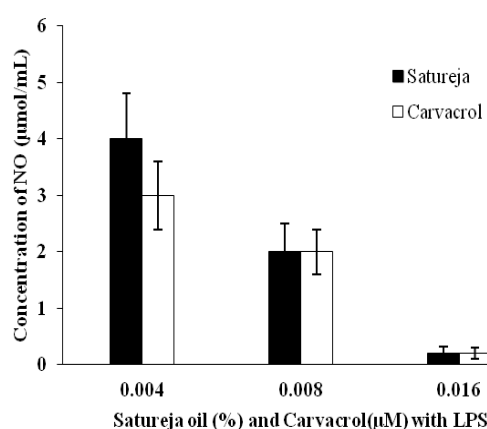


Figure 1. Comparison between the effects of different doses of *satureja* essential oil and carvacrol on J774A.1 macrophage cell line with respect to nitric oxide production, in the presence of lipopolysaccharide, after 12 hours

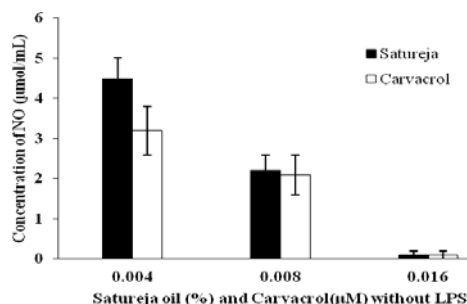


Figure 2. Comparison between the effects of different doses of Satureja essential oil and carvacrol on J774A.1 macrophage cell line with respect to nitric oxide production, in the absence of lipopolysaccharide, after 24 hours

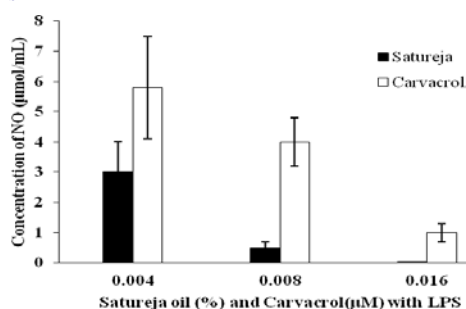


Figure 3. Comparison between the effects of different doses of Satureja essential oil and carvacrol on J774A.1 macrophage cell line with respect to nitric oxide production, in the presence of lipopolysaccharide, after 24 hours

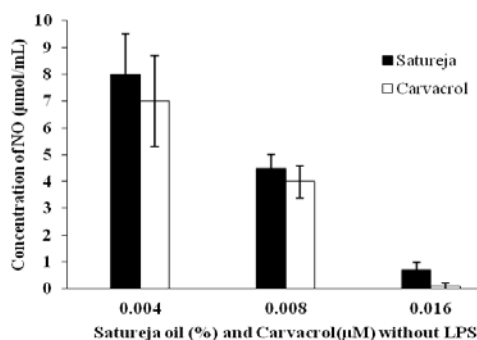


Figure 4. Comparison between the effects of different doses of Satureja essential oil and carvacrol on J774A.1 macrophage cell line with respect to nitric oxide production, in the absence of lipopolysaccharide, after 48 hours

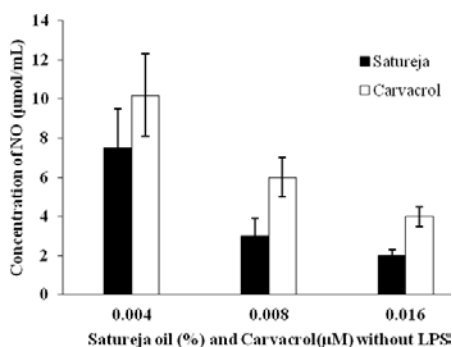


Figure 5. Comparison between the effects of different doses of Satureja essential oil and carvacrol on J774A.1 macrophage cell line with respect to nitric oxide production, in the presence of lipopolysaccharide, after 48 hours

Discussion

It has been reported in the previous studies that anti-inflammatory effect of satureja essential oil on the treatment of inflammatory bowel disease-induced rats is significantly higher than that of prednisolone [12]. In addition, important anti-inflammatory effects of *Satureja khozestanica*, compared with indomethacin and morphine, have been published [13]. In the present study, the effect of *Satureja khozestanica* essential oil on the production of nitric oxide, as an important inflammatory factor in J774A.1 cell line, in vitro condition has been investigated. The results from the present study demonstrated that *Satureja khozestanica* essential oil over 12, 24, and 48 h intervals, in the presence and absence of lipopolysaccharide caused reduced production of nitric oxide in J774A.1 macrophage cell line. In this study, the effect of *Satureja khozestanica* on reduction of nitric oxide during 12, 24, and 48 hours of incubation was significant, indicating the role of satureja essential oil in treatment of inflammation through decreasing nitric oxide production.

The effect of *Satureja khozestanica* on reduction of nitric oxide production, in the presence of lipopolysaccharide, is higher than carvacrol. Regarding that carvacrol is the major compound of this plant, therefore it can be said that some compositions in *Satureja khozestanica* are involving in reduction of nitric oxide production. A large number of studies have been published on the properties of *Satureja khozestanica* essential oil: A study has introduced *Satureja khozestanica* essential oil with several antioxidant properties [14]; another study used cyclophosphamide to induce cystic hemorrhagic in the rats, and then they were treated with *Satureja khozestanica* essential oil, leading to the reduction of free radicals production and protection against hemorrhagic [15]. Improvement of fertility indicators in the male and female rats may be due to antioxidant effect of SKEO [4]. The effect of *Satureja khozestanica* essential oil on the liver of diabetic rats led to significant reduction of phosphoenol pyruvate carboxycausekinase activity and so lowering of plasma glucose. It seems that this effect is associated with antioxidant properties of SKEO [16]. The protective impact of *Satureja khozestanica* extract on inhibition of glomerular changes in diabetic-unilateral nephrectomy rats is attributed to its antioxidant properties [17].

Regarding the previous studies mentioned earlier with respect to several properties of *Satureja khozestanica* essential oil, the majority of them emphasize on its strong antioxidant property. Given that nitric oxide is a free radical and considered to be an active species of nitrogen, it can be expected to be neutralized by antioxidant compounds. In addition, increase in the activity of oxidant substances is one of the important causes of inflammations [18, 19]. Therefore, it is possible that, due to anti-oxidative properties, *Satureja khozestanica* essential oil reduces inflammation through weakening the

factors involved with oxidative stress dependent signaling pathway.

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Authors' Contributions

Jalalvand M. performed all experiments. Shahsavari G. extracted *Satureja* oil and wrote the paper and Mosayebi G. designed and supervised the project.

Conflict of Interest

The authors declare no conflict of interest.

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