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Effect of Satureja mutica extract on serum nitric oxide levels in rats: potential role of quercetin

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Abstract

Introduction: This study aimed to investigate the impact of the hydroalcoholic extract of Satureja mutica (S.mutica), a commonly used plant for cardiovascular diseases in Northern Iran, on nitric oxide levels in the blood.

Materials and methods: Male Wistar rats were divided into three groups, each consisting of 5 rats. The groups included a control group, a group that administered normal saline, and a group that received an extract at a dosage of 100 mg/kg. The normal saline and extract were administered through intraperitoneal injection (IP) once a day for a week. Blood samples were gathered from the heart in order to analyze the serum level of nitric oxide using spectrophotometric analysis.

Results: The serum level of nitric oxide in the groups receiving normal saline did not change significantly compared to the control group, but the serum level of Nitric oxide decreased significantly only in the rats receiving the *Satureja mutica* extract compared to the control group ($P<0.001$). HPLC-PDA results show that the most phenolic compounds present in the extract are Gallic acid, 2,5-Dihydroxybenzoic Acid, Cinnamic Acid, Quercetin and Apigenin. The highest content and percentage of phenolic compounds is Quercetin.

Conclusions: Hydroalcoholic extract of *S. mutica* reduces serum NO levels in rats. Quercetin may contribute to this effect; however, confirmatory studies using isolated compounds are required. Although these findings are promising, more human studies are needed to determine whether this compound could be an alternative or complementary treatment.

Keywords: Hydroalcoholic extract, Satureja mutica, Nitric oxide, Male rats, Blood level

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Introduction

Nitric oxide (NO) is a small molecule with a short half-life and lipophilic properties, and many living cells can to make this molecule (1). In mammals, the production and formation of this molecule vary among different species. In Wistar rats, the production of nitric oxide is reported to be between 0.85-0.33 µmol/kg/h (2). The molecule has a very short half-life in the body, measuring less than 0.1 seconds (3).

The genus *Satureja* (family *Lamiaceae*) contains over 200 species, with several endemic to mountainous areas of Iran. Various biological activities have been reported for *Satureja* species, including antimicrobial, anti-inflammatory, and antioxidant effects (4). Nitric oxide (NO) is a small, short-lived, lipophilic molecule produced by many cell types. NO plays essential roles in vascular homeostasis, including vasodilation, inhibition of platelet aggregation, and regulation of blood pressure. Excessive NO production occurs in pathological conditions such as hypertension, atherosclerosis, septic shock, and ischemia (5-8). Among the biological processes in which NO plays a role are the regulation of reproductive actions (8), lipolysis, and regulation of energy balance (9). However, there is limited scientific evidence on the impact of *Satureja mutica* extract on NO modulation, particularly in vivo. This study addresses this gap by evaluating the extract's effect of *S.mutica* on serum NO levels in rats and identifying its major phenolic constituents. Therefore, this study was conducted in response to the abundance of evidence in Guilan province's traditional medicine indicating that regular use of *Satureja mutica* can enhance heart function.

Materials and methods

Samples

The present study follows an experimental laboratory approach, where samples under treatment are compared to a control group**.** Male Wistar rats, which were adults and weighed 190 ± 10 grams, were sourced from the Pasteur Institute of Iran. The rats were housed in a special animal room at a temperature of 25°C±2°C degrees Celsius, with a 12-hour light and 12-hour dark cycle.

Animals Grouping

Animals were randomly assigned to three groups (n=5 each): (1) control (no treatment), (2) saline group (intraperitoneal normal saline), and (3) treatment group (*S. mutica* extract 100 mg/kg/day, IP, for 7 days). They had access to unlimited food and water, which provided in the form of ready-made mouse feed from Pars Animal Factory. The rats randomly divided into groups, and each group was assigned a number for identification. The rats were adapted to the presence of the researcher. All procedures complied with the Institutional Animal Care and Use Committee guidelines at Guilan University of Medical Sciences. Animals were humanely euthanized under anesthesia before blood collection.

Extract preparation and administration

The botanist Dr. Mahdavi collected and identified the *S. mutica* plant in the vicinity of Rostam Abad city in Guilan province. The plant was given the code Herbarium 7011 and then taken to the Herbarium at the Guilan Agricultural Education and Natural Resources Research Center. Initially, the plant's leaves were cleaned before being left to dry in the shade for a week. Subsequently, the dried leaves were ground into powder using an electric mill, then the powder was dissolved in 80% ethanol. Following filtration of the solution, a rotary machine was utilized to separate the solvent from the extract. Finally, the extract was dried and an aqueous solution was obtained by adding normal saline. The intraperitoneal injections of the extract were administered once daily at 10 am for a duration of 7 days. Upon finishing the experiments at the end of a week, blood samples were obtained from the animals. The animals were anesthetized with ether before blood collection, and blood was drawn from the heart. Subsequently, serum was separated from the blood samples using a routine method. The serum nitric oxide level was measured through the spectrophotometry method. Spectrophotometric analysis of nitric oxide and nitrites in biological samples using the Griess reagent relies on a series of chemical reactions. These reactions involve diazotization followed by coupling. The resulting azo compound from Griess reactions exhibits absorbance in

the ultraviolet and visible spectrum, ranging from 300 to 700 nm (10).

HPLC-PDA separation

A new method using high-performance liquid chromatography with a photodiode array detector (HPLC/PDA) was used to measure phenolic compounds in extract of *S. mutica* accurately. HPLC-PDA was used to examine organic compounds. HPLC separates these compounds by their interaction with the stationary phase, while PDA measures absorbance at various wavelengths, offering details on the quality and quantity of the compounds in the sample.

Separation of phenolic compounds extracted from *Satureja mutica* extract was performed by using an ethanol/methanol/formic acid/water solution with HPLC-PDA, detected at 280 nm (A) and 520 nm (B). The column used was Luna RP-C18(2) (250 × 2.0 mm I.D., 5 µm) with a C18 guard cartridge column (4 × 2.0 mm I.D.) from Phenomenex. The compounds were eluted using a multi-segment linear gradient, with a flow rate of 0.2 mL per minute.

Statistics

The statistical analysis was conducted using the one-way analysis of variance (ANOVA) method in SPSS version 17. This approach was selected to determine whether there were statistically significant differences among the means of the different experimental groups. Following the ANOVA test, Benferoni's post hoc test was applied to perform multiple pairwise comparisons between groups, thereby identifying the specific group differences responsible for the overall statistical significance. Throughout the analysis, a p-value of less than 0.05 ($p < 0.05$) was considered the threshold for statistical significance, indicating that the observed differences were unlikely to have occurred by random chance.

Results

Table 1 presents the nitric oxide levels in the analyzed groups. The findings suggest that the nitric oxide levels in the group that was administered normal saline did not exhibit a notable alteration in comparison to the control group. However, there was a considerable reduction in nitric oxide levels in the rats that were given the extract when contrasted with the control group, only following the administration of *Satureja mutica* extract ($P<0.001$).

Figure 1 shows peaks identification: Galic acid; 3.,4 dhb; Chlorogenic acid; Cathechin Caffeic acid; Vanilic acid; 2.5 dhb; Syrginic acid; P-cumaric acid; Ferrulic acid; Rutin; Salycilic acid; Rosmarinic acid; Cinamic acid; Quercetin; Kaempferol and Apigenin.

The chromatogram (Figure 1) displays the separation and detection of chemical compounds present in the *S. mutica* extract using High-Performance Liquid Chromatography (HPLC) coupled with a Photodiode Array (PDA) detector. Each peak represents a distinct compound, with the X-axis indicating retention time (compound separation time) and the Y-axis showing signal intensity (relative concentration). Based on the plant's phytochemical profile, major peaks likely correspond to terpenes (e.g., carvacrol, thymol) and phenolic compounds such as quercetin, rutin, or rosmarinic acid. The varying peak heights and widths suggest differences in compound abundance and purity, with sharper peaks indicating well-separated components**.** This HPLC-PDA analysis serves as a fingerprint for the extract's chemical composition, highlighting bioactive phenolics like quercetin, which may explain the biological activities observed in Figure 2. The method's precision allows for qualitative identification of compounds, though exact confirmation would require comparison with reference standards or mass spectrometry (MS) data. The presence of these compounds underscores the extract's potential pharmacological value, particularly in studies involving antioxidant or anti-inflammatory effects. Further analysis could quantify specific compounds and explore their synergistic interaction**s**.

The information is presented as "mean ± standard deviation". P values obtained from one-way analysis of variance are used to compare and indicate differences from the control group. The label NS indicates that there is no statistically significant difference compared to the control group.

Table 1. NO serum level in the different groups following the administration of *Satureja mutica* extract.

P value	NO(SEM) (µmol/L)	Group
-	99.37 ± 9.72	Control
NS	55.28 ± 6.2	Normal Saline
P<0.001	10.87±44.12	Extract (100 (mg/kg

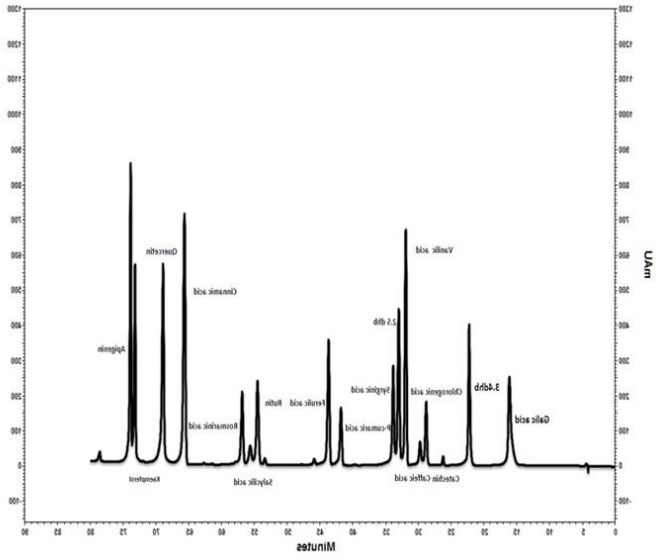

Figure 1. Chromatogram of *Satureja mutica* extract in HPLC-PDA.

Discussion

The Lamiaceae family consists of over 200 species in the genus Satureja L., primarily found in the Mediterranean region. Among them, eight species are unique to mountainous areas in Iran, especially Guilan provinve, north part of Iran (11). The results demonstrated that there was no significant change in the serum nitric oxide level in the group treated with normal saline compared to the control group. This suggests that administering the extract through injection did not impact the test outcomes. In contrast, it was found that the serum nitric oxide level in rats treated with the hydroalcoholic extract of *S. mutica* significantly decreased in comparison to the control group (P<0.001).

Several studies have shown that the essential oil of *Satureja* species contains high levels of monoterpenoids and phenolic compounds like carvacrol, γ-terpinene, thymol, and p-cymene. The antimicrobial properties of the essential oil and extract of particular *Satureja species* have been documented (12). While extracts, bioactive fractions, or compounds derived from medicinal plants serve various purposes, the techniques employed to obtain them remain mostly consistent regardless of the intended biological testing. The core steps in obtaining high-quality bioactive molecules include selecting a suitable solvent, utilizing extraction methods, conducting phytochemical screening procedures, employing fractionation methods, and utilizing identification techniques. In this research, two frequently used polar solvents (water and alcohols) were utilized for Soxhlet extraction (10).

Nitric oxide synthase (NOS) comprises three different isozymes that play a role in producing NO: the constitutive endothelial (eNOS) and neuronal (nNOS) isozymes, as well as the inducible isozyme. It is understood that the inducible isozyme (iNOS) is present in various cell types, including cardiac myocytes. iNOS is typically activated in response to a range of physiological and pathophysiological triggers, such as vigorous exercise and hypoxia(13-14). *Echinodorus grandiflorus*, also known as Burhead, is utilized in traditional Brazilian medicine as a diuretic treatment. The herb stimulates prolonged urine production and lowers blood pressure by interacting with muscarinic and bradykinin receptors, affecting pathways related to prostaglandins and nitric oxide (15). *Stephania tetrandra* can help control hypertension by decreasing the expression of inducible nitric oxide synthase (iNOS) and inhibiting Ca^{2+} channels. The alkaloid tetrandrine, found in this plant, possesses anti-inflammatory and antioxidant properties that likely contribute to its ability to lower blood pressure (16). The Tianma methanolic extracts (at a concentration of 0.02 ml/g) demonstrated anti-inflammatory effects by reducing iNOS expression and levels of NO (17).

The highest percentage of phenolic compounds in the *Satureja mutica* extract was quercetin. Quercetin, a flavonoid present in various fruits, vegetables, and grains, possesses potent antioxidant and anti-inflammatory properties. Some research suggests that quercetin can boost the activity of endothelial nitric oxide synthase (eNOS), the enzyme responsible for generating nitric oxide in blood vessels, thereby assisting in improving endothelial function and promoting vasodilation (13-14). Acting as a robust antioxidant, quercetin could safeguard nitric oxide from degradation by reactive oxygen species (ROS), preserving its availability and efficacy. The results of this study probably suggest that if a high percentage of a plant's polyphenolic compounds is quercetin, changes or effects on the cardiovascular system can be expected. Nonetheless, further research is necessary to fully comprehend the extent and mechanisms of quercetin's impact on nitric oxide and overall cardiovascular function. Inhibition of nitric oxide production can be achieved through pharmacological means, such as blocking NOS activity or downstream signaling molecules. Pharmacological and non-pharmacological approaches are not considered in this study.

Mechanisms by Which Quercetin May Decrease NO Levels

1) **Inhibition of Inducible Nitric Oxide Synthase (iNOS)**

In inflammatory conditions, quercetin has been shown to suppress the expression of iNOS, an enzyme responsible for high-output NO production. This suppression occurs through the inhibition of the NF-κB signaling pathway, leading to reduced NO synthesis in activated immune cells**.**

2) **Suppression of Endothelial Nitric Oxide Synthase (eNOS) Expression**

Some studies suggest that quercetin can downregulate eNOS expression in endothelial cells, particularly under pro-inflammatory stimuli like TNF-α. This downregulation may result in a decrease in NO production, affecting vascular tone and blood pressure regulation.

3) **Modulation of Neuronal Nitric Oxide Synthase(nNOS)**

Quercetin's effects on nNOS are less well-defined, but there is evidence indicating that it may influence nNOS activity, potentially impacting NO levels in neuronal tissues.

The observed upregulation of iNOS mRNA and protein by quercetin, coupled with decreased NO production, holds significant implications for cancer biology. iNOS-derived NO plays a dual role in cancer progression, acting as either a pro-tumor or anti-tumor agent depending on concentration and context. At high levels, NO can promote DNA damage and angiogenesis, fueling tumor growth, while at low levels, it may suppress immune responses. Quercetin's ability to modulate this balance suggests its potential as a chemopreventive agent, particularly in cancers where chronic inflammation drives tumorigenesis, such as colorectal or breast cancer. Targeting the iNOS/NO pathway with phenolic compounds like quercetin could offer a strategic approach to cancer therapy. By reducing excessive NO, quercetin may mitigate inflammation-induced carcinogenesis while preserving anti-tumor immunity. However, the paradoxical effects increased iNOS expression but decreased NO, warrant further investigation to optimize dosing and avoid unintended pro-tumor effects. Clinical studies are needed to validate these mechanisms in human models and explore synergies with conventional therapies, potentially positioning quercetin as an adjunct in precision oncology (Figure 2).

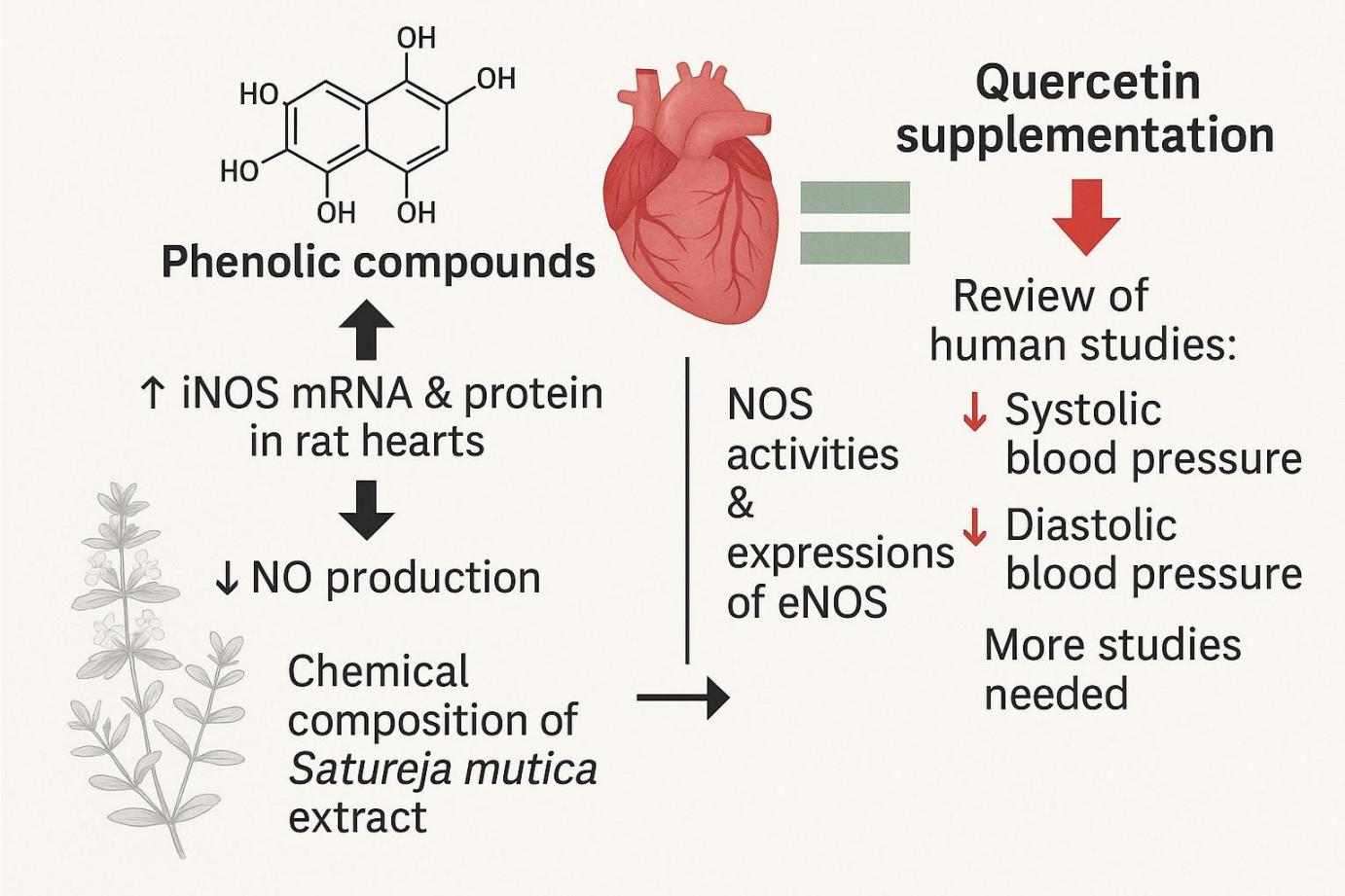

Figure 2. It is likely that phenolic compounds, mainly quercetin, significantly increased the levels of iNOS mRNA and protein in rat hearts, indicating a rise in the baseline expression of iNOS mRNA and protein, which led to a decrease in NO production.

Limitations

While this study provides valuable insights into the NO-modulating effects of *Satureja mutica* extract, several limitations must be acknowledged**:**

Small Sample Size: The study used only 5 rats per group, which may limit the statistical power and generalizability of the findings. Larger cohorts are needed to validate the observed effects**.**

Short-Term Administration: The 7-day treatment period may not reflect the long-term physiological impacts of *S. mutica* extract. Chronic exposure studies could reveal cumulative or adaptive effects on NO metabolism**.**

Lack of Mechanistic Depth: Although quercetin was identified as a major phenolic compound, the exact molecular pathways (e.g., iNOS/eNOS modulation, NF-κB inhibition) were not experimentally verified. Isolating quercetin or using knockout models would clarify its specific role**.**

Single-Dose Testing : Only one dose (100 mg/kg) was evaluated. A dose-response analysis would help determine optimal efficacy and potential toxicity thresholds**.**

Species and Model Constraints: Findings in healthy Wistar rats may not translate to diseased models (e.g., hypertensive or inflammatory conditions) or humans**.**

Technical Limitations: Spectrophotometric NO measurement via Griess assay detects only stable metabolites (nitrite/nitrate), potentially underestimating total NO dynamics. Advanced techniques like chemiluminescence or ESR spectroscopy could improve accuracy**.**

Future Research Directions

To address these gaps and expand on the current findings, future studies should elucidate mechanism and investigate quercetin's direct effects using purified compounds and siRNA/iNOS inhibitors**.**

Assess transcriptional regulation of NOS isoforms (iNOS, eNOS) via qPCR/Western blot**.**

Expand Experimental Design : Include multiple doses (e.g., 50–200 mg/kg) and longer treatment durations**.**

Test in disease models (e.g., hypertension, atherosclerosis) to evaluate therapeutic potential**.**

Enhance Analytical Methods :Employ LC-MS/MS to quantify quercetin and other phenolics in serum/tissues**.**

Use real-time NO sensors to capture dynamic changes**.**

Clinical Translation : Conduct pharmacokinetic studies to assess bioavailability and safety in humans**.**

Explore synergistic effects with standard cardiovascular therapies**.**

Broader Phytochemical Profiling **:**Investigate interactions between quercetin and other S. mutica compounds (e.g., apigenin, rosmarinic acid) to identify additive or antagonistic effects**.**

By addressing these limitations, future work could solidify *S. mutica* extract's role in NO modulation and its potential as a complementary therapy for cardiovascular diseases.

Conclusion

In this study, the protective effects of both quercetin and gallic acid by gavage on aluminum-induced brain damage were investigated. As the results show, the nanocapsule form of both quercetin and gallic acid by gavage at a lower dose can protect against aluminum-induced damage by generating a protective effect, and the nanocapsule form does not have the adverse effects that the free form of the drug causes. Therefore, the nanocapsule form of quercetin and gallic acid may offer a promising direction for future preclinical studies to protect against the adverse effects of aluminum.

Author contribution

AJ and **EMF** were responsible for the study's concept, design, and thorough review of the manuscript to ensure its intellectual significance. **AAF** re-evaluated the data. **AJ** made revisions to the manuscript and incorporated additional professional insights.

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Conflicts of interest

There are no conflicts of interest.

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