



# Hypoglycemic effect of *Satureja montanum* L. hydroethanolic extract on diabetic rats

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## ABSTRACT

**Introduction:** Diabetes is one of the most prevalent metabolic disorders which is associated with several complications in different organs. Nowadays, medicinal herbs are being widely used to treat diseases. This study was conducted to study the hypoglycemic effect of *Satureja montanum* in diabetic male rats.

**Methods:** In this study 42 male Wistar rats were randomly assigned to 7 equal groups including control, diabetic control, treatments 1, 2, and 3, metformin-treated diabetic, and healthy treated with *Satureja montanum*. To induce diabetes streptozotocin (STZ) at 60 mg/kg was intraperitoneally (ip) administered. The animals were treated daily with *Satureja montanum* extract (ip) for one week and their blood glucose was measured daily.

**Results:** *Satureja montanum* extract could significantly decrease blood glucose. The greatest effect of the extract was seen on day 8 at 800 mg/kg ( $P < 0.001$ ). *Satureja montanum* extract caused a significant increase in serum insulin compared to the control group ( $P < 0.001$ ).

**Conclusion:** The findings of this study indicate that *Satureja montanum* hydroethanolic extract is able to significantly decrease blood glucose of diabetic rats possibly with a stimulatory effect on beta cells.

## Implication for health policy/practice/research/medical education:

*Satureja montanum* hydroethanolic extract can significantly enhance blood insulin of diabetic rats, decreasing blood glucose, hence, it might be beneficial in diabetic patients.

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## Introduction

Diabetes is one of the most prevalent metabolic disorders which is associated with several complications in different organs (1). This disease is characterized by hyperglycemia and disorder of metabolism of carbohydrates, lipids, and proteins. Diabetes is one of the most prevalent and costly chronic diseases worldwide and is increasing in prevalence because of variations in lifestyle and also improved health-care in communities, leading to enhanced survival (2).

Insulin is one of the hormones contributing differently to metabolism and other body processes. Insulin controls the storage and metabolism of carbohydrates, proteins, and lipids. It also enhances active transfer of glucose from tissue cell membrane of lipid and muscle, is involved in converting glucose and intracellular free fatty acids into reserves of glycogen and triglycerides, increases conversion of hepatic glucose into glycogen, and inhibits glucose

output from liver. Intravenous administration of insulin causes stimulation of growth hormone through decreasing glycemia (3).

Inadequate monitoring of blood sugar, genetic predisposition including family history, smoking, obesity, physical inactivity, hypertension, and hyperlipidemia are some of the risk factors for diabetes (4).

Free radicals are chemically active molecules. The production of free radicals in cells is a natural process of body metabolic reactions. Normally, these radicals are removed by the enzymes and antioxidant compounds in the body. Lack of balance between contribution of antioxidant defense and increased production of free radicals leads to conditions called oxidative stress. The diabetes-derived hyperglycemia causes intensified indices of oxidative stress such as membrane lipids peroxidation (5).

Medicinal herbs are considered as a very suitable alterna-

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tive to synthetic drugs because of easy access, fewer side effects, and less cost. Plant-derived biological substances are a subdiscipline of modern pharmacotherapy of diseases (6). Summer savory, scientifically called *Satureja montanum*, is a medicinal herb from Lamiaceae.

This genus consists of approximately 30 species, of which 12 occur in and 8 are exclusively unique to Iran (7). In traditional medicine, analgesic and disinfectant properties have been mentioned for *Satureja montanum* (8). *Satureja montanum* is used to treat thoracic diseases, cough, thinness, and rheumatologic and neurological pains (9). Use of *Satureja montanum* leaf extract causes decrease in hypercholesterolemia (10). Some studies have demonstrated its anti-inflammatory and antimicrobial properties, as well (11,12).

Aerial parts of *Satureja montanum* have many applications in food and pharmacologic industries and could be used as a strong and natural alternative to synthetic antioxidants (13).

Studies have demonstrated that *Satureja montanum* extract protects reproductive system against cyclophosphamide toxicity through antioxidant and androgenic activities and is used as a supplement in patients with hepatitis (14). Zeidán-Chuliá et al study demonstrated antioxidant, antimicrobial, and anti-inflammatory properties of *Satureja montanum* (15).

In light of the significance of study of diabetes and no investigation of *Satureja montanum* hydroalcoholic extract on diabetes as a significant and prevalent disease, this study was conducted to study the hypoglycemic effect of *Satureja montanum* extract in diabetic male Wistar rats.

## Materials and methods

### Plant preparation and extraction

*Satureja montanum* was obtained from the Herbarium unit of Agriculture of Jihad Organization of Hamadan province and was transferred to laboratory after identification by a botanist in Agricultural Research Center of Hamadan province. After the plants were cleaned, they were shadow-dried in air. The leaves were separated and pulverized by a meal. Two hundred grams of the obtained powder was stored in ethylic alcohol 80% in refrigerator for a week. Then the contents were filtered by a filter paper and the obtained solution was concentrated by rotary at 55 rpm at 60°C. After concentration, the resulting extract was placed under the hood for two days to completely dry. The prepared extract was kept in freezer at -20°C for later uses.

### Laboratory animals

In this study, 42 animals were kept in Animal House of Research Laboratory of Faculty of Sciences, Bu-Ali Sina University, Hamadan at 22±2°C under 12 hours dark/light cycles with free access to water and food. For adaptation to laboratory conditions, the animals were kept in the laboratory for one week before the study. The protocol was

confirmed in Ethical Committee of Bu-Ali Sina University and all ethical standards of animal experimentation (National Institutes of Health, 1985) were followed in the present study.

The rats were randomly assigned to eight equal groups consisting of healthy control, diabetic control intraperitoneally (i.p) administered with streptozotocin (STZ) at 60 mg/kg once, healthy group administered with the extract at 400 mg/kg, diabetic groups i.p administered with various doses of the extract (at 200, 400, and 800 mg/kg) daily for 8 days, and diabetic group treated with gavage metformin at 500 mg/kg daily.

To induce diabetes, STZ (Sigma Co.) at 60 mg/kg was administered i.p. Three days after administration, the rats' glycemia was measured and those with blood glucose over 250 mg/dL were considered as diabetic. The diabetic rats had diabetes symptoms such as polydipsia, diuresis, and weight loss.

After the diabetes was confirmed in the groups of interest, the animals were treated with the extract for one week. The animals were i.p administered with the extract at specified doses according to the grouping on a daily basis. Glycemia was measured and recorded at 3 steps, prior to STZ administration, after diabetes induction on a daily basis for one week, and at the completion of tests. All the animals were sacrificed with no suffering according to ethical principles of animal experimentation and their blood samples were collected. The level of serum insulin was measured by radioimmunoassay kit (Diasorin, Italy). The used kit was compatible with rats' insulin.

The data of studied groups were analyzed by SPSS and expressed as mean ± SEM. For comparison, one-way analysis of variance (ANOVA) was used and for inter-group comparisons Tukey test was used. The level of significance was considered  $P < 0.05$ .

## Results

Seventy-two hours after STZ administration, glycemia increased significantly in the groups administered with STZ compared to the control group not receiving STZ ( $P < 0.001$ ). Treatment with *Satureja montanum* leaf hydroethanolic extract could significantly decrease glycemia in diabetic rats in a dose-dependent manner.

These findings indicated that glycemia decreased significantly in the diabetic rats treated with *Satureja montanum* leaf hydroethanolic extract at 200 mg/kg on day 5 compared to the diabetized control group ( $P < 0.01$ ). Moreover glycemia decreased significantly in the diabetic rats treated with *Satureja montanum* leaf hydroethanolic extract at 400 and 800 mg/kg compared to the diabetic control group ( $P < 0.001$ ). The difference in glycemia between the diabetic group treated with the extract at high dose and the control group was not significant, between metformin-treated diabetic group and control group was significant ( $P < 0.001$ ), and between metformin-treated diabetic group and the groups treated with *Satureja montanum*

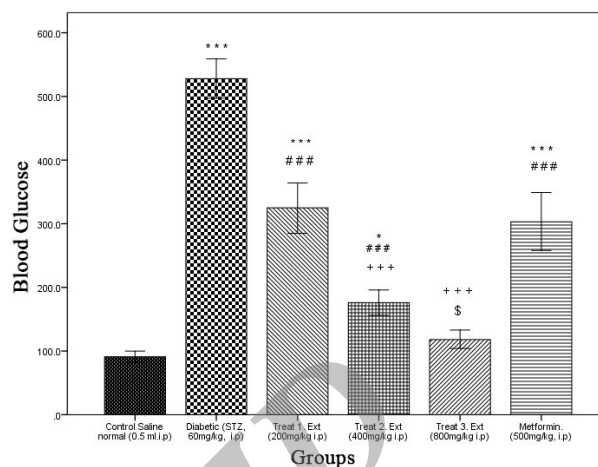
leaf hydroethanolic extract at low and moderate doses was not significant. On the other hand, the difference between metformin-treated group and control group was derived significant ( $P < 0.001$ ; Figure 1).

These findings indicated that glycemia decreased significantly in the diabetic rats treated with *Satureja montanum* leaf hydroethanolic extract at 200 mg/kg on day 6 compared to the diabetic control group ( $P < 0.01$ ). The difference remained significant compared to the control group ( $P < 0.001$ ). Moreover, glycemia decreased significantly in the diabetic rats treated with *Satureja montanum* leaf hydroethanolic extract at 400 or 800 mg/kg compared to the diabetic control group and the group treated with the extract at 200 mg/kg ( $P < 0.001$ ), but the difference in glycemia between the group treated with the extract at 400 mg/kg and the control group with increased glycemia remained significant ( $P < 0.001$ ). The difference in glycemia between the diabetic group treated with the extract at high dose and the control group was not significant, between metformin-treated diabetic group and control group was significant ( $P < 0.001$ ), and between metformin-treated diabetic group and the groups treated with *Satureja montanum* leaf hydroethanolic extract at low and moderate doses was not significant. On the other hand, the difference between metformin-treated group and control group was derived significant ( $P < 0.001$ ; Figure 2).

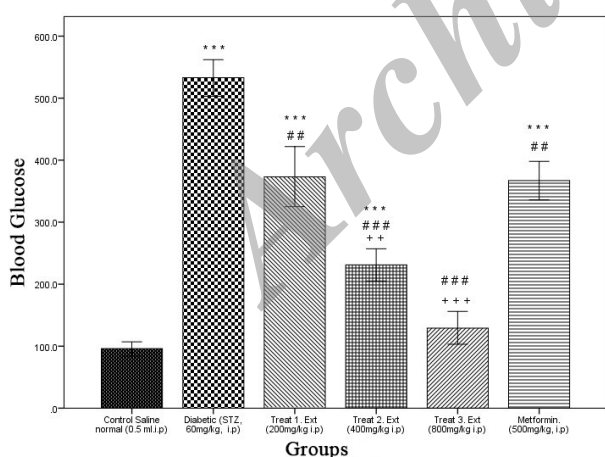
These findings indicated that glycemia decreased significantly in the diabetic rats treated with *Satureja montanum* leaf hydroethanolic extract at different doses on day 8 were similar to day 6 (Figure 3).

The variations in glycemia in any studied groups at different days indicated that *Satureja montanum* leaf hydro-

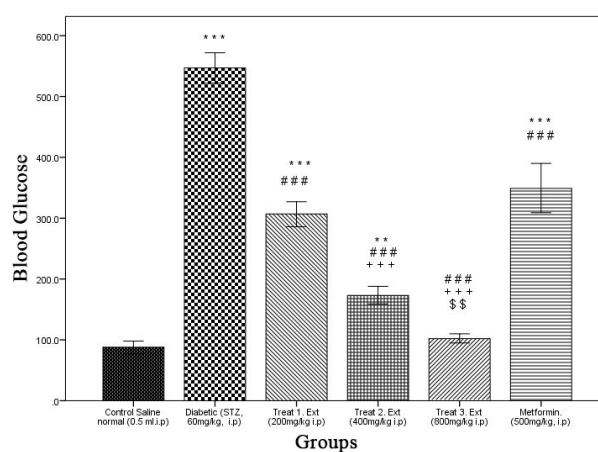
ethanolic extract caused a significant, dose-dependent decrease in glycemia in the rats. This decrease was obtained highest for metformin at day 6, and a significant increase in the rats' glycemia was seen from day 7 afterwards com-



**Figure 2.** Comparison of glycemia in different groups at day 6 of the study; mean  $\pm$  standard error of measurement express the data of male Wistar rats ( $n = 6$ ); \* represents the significance of variation in glycemia of the animals in different groups after treatment in comparison to the control group (\*\*\*  $P < 0.0001$ , \*  $P < 0.05$ ); # represents the significance of variation in glycemia of the animals in different groups in comparison to the untreated diabetized group (###  $P < 0.001$ ); + represents the significance of variation in glycemia of the animals in different groups in comparison to the group administered with the extract at low dose (200 mg/kg) (\*\*\*  $P < 0.001$ ); \$ represents the significance of variation in glycemia of the animals in the group administered with the extract at high dose (800 mg/kg) in comparison to those in the group administered with the extract at moderate dose (400 mg/kg) ( $P < 0.05$ ).

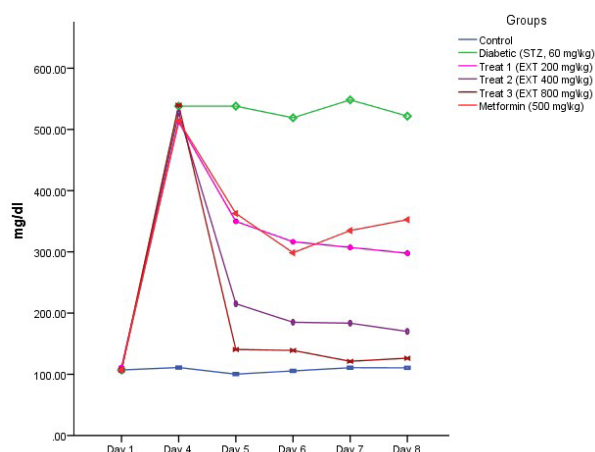


**Figure 1.** Comparison of glycemia in different groups at day 5 of the study; mean  $\pm$  standard error of measurement express the data of male Wistar rats ( $n = 6$ ); \* represents the significance of variation in glycemia of the animals in different groups after treatment in comparison to the control group (\*\*\*  $P < 0.001$ ); # represents the significance of variation in glycemia of the animals in different groups in comparison to the untreated diabetized group (###  $P < 0.01$ , ###  $P < 0.001$ ); + represents the significance of variation in glycemia of the animals in different groups in comparison to the group administered with the extract at low dose (200 mg/kg) (\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).

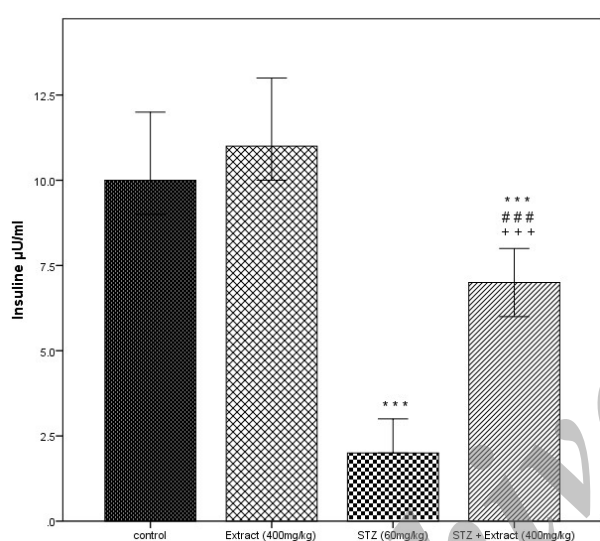


**Figure 3.** Comparison of glycemia in different groups at day 8 of the study; mean  $\pm$  standard error of measurement express the data of male Wistar rats ( $n = 6$ ); \* represents the significance of variation in glycemia of the animals in different groups after treatment in comparison to the control group (\*\*\*  $P < 0.001$ ); # represents the significance of variation in glycemia of the animals in different groups in comparison to the untreated diabetized group (###  $P < 0.001$ ); + represents the significance of variation in glycemia of the animals in different groups in comparison to the group administered with the extract at low dose (200 mg/kg) (\*\*\*  $P < 0.001$ ).





**Figure 4.** Comparison of glycemia in different groups at different days of the study.



**Figure 5.** Comparison of serum insulin among control group, the group treated with extract at moderate dose, untreated diabetic group, and diabetic group treated with extract at moderate dose; mean  $\pm$  standard error of measurement express the data of male Wistar rats ( $n=6$ ); \* represents the significance of variation in serum insulin of the animals in groups in comparison to the control group (\*\* $P < 0.001$ ); # represents the significance of variation in serum insulin of the animals in different groups in comparison to the healthy, extract-treated group (###  $P < 0.001$ ); + represents the significance of variation in serum insulin of the animals in different groups in comparison to diabetic, untreated group (200 mg/kg) (+++  $P < 0.001$ ).

pared to the groups treated with *Satureja montanum* leaf hydroethanolic extract at low, moderate, and high doses ( $P < 0.001$ ; Figure 4). The variations in serum insulin in the groups treated with *Satureja montanum* leaf hydroethanolic extract indicated that *Satureja montanum* leaf hydroethanolic extract at 400 mg/kg caused no significant difference in the treated healthy group. However, *Satureja montanum* leaf hydroethanolic extract at 400 mg/kg caused a significant increase in serum insulin in diabetic group compared to the diabetic control group ( $P < 0.001$ ). However the increase in insulin in this group decreased

significantly compared to the control group and the control group treated with the extract at 400 mg/kg ( $P < 0.001$ ; Figure 5).

## Discussion

The findings of this study indicated that i.p administration of *Satureja montanum* hydroalcoholic extract in short-term significantly affected the glycemia in the studied animals in a positive manner. *Satureja montanum* hydroethanolic extract at low, moderate, and high doses caused a significant decrease blood glucose of the rats with STZ-induced diabetes in a dose-dependent manner.

The studies have indicated oxidative stress and inflammatory damages are caused by hyperglycemic conditions in diabetes (16). The incidence of oxidative stress is higher in the patients with diabetes than the individuals in control group. The production rate of free radicals increases in the patients with diabetes while antioxidant resistance declines in them. As a result, oxidative stress increases in these patients (12). The induction of diabetes in rats caused decline in activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase, in the studied groups. Prolonged diabetes caused decline in catalase, glutathione reductase, GSH-Px and SOD (17).

In traditional medicine, *Satureja montanum* is used as a supplement for patients with diabetes. Studies have indicated that *Satureja montanum* essence could decline free radicals (17,18). In addition, antioxidant, antimicrobial, and anti-inflammatory properties have been confirmed for *Satureja montanum* (12,13,19). Containing several phenolic compounds with strong antioxidant and anti-inflammatory effects, *Satureja montanum* can reduce diabetes-derived oxidative stress. A study found that *Satureja montanum* exerted stronger antioxidant properties than *Mentha piperita* extract. Many studies have pointed to anti-inflammatory and antimicrobial properties of *Satureja montanum* (20).

A study has demonstrated that *Satureja montanum* contains mucilage compounds, resin, and tannin. Moreover limonene 1 and 8 and cineole are other single ring monoterpenes in *Satureja montanum*. Borneol and camphor have been reported as other monoterpenes in this plant (21). Antioxidant property of *Satureja montanum* ethanol extract, which is enhanced as the extract's concentration increases, has been also demonstrated (22). Furthermore some studies have found that *Satureja montanum* essence's antioxidant property is enhanced as its concentration increases (23).

Studies have indicated that there are phenolic monoterpenes such as thymol, and gamma-terpinene in *Satureja montanum*. In addition, *Satureja montanum* extract has been reported to contain carvacrol (24,25). A study has demonstrated that *Satureja montanum* essence' ability to prevent linoleic acid oxidation was very high and approximately equal to BTH's (26).

In light of the present study's and other studies' findings,

*Satureja montanum* could exert anti diabetic properties because of strong antioxidant and anti-inflammatory properties and suppressing oxidative stress following diabetes. As mentioned previously, as the *Satureja montanum* extract concentration increases, its antioxidant property is enhanced. This finding is consistent with the present study as the *Satureja montanum* extract anti diabetic property was enhanced with increase in its concentration. Hence *Satureja montanum* could prevent increase in glycemia in the studied rats through high antioxidant and anti-inflammatory activity.

As mentioned above, *Satureja montanum* antioxidant effects and hence high anti-inflammatory activity demonstrated in several studies could contribute to diabetogenic process of STZ and prevent its further effects, facilitating the process of recovery and rearrangement of insulin-producing cells. These findings demonstrated that the rate of insulin secretion in the diabetic animals treated with *Satureja montanum* leaf extract increased significantly compared to the diabetic group. Since the destruction of pancreatic islets beta cells and cell DNA is caused after STZ administration, the *Satureja montanum* extract is thought to contribute to protecting or repair damaged pancreas tissue against inflammation and necrosis due to STZ by increasing the activity of dismutase superoxide and glutation peroxidase, and therefore cell stimulation was furthered and pancreatic islets tissue was restored. The findings of the present study indicated that *Satureja montanum* could significantly affect the diabetes course and prevent increase in glycemia and insulin in STZ-diabetic adult male rats. However further and more advanced equipment lacking in the present study is needed to explain the cell and enzyme mechanisms, and further studies are strongly recommended to remove the limitations of the present study. Altogether, if the potentially involved mechanisms in this process are explained, *Satureja montanum* could be used as a strong anti-diabetic medication and/or natural anti diabetic drugs with fewer side effects could be supplemented by extraction of *Satureja montanum* effective compounds.

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### Authors' contributions

All the authors wrote the manuscript equally.

### Conflict of interests

The authors declared no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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