Hypoglycemic effects of aqueous extract of *Salvia mirzayanii* Rech. F& Esfand in diabetic patients; a randomized controlled trial study

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**ABSTRACT**

*Background:* Medicinal plants are used in diabetes treatment. *Salvia mirzayanii* Rech. F& Esfand was used for diabetes treatment in Iranian traditional medicine.

*Objectives:* The main objective of this study is to investigate the antidiabetic effects of aqueous extract of *S. mirzayanii* in diabetic patients.

*Patients and Methods:* In the present randomized controlled trial study, the patients were divided into trial (n=27) and control (n=25) groups. The trial group received daily one capsule containing 450 mg of aqueous extract of *Salvia mirzayanii*; the control group received daily one capsule containing 450 mg of caramelized flour and 5% aqueous extract of *S. mirzayanii*. Fasting blood samples were collected at the beginning of the study and after 3 months, fasting blood sugar (FBS), HbA₁C, insulin, HOMA-IR index, alanine transaminase (ALT), aspartate transaminase (AST), and lipid profile were evaluated.

*Results:* Types of used drugs, gender, age, and body mass index (BMI) were not significantly different between trial and control groups before treatment. Additionally, after treatment, BMI did not significantly change between these two mentioned groups. The results showed that FBS and HbA₁C levels did not change significantly in the trial group after treatment; however, in this group, insulin secretion was increased significantly (*P* = 0.015). Nevertheless, after treatment, HOMA-IR index did not significantly change between the two mentioned groups. In the trial group, after treatment, liver enzymes (ALT and AST) levels did not change significantly. After the treatment, LDL-C was changed in the trial group.

*Conclusion:* The herbal drug used in the present study enhanced insulin secretion, and improved LDL-C level in diabetic patients.

*Trial Registration:* Registration of trial protocol has been approved in the Iranian Registry of Controlled Trials website (identifier: IRCT2015122725712N1; https://en. irct. ir/trial/21454, Ethical code# HUMS.REC.1394.53).

**Implication for health policy/practice/research/medical education:** In a randomized controlled trial study on a group of diabetic patients who were divided into, two trial (n=27) and control (n=25) groups, we found that aqueous extract of *Salvia mirzayanii* Rech. F& Esfand enhanced insulin secretion and improved LDL-C level in diabetic patients.


1. **Background**

Type 2 diabetes mellitus (T2DM) is one of the most common disorders in the world since it affects approximately 120 million people worldwide, which could be doubled in the next 10 years (1). The metabolic aspect of diabetes is characterized by moderate to severe hyperglycemia and impaired metabolism of macromolecules including carbohydrates, proteins, and lipids (2).

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T2DM is the main cause of renal disorder, blindness, and non-traumatic amputations. In this regard, a correlation has been reported between type 2 diabetes with inadequate metabolic regulation and the high prevalence of mortality because of cardiovascular illness and nephropathy. The appropriate regulation of plasma glucose concentrations inhibits microvascular complications. Accordingly, the main effect of medical treatment in patients with T2DM is the regulation of metabolic pathways (3). T2DM is a multifactorial heterogeneous group of metabolic disorder with hyperglycemia and decreased insulin action and/or insulin secretion (4). Nowadays, the use of plants for treating diabetes is favored. However, the medicinal use of herbs is effective in the treatment of diabetes as a multifactorial disorder. The side effects of insulin and oral hypoglycemic factors, increase interests among diabetic patients for using natural products with antidiabetic effects (5). Hence, finding natural compounds for treating diabetes can be considered as a favorable field (6). To treat T2DM, more than 1000 plant species have been consumed worldwide (7). *Salvia* species (sages) are consumed in many parts of the world for treating several disorders. In the present study, the effect of aqueous extract of *Salvia mirzayanii* Rech. F& Esfand in patients with T2DM is evaluated. As the largest genus of Labiatae family, *Salvia* contains approximately 900 species, 58 of which are located in Iran (7,8). Plants of this genus grow all over the world (9-12) and a large number of them have been used for medical purposes (11-14), such as anti-stomachache and anti-diabetes (15) in various countries (12-14). In a study, it was reported that n-butanol fraction of *S. mirzayanii* leaves extract, inhibits an enzyme called α-glucosidase that increases carbohydrate digestion rate and it has the potential to induce the occurrence of T2DM (16).

The effect of *S. mirzayanii* extract has already been investigated in animal models. For instance, the methanol extract of *S. mirzayanii* could repair and increase blood supply of pancreatic cells in diabetic rats. Additionally, the acute and chronic anti-hyperglycemic effects of methanol extract of *S. mirzayanii* was detected in rats (17), since methanol extract of *S. mirzayanii* could reduce blood glucose of experimental animals (18).

**Objectives**

The main purpose of the present study is to determine the hypoglycemic effect of aqueous extract of *S. mirzayanii* leaves in diabetic patients. Note that this work is the first clinical trial study carried out on human subjects. In the present study, fasting blood glucose, HbA1C, and insulin levels, ALT (alanine transaminase) and AST (aspartate transaminase) levels, lipid profile, and blood pressure were determined.

**Patients and Methods**

**3.1 Protocol**

This double-blind study was conducted from September 2017 to July 2017 by Department of Biochemistry, Hormozgan University of Medical sciences. The diabetic patients were randomly divided into interventional (n=27) and control (n=25) groups. The interventional group received the capsule containing 450 mg/daily of concentrated aqueous extract of *S. mirzayanii* and the control group received the placebo capsule containing 450 mg/daily of caramelized flour and 5% of aqueous extract of *S. mirzayanii*. The demographic information of all subjects including age, gender, height, weight, body mass index (BMI), systolic and diastolic pressure, and drugs consumption was recorded. Aqueous extract of *S. mirzayanii* in placebo capsule used as coloring and flavoring and this amount of (5%) the extract had no significant effects on the obtained results.

**Extraction preparation**

Aerial parts of *S. mirzayanii* plants were collected from Genu Mountains in northeast of Bandar-Abbas city Hormozgan province, Iran. They were identified by MM (The third author). Voucher specimen has been preserved at the herbarium of Medicinal Plants Processing Research Center (Voucher # MPRCM 94-84), Shiraz University of Medical Sciences. Herbal drugs were prepared by Department of Phytopharmaceutical (traditional pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

**Biochemical experiments**

The biochemical experiments included determination of fasting blood sugar (FBS), glycosylated hemoglobin (HbA1C), liver enzymes (including AST and ALT), lipid profile (including cholesterol, triglyceride, and LDL-C and HDL-C levels) and serum insulin conducted at the beginning of the study and after three months. Serum was separated from 5 cc blood of fasting patients, the aforementioned measurements were performed and the results were compared in the interventional and control groups. Insulin was measured by enzyme-linked immunosorbent assay (ELISA) using Monobind kit (Monobind Inc, lake Forest, CA, USA), and for this measurement, Awareness instrument (USA) was used. HbA1C was measured by Axis-Shield HbA1C calibrator kit, and other factors were measured by an auto-analyzer (BT 3500, Italy). HOMA-IR (Homeostasis Model Assessment of Insulin
Resistance) on the development of type 2 diabetes was measured using the following formula: HOMA-IR = Insulin (µU/dL) * Glucose (mmol/L)/22.5 (11).

Inclusion and exclusion criteria
Inclusion criteria were as follows: patients within the age range of 18 to 65 years, patients having type II diabetes mellitus based on the guidelines of the (WHO 2018), patients with BMI >19 kg/m² and <30 kg/m², patients without insulin therapy and any other complications. All the selected patients signed the informed consent form.

Exclusion criteria were as follows: pregnant women or women intended to become pregnant, patients with active infections or hepatitis C, B and HIV or TB (or in TB therapy), cancer patients (except patients with skin cancer), using prednisone for the treatment of a chronic disease, smoking cigarettes and drinking alcohol, blood pressure higher than 160/90 mm Hg, taking contraceptive pill by women; moreover, patients with malabsorption, chronic diarrhea, liver and kidney diseases, ischemic heart disease, anemia, heart attack, peripheral vascular disease and diabetic nephropathy were excluded from the study.

Sample size calculation
Two-sample parallel design

\[ H_0: \mu_1= \mu_2 \text{ vs } H_1: \mu_1 \neq \mu_2, \text{ (H1-2)} \]

Assessed for eligibility (n=52)

Excluded (n=0)

Randomized (n=52)

Allocated to intervention (n=25)
  • Received allocated intervention (n=25)
  • Did not receive allocated intervention (n=0)

Allocated to intervention (n=27)
  • Received allocated intervention (n=27)
  • Did not receive allocated intervention (n=0)

Lost to follow-up (n=0)
  Discontinued intervention (n=0)

Lost to follow-up (n=0)
  Discontinued intervention (n=0)

Analysed (n=25)
  • Excluded from analysis (n=0)

Analysed (n=25)
  • Excluded from analysis (n=0)

Figure 1. CONSORT (consolidated standard of reporting trial) chart for study.
Results
At the beginning of this study there was no significant difference between age ($P=0.369$, Table 1), BMI ($P=0.374$), systolic ($P=0.500$) and diastolic pressures ($P=0.295$, Table 2), ratio of gender ($P=0.430$, Table 1), ratio of drug use such as metformin ($P=0.183$), atorvastatin ($P=0.071$), aspirin ($P=0.492$), losartan ($P=0.845$) and repaglinide ($P=0.488$) between trial and control groups (Table 3). Moreover, after the treatment, no significant change was observed regarding BMI and systolic and diastolic blood pressures between trial and control groups.

We found that fasting serum HbA1C and fasting blood glucose levels were not changed significantly in the trial group after the treatment, while insulin level was increased in the trial group after treatment (Table 4). HOMA-IR Index in control group before (10.1±7.5 µU/dL) and after intervention (13.5±11.4 µU/dL) and after intervention (13.5±11.4 µU/dL) was not significantly changed ($P>0.05$), also this index did not significantly change in trial group before (4.8±2.26 µU/dL) and after (8.84±5.04 µU/dL) intervention ($P<0.05$).

After the intervention, LDL-C level (Table 5) was reduced in the trial group ($P<0.199$). In the trial group after treatment, there were no significant differences in AST ($P=0.093$) and ALT ($P=0.135$) levels (Table 6).

Discussion
In the present study, we investigated the hypoglycemic effect of aqueous extract of $S.$ mirzayanii in diabetic patients.

The results of other studies revealed that species of $Salvia$ containing three flavonoids, namely, luteolin 7-O-glucoside, luteolin 7-O-glucuronide, and diosmetin 7-O-glucuronide which have inhibitory effects on α-glucosidase with IC$_{50}$ values similar to that of acarbose as a standard (22). In addition, compounds like catechins and rosmarinic acid were isolated from $Salvia$ species such as $Salvia$ choloroleuca, $Salvia$ santolinifolia, and $Salvia$ mirzayanii by HPLC analysis (23).

In clinical trial studies of diabetes, probably some symptoms of DM such as polydipsia, polyphagia, body weight loss, (24) and blood pressure should be considered as significant factors for managing DM and related complications (24).

In the current study, diastolic and systolic pressures and BMI were not significantly different between trial and control groups before and after treatment with herbal drug, ($P=0.473$, $P=0.641$ and $P=0.987$, respectively).

HbA1C is a gold standard in the evaluation of diabetes while numerous studies showed that the level of HbA1C is strongly correlated with the glycemic control over a period of 2 to 3 months (25). However, in some conditions HbA1C level may not reliably reveal the glycemic control.

Table 1. Gender, age, distributions of the case and control groups before and after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gender</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44% male</td>
<td>55.4±8.48</td>
</tr>
<tr>
<td></td>
<td>56% female</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>33.3% male</td>
<td>53.37±7.68</td>
</tr>
<tr>
<td></td>
<td>66.7% female</td>
<td></td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.43</td>
<td>0.369</td>
</tr>
</tbody>
</table>

Table 2. BMI distribution and systolic and diastolic blood pressures of the case and control groups before and after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>BMI (kg/m²) Before treatment</th>
<th>After treatment</th>
<th>$P$ value</th>
<th>Diastolic (mm Hg) Before treatment</th>
<th>After treatment</th>
<th>$P$ value</th>
<th>Systolic (mm Hg) Before treatment</th>
<th>After treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.84±3.9</td>
<td>25.1±0.66</td>
<td>0.3</td>
<td>129.6±20</td>
<td>123.80±19.5</td>
<td>0.206</td>
<td>76±7.07</td>
<td>73.00±9.46</td>
<td>0.146</td>
</tr>
<tr>
<td>Case</td>
<td>24.92±3.37</td>
<td>25.20±3.51</td>
<td>0.47</td>
<td>123.46±15.48</td>
<td>121.30±14.71</td>
<td>0.234</td>
<td>77.31±6.04</td>
<td>73.00±8.50</td>
<td>0.092</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.376</td>
<td>0.987</td>
<td></td>
<td>0.295</td>
<td>0.743</td>
<td></td>
<td>0.50</td>
<td>0.641</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Percentage of drugs use in the case and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Metformin</th>
<th>Atorvastatin</th>
<th>ASA (aspirin)</th>
<th>Losartan</th>
<th>Repaglinide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Use 84%</td>
<td>Use 72%</td>
<td>Use 76%</td>
<td>Use 20%</td>
<td>Use 28%</td>
</tr>
<tr>
<td></td>
<td>Not use 16%</td>
<td>Not use 38%</td>
<td>Not use 24%</td>
<td>Not use 80%</td>
<td>Not use 72%</td>
</tr>
<tr>
<td>Case</td>
<td>Use 96.3</td>
<td>Use 92.6</td>
<td>Use 85.2</td>
<td>Use 22.2%</td>
<td>Use 37%</td>
</tr>
<tr>
<td></td>
<td>Not use 3.7</td>
<td>Not use 7.4%</td>
<td>Not use 14.8</td>
<td>Not use 77.8%</td>
<td>Not use 63%</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.183</td>
<td>0.071</td>
<td>0.492</td>
<td>0.845</td>
<td>0.488</td>
</tr>
</tbody>
</table>
salvia mirzayanii in diabetic patients

Table 4. FBS, HbA1C, insulin levels before and after treatment in the case and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBS (mg/dL) Before treatment</th>
<th>FBS (mg/dL) After treatment</th>
<th>P value</th>
<th>HbA1C (percentage) Before treatment</th>
<th>HbA1C (percentage) After treatment</th>
<th>P value</th>
<th>Insulin (µI/µL) Before treatment</th>
<th>Insulin (µI/µL) After treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>167.2±53.6</td>
<td>157.08±51.3</td>
<td>0.479</td>
<td>7.25±1.07</td>
<td>7.15±1.3</td>
<td>0.729</td>
<td>10.1±7.5</td>
<td>13.5±11.4</td>
<td>0.375</td>
</tr>
<tr>
<td>Case</td>
<td>190.09±51.6</td>
<td>191.5±74.3</td>
<td>0.464</td>
<td>7.66±1.32</td>
<td>7.9±1.5</td>
<td>0.0697</td>
<td>9.3±5.9</td>
<td>15.6±12.3</td>
<td>0.015</td>
</tr>
<tr>
<td>P value</td>
<td>0.124</td>
<td>0.342</td>
<td></td>
<td>0.229</td>
<td>0.003</td>
<td>0.379</td>
<td>0.530</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

µI/µ: micro international unit.

Table 5. HDL-C, LDL-C and total cholesterol levels before and after treatment in the case and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL (mg/dL) Before treatment</th>
<th>HDL (mg/dL) After treatment</th>
<th>P value</th>
<th>LDL (mg/dL) Before treatment</th>
<th>LDL (mg/dL) After treatment</th>
<th>P value</th>
<th>Cholesterol (mg/dL) Before treatment</th>
<th>Cholesterol (mg/dL) After treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.9±8.03</td>
<td>48.40±13.37</td>
<td>0.02</td>
<td>89.56±31.49</td>
<td>97.4±34.36</td>
<td>0.460</td>
<td>157.1±37.15</td>
<td>164.4±43.05</td>
<td>0.532</td>
</tr>
<tr>
<td>Case</td>
<td>39.9±11.43</td>
<td>45.37±10.85</td>
<td>0.004</td>
<td>108.78±30.86</td>
<td>99.8±37.46</td>
<td>0.199</td>
<td>171.2±53.5</td>
<td>167.7±54.3</td>
<td>0.772</td>
</tr>
<tr>
<td>P value</td>
<td>0.714</td>
<td>0.372</td>
<td></td>
<td>0.031*</td>
<td>0.81</td>
<td></td>
<td>0.279</td>
<td>0.522</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. ALT, AST and TG levels before and after treatment in the case and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L) Before treatment</th>
<th>ALT (U/L) After treatment</th>
<th>P value</th>
<th>AST (U/L) Before treatment</th>
<th>AST (U/L) After treatment</th>
<th>P value</th>
<th>Triglyceride (mg/dL) Before treatment</th>
<th>Triglyceride (mg/dL) After treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.2±10.25</td>
<td>27.76±12.4</td>
<td>0.057</td>
<td>21.32±4.2</td>
<td>24.5±6.17</td>
<td>0.035</td>
<td>136.2±70</td>
<td>112.6±62.1</td>
<td>0.354</td>
</tr>
<tr>
<td>Case</td>
<td>26.09±16.8</td>
<td>28.5±15.26</td>
<td>0.135</td>
<td>22±7.5</td>
<td>23.6±8.9</td>
<td>0.093</td>
<td>142.9±80</td>
<td>125.8±70.2</td>
<td>0.075</td>
</tr>
<tr>
<td>P value</td>
<td>0.214</td>
<td>0.845</td>
<td></td>
<td>0.683</td>
<td>0.68</td>
<td></td>
<td>0.816</td>
<td>0.574</td>
<td></td>
</tr>
</tbody>
</table>

High doses of antioxidant agents such as vitamins C and E have been reported to decrease the level of HbA1C by reducing the rate of hemoglobin glycation; however, the pharmacological doses of antioxidant agents in which this event occurs, is not clear (25-27).

The results of this study presented no significant differences between FBS and HbA1C levels in trial and control groups before and after treatment. Similar results were reported in another clinical trial study, in which the trial group was treated with aqueous extract of Salvia officinalis (28). In this study, unreduced blood glucose and HbA1C levels in the trial group may indicate that S. mirzayanii extract has no effect on gluconeogenesis and postprandial blood glucose, respectively.

However, in the present study, the herbal drug increased the secretion of insulin in the trial group, but HOMA-IR is not altered before and after intervention in both control and trial groups. It is reported that elevation in HOMA-IR had a strong effect on the development of type 2 diabetes among subjects (29).

Elsewhere, it was reported that extract of Momordica charantia L (25) was agonist to, insulin; a finding obtained from animal pancreas revealed a consistent hypoglycemic effect in diabetic patients.

In another study, aldose reductase inhibitory activity (30) of rosmarinic acid derived from the root of Salvia grandifolia was reported (31). Aldolase reductase is a key enzyme in polyol pathway that converts glucose to sorbitol and accumulates reactive oxygen species in various tissues of diabetic patients (32).

In diabetic status, high levels of serum lipids are attributed to the increased function of lipolytic in adipose tissue (31,32). We found that the mean total cholesterol, HDL-C, and TG levels after the intervention had no significant differences in the trial and control groups. However, a significant difference is observed between LDL-C levels of the trial group compared to the placebo group. In other words, LDL-C level of the trial group (89.6±31.5 mg/dL) is more than the control group (108.8±30.9 mg/dL). Since this difference is not observed after treatment, it is suggested that the administration of the S. mirzayanii capsule, decreased LDL-C levels.

In another study, it was reported that the mean triglyceride, LDL-C, and HDL-C levels in the trial group had no significant differences before and after treatment by S. officinalis. However, administration of S. officinalis extracts reduced total cholesterol of trial group, which can
be an indicator of the beneficial effect of this extract on patients with hyperlipidemia (27).

In our study, there were no significant differences in the levels of ALT and AST before and after treatment in the trial group. However, AST level was increased in the control group from 21.3±4.2 mg/dL to the normal value of 24.5±6.2 mg/dL.

Hence, it can be stated that the drug has no adverse effects on liver enzymes, suggesting its safe application.

Conclusions
Considering the results obtained in the present work, lack of a change in FBS and HbA, C levels may be explained by the fact that patients do not take the drug regularly. To obtain better results, we suggest using a larger study population and administering the patients with two herbal capsules every day. In fact, higher doses might be needed to decrease fasting blood glucose and glycosylated hemoglobin levels. Using capsule containing S. mirzayanii extract decreases LDL-C level that can be an indicator of favorable effects of this drug in hyperlipidemic patients. The results showed that this drug had no unwanted and toxic effect on the liver, implying that it is safe for the liver. Further controlled studies may be needed on this subject.

Study limitations
There was a limitation in finding diabetic patients who accepted to participate in the current study and consumed regularly capsule containing S. mirzayanii extract. If this study was performed with a larger population with the administered capsules (herbal capsules) containing more S. mirzayanii extract, the obtained results could be impressive more.

Acknowledgments
The authors thank the personnel of Diabetes Clinic of Shahid Mohammadi hospital. The authors also express their thanks to Hormozgan University of Medical Sciences for the financial support (Grant # 9437).

Authors’ contribution
MM and SM designed the study. PS performed the experiments. RM collected data from patients and helped in performing the experiments. MZ prepared the capsules. SM wrote and revised the article.

Conflicts of interest
There are no conflicts of interest related to various aspects such as financial support by commercial firms.

Ethical considerations

Funding/Support
Hormozgan University of Medical Sciences supported financially the study (Grant #9437).

References
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