THE EFFECT OF SALVIA OFFICINALIS LEAF EXTRACT ON BLOOD GLUCOSE IN STREPTOZOTOCIN-DIABETIC RATS

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Summary

Salvia officinalis L. is reported to have a wide range of biological activities, and was suggested some of its extracts have hypoglycemic effects in normal and diabetic animals. The purpose of the current study was to examine the antidiabetic effects of subchronic administration of aqueous and ethanol extracts of Salvia officinalis leaves in streptozotocin (STZ)-induced diabetic rats. The animals were rendered diabetic by a single intraperitoneal injection of 50 mg/kg streptozotocin. The aqueous and ethanolic extracts were injected intraperitoneally at a dosage of 430 mg/kg for 6 days since the day after diabetes confirmation. Blood samples were obtained from retro-orbital sinus on day 6, after 2 and 4h of extract administration. The serum glucose was measured by the enzymatic method of glucose oxidase. The results showed that the aqueous and alcoholic extract of Salvia officinalis at dose of 430 mg/kg (ip) does not possess the hypoglycaemic activity on subchronic treatment in STZ-diabetic rats. Further studies are necessary to prove the antidiabetic effect of salvia officinalis and its active components.

Key words: Salvia officinalis, Diabetes mellitus, Hyperglycaemia, Rat.

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Introduction

Diabetes Mellitus is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion, action or both. Chronic hyperglycemia in diabetes is associated with long term damages, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves and cardiovascular system (1). Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, metformin and α-glucosidase inhibitors. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the above oral agents suffers from a number of serious adverse effects (2,3). Thus, it appears useful to look for new methods in treatment of diabetes. Medical plants are world widely used and there is also an interest in studying them in order to provide a scientific explanation for their beneficial effect (4-8).

Salvia officinalis L. (sage), a member of the family of Lamiaceae, has been reported to have a wide range of biological activities, such as antioxidant (9), antibacterial (10), hypoglycemic (11) and anti-inflammatory (12) properties. Other experimental studies on sage extracts or sage essential oil have shown hypotensive properties, central nervous system-depressant actions and anti-spasmodic activity (13). The constituents reported in this plant are rosmarinic acid, phenolic acids, carnosic compounds and flavonoids or their derivatives (14-17).

Salvia officinalis L. is among the plants that are claimed to be beneficial to diabetic patients, and previous studies have suggested that some of its extracts have hypoglycemic effects in normal and diabetic animals. The purpose of the current study was to examine the hypoglycemic effects of subchronic administration of aqueous and ethanol extracts of Salvia officinalis leaves in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Preparation of the plant extract

Sage leaves were obtained from Iman Pharmaceutical Company, division of herbal plants (Mashhad, Iran) and verified in Pharmacy Faculty (Mashhad, Iran). The leaves were finely powdered. For preparation of ethanolic extract, the powder (30g) was extracted with 450 mL ethanol in a Soxhlet apparatus for 13h. After extraction, the solvent was filtered and evaporated to get the extract at a concentration of 50g/100 ml.

For preparation of aqueous extract, the powder (25g) were boiled in 500 ml distilled water for half an hour. Then the mixture was filtered and evaporated to get the extract at a concentration of 25gr/100ml. At the end, both extracts were centrifuged for 15 min at 2500 rpm to remove particulate substances.

Animals

Male albino N.MRI rats (Razi's Institute, Mashhad, Iran) weighing 220-280g were housed in an air-conditioned colony room at 23 ± 2°C on a standard pellet diet and tap water at libitum.
The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by Mashhad University of Medical Sciences.

Experimental protocol

The overnight fasted rats were rendered diabetic by a single intraperitoneal injection of 50 mg/kg STZ freshly dissolved in cold normal saline. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria and weight loss. After 72h of the STZ injection, blood samples were collected and serum glucose concentrations was measured using glucose oxidation method (Zistshimi, Tehran). Only those animals with serum glucose higher than 250 mg/dl were selected as diabetics for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. The rats were randomly allocated and similarly grouped into four groups: normal saline-treated control (n=6), diabetic (n=7), aqueous extract-treated diabetics (n=8), ethanol extract-treated diabetics (n=7). Extracts were administered intraperitoneally at a dosage of 430 mg/kg body weight/day since day 1 for 6 days. Changes in body weight, food consumption and water intake were regularly recorded during the experimental period.
At the end of the experiment (day 6), rats were fasted overnight and blood samples were obtained after 2 and 4h of the extract injection. Blood was allowed to clot and serum separated by centrifugation at 3500 rpm for 10 min. Serum glucose levels were spectrophotometrically measured using appropriate kits (Zistshimi, Tehran).

Statistical analysis

The data were expressed as mean ± S.E.M. Statistical analysis was carried out using one-way ANOVA and unpaired *t*-test. A statistical *p* value less than 0.05 was considered significant.

Results

Body weight and serum glucose measurements indicated that before diabetes induction, there were no significant differences among animals in each group. After diabetes induction, the weight of diabetic rats was significantly decreased as compared to control rats. Diabetic rats also showed the symptoms, such as hyperglycemia, polyphagia, polydipsia and polyuria.

Untreated diabetic rats also had an elevated serum glucose level over those of control rats, after 72 h of the STZ injection (Fig.1, $p < 0.001$).

Treatment of diabetic rats for 6 days with the aqueous extract of Salvia did not change the serum glucose concentration, after 2 and 4h of the last extract injection, in comparison to untreated diabetic rats (Fig.1).

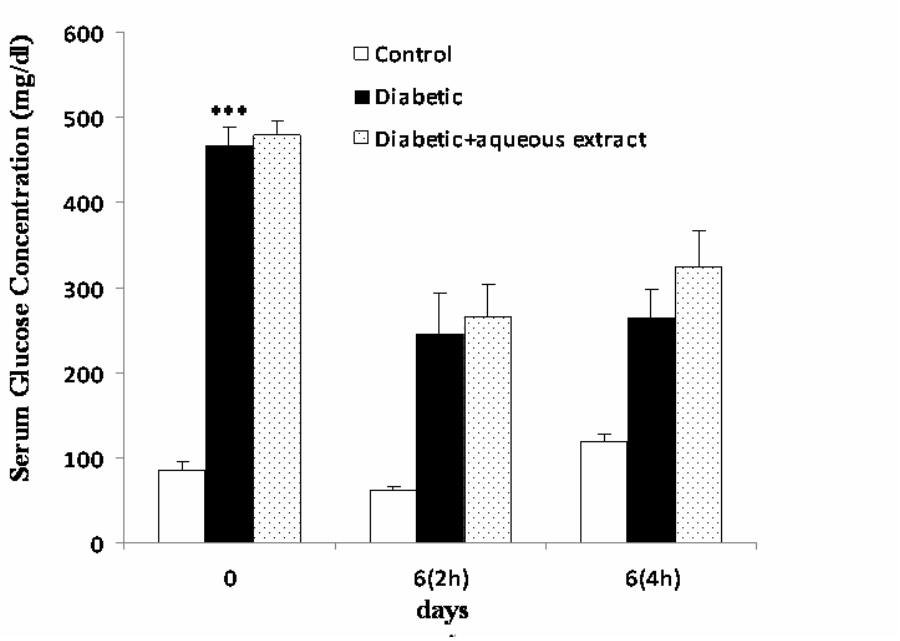

Fig. 1. Serum glucose concentration in control and streptozotocin rats without or with aqueous extract of Salvia treatment (430 mg/kg, ip) on day 0 and 6 (after 2 and 4h of the extract

injection). Data were expressed as mean ± SEM. *** p<0.001 (as compared to control group in the same day).

Also, treatment of diabetic rats with the ethanolic extract of Salvia for 6 days, did not produce any significant reduction in serum glucose level as compared to diabetic rats (Fig.2).

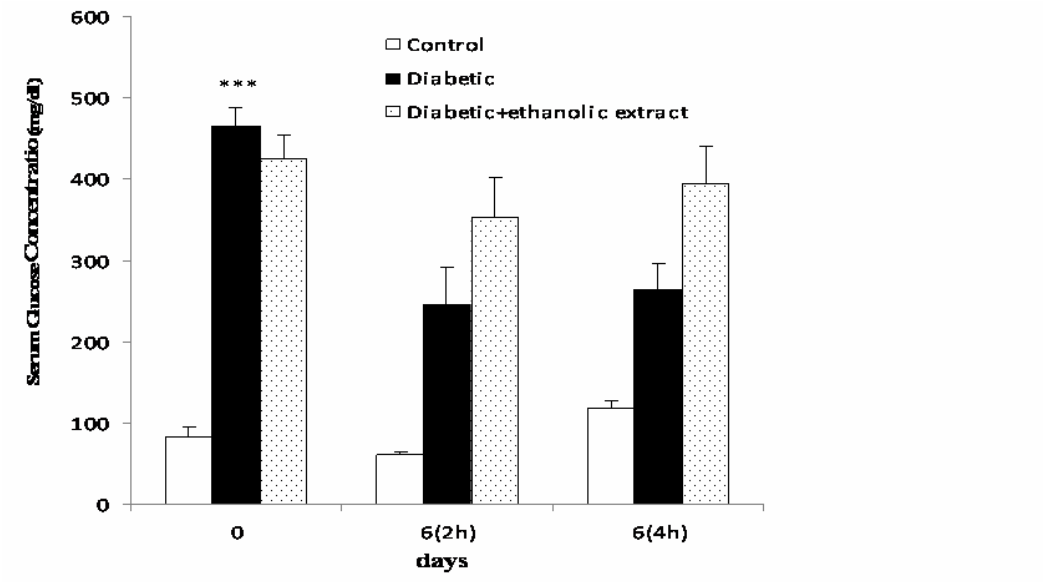

Fig 2. Serum glucose concentration in control and streptozotocin rats without or with ethanolic extract of Salvia treatment (430 mg/kg, ip) on day 0 and 6 (after 2 and 4h of the extract injection). Data were expressed as mean ± SEM. *** p<0.001 (as compared to control group in the same day).

Discussion

The single high-dose STZ-induced diabetic rat is one of the animal models of type I diabetes mellitus. In this model, diabetes arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion. In this type I model of diabetes, insulin is markedly depleted, but not absent (18).

In the present study, administration of STZ to rats, as expected, resulted in hyperglycemia and decreased body weight. STZ is taken up by pancreatic β cells via glucose transporter GLUT2. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound. However, production of NO and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effects of STZ (19).

Our present data showed that the aqueous and alcoholic extract of Salvia officinalis at dose of 430 mg/kg does not possess the hypoglycaemic activity on subchronic treatment in STZ-diabetic rats.

The effect of sage extract on hyperglycemia have previously been investigated by other researchers using a different extract and experimental methodology (11,20,21). Alarcon-Aguilar and collaborators (2002) showed that, 4 hours after an ip injection of a sage water- ethanolic extract, blood glucose decreased significantly in fasted normal mice and in fasted mildly alloxan diabetic mice but not in fasted severely alloxan-diabetic mice (11). Additionally, Eidi and co-workers (2005) showed that, 3 hours after an ip injection of a sage methanolic extract, blood glucose decreased significantly in fasted STZ-diabetic rats but not in fasted normal rats (20).

Sage has a high essential oil content (22), that has also been tested and proved to be hypoglycemically active in normal and in alloxan-induced diabetic rats (23) but not in streptozotocin-induced diabetic rats (20).

In another report, Lima and collaborators (24) have reported that sage tea drinking for 14 days has a lowering effect on fasting glucose levels in normal mice and metformin-like effects on rat hepatocytes, but it does not possess antidiabetic effects at this level. Also, Sa and co-workers (2009) have shown that four weeks sage tea treatment had no effects on plasma glucose in healthy humans (25).

One possible explanation of the results in the current study and others may be related to the lack of standardisation of herbal extracts. Environmental conditions lead to considerable variability between examples of the same species grown in different countries or indeed in the same location in different years (26). It may be the case that the aqueous or ethanol extract employed in this study had a different constitution to that used in other research, such that it was low in levels of the active compounds.

An alternative explanation might be related to the pharmacokinetics of any active components. The bioavailability of such compounds in orally administered herbs and herbal extracts is dependent of a number of factors that influence absorption and first pass metabolism (27).

Conclusion

In conclusion, This study showed that the aqueous and alcoholic extract of Salvia officinalis at dose of 430 mg/kg (ip) does not possess the hypoglycaemic activity on subchronic treatment in STZ-diabetic rats. Thus, the antidiabetic effect of salvia officinalis and its active components remains to be elucidated by further examinations.

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