

Hypoglycemic Effects of a Standardized Extract of *Salvia miltiorrhiza* Roots in Rats

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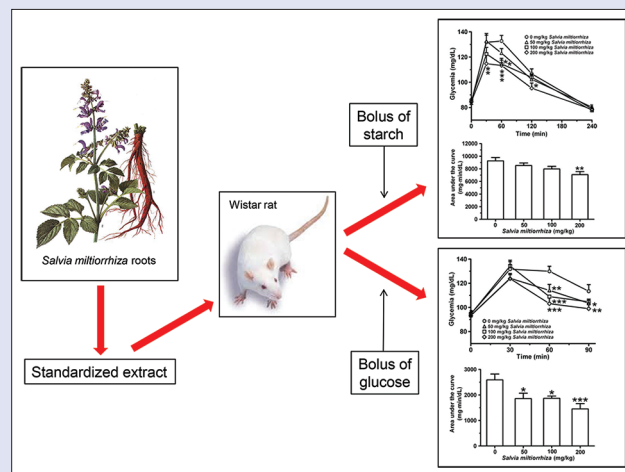
ABSTRACT

Background and Aims: *Salvia miltiorrhiza* Bunge (Labiatae) is a Chinese medicinal plant, the dried roots of which (known as Dan-Shen) have been used for hundreds of years in the treatment of a series of ailments, including hyperglycemia. This study was designed to evaluate the hypoglycemic effect of a new, standardized extract of *S. miltiorrhiza*. **Materials and Methods:** *S. miltiorrhiza* extract (containing 21% total tanshinones and 3.7% tanshinone IIA) was administered acutely and intragastrically at the doses of 0, 50, 100, and 200 mg/kg to male, healthy, fasted Wistar rats 60 min before the intragastric infusion of a bolus of starch (3 g/kg; a semi-naturalistic experimental condition) (Experiment 1) or glucose (2 g/kg) (Experiment 2). **Results:** In both experiments, treatment with *S. miltiorrhiza* extract produced a dose-related decrease in glycemia, evidenced in terms of reduction of peak value and/or area under the curve of the time-course of glycemia. The effect of *S. miltiorrhiza* extract occurred at doses devoid of any behavioral toxicity in rats. **Conclusion:** The results of this study suggest that the hypoglycemic effect of *S. miltiorrhiza* extract was likely secondary to an action on carbohydrate metabolism. These results are consistent with several preclinical and clinical data and add further support to the hypothesis that *S. miltiorrhiza* extracts may act as effective *anti*-hyperglycemic remedies.

Key words: Rats, standardized extract of *S. miltiorrhiza* roots, starch- and glucose-induced rise in glycemia, type-2 diabetes

SUMMARY

- Acute treatment with *Salvia miltiorrhiza* extract reduced the glycemia rise induced by a bolus of starch in rats
- Acute treatment with *Salvia miltiorrhiza* extract reduced the glycemia rise induced by a bolus of glucose in rats
- This effect relates to both glycemia peak value and area under the curve of the time-course of glycemia.



Abbreviations used: Intragastric: i.g., Least significant difference: LSD test.

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INTRODUCTION

Recent research and re-evaluation of knowledge deriving from traditional medicines suggest the potential effectiveness of herbal remedies in the treatment of diabetes and its complications; a series of herbal extracts have been described to decrease hyperglycemia and prevent diabetes-induced ailments in animal models, clinical surveys, and anecdotal reports.^[1,2]

Salvia miltiorrhiza Bunge (Labiatae) is a medicinal plant, the dried roots of which (known as Dan-Shen) are officially listed in the Chinese Pharmacopoeia; these roots have been used for hundreds of years in Chinese folk medicine in the treatment of a series of pathological conditions, including cardiovascular disease, hematological abnormalities, hyperlipidemia, hepatitis, hemorrhage, menstrual disorders, miscarriage, edema, and insomnia.^[3-6] More recently, the potential for the treatment of alcohol use disorders has been proposed.^[7,8] In addition, several preclinical and clinical Chinese studies have reported the efficacy of *S. miltiorrhiza* extracts in decreasing hyperglycemia as well as its traditional use for treating diabetes mellitus and its complications.^[1,2,9]

This study was aimed at providing an additional contribution to the anti-diabetes potential of *S. miltiorrhiza* extracts; to this end, the present

study evaluated the capacity of a new, standardized extract of *S. miltiorrhiza* roots to lower the rise in glycemia produced by administration of a bolus of starch (Experiment 1) and glucose (Experiment 2) in healthy, adult rats.

MATERIALS AND METHODS

The experimental procedures employed in the present study were in accordance with the Italian Law on “Protection of animals used for scientific reasons.”

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Animals

Healthy, adult male Wistar rats (Charles River Laboratories, Calco, Italy), weighing approximately 250 g at the start of each experiment, were used. In both experiments, starting from the age of 60 days, rats were individually housed in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12:12 h light/dark cycle (lights on at 9:00 pm), at a constant temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of approximately 60%. Standard rat chow (Mucedola, Settimo Milanese, Italy) and tap water were available *ad libitum* in the homecage, except as noted below. Rats were extensively habituated to handling and intragastric (i.g.) infusion. Independent groups of rats were used in each experiment. In both experiments, rats were food-deprived for 12 h before the test.

Extract preparation

S. miltiorrhiza roots were collected in 2005 in the Eastern part of China and identified using the genus treatment found in Flora of China^[10] and Chinese Pharmacopoeia.^[5] A voucher specimen is kept at Indena S.p.A., Milan, Italy, under registry no: 137127.

Roots of *S. miltiorrhiza* were dried and then extracted with acetone. After concentration under vacuum, the residue was re-dissolved in alcohol and the solution defatted with *n*-hexane. The aqueous layer was concentrated and extracted with ethyl acetate. The final extract (named IDN5655) was obtained after removal of ethyl acetate by alcohol and drying under vacuum at 50°C for approximately 48 h. The *S. miltiorrhiza* extract used in the present study contained 21% total tanshinones and 3.7% tanshinone IIA.

Experimental procedures

Experiment 1: Effect of *Salvia miltiorrhiza* extract on starch-induced rise in glycemia

On the test day (occurring approximately 15 days after the start of the single-housing period), rats were divided into 4 groups of $n = 12$, matched for body weight, and treated acutely with 0, 50, 100, and 200 mg/kg *S. miltiorrhiza* extract. *S. miltiorrhiza* extract (code: IDN5655) was dissolved in a 1:1 mixture of polysorbate 80 plus polyethylene glycol 600. The solution was kept under continuous agitation, at room temperature and in a dark container, for at least 12 consecutive h before administration. *S. miltiorrhiza* extract was administered i.g. (infusion volume: 4 mL/kg) 60 min before lights off. Doses of *S. miltiorrhiza* extract were chosen on the basis of preliminary data (this laboratory, unpublished results).

At lights off, rats were treated i.g. with 3 g/kg corn starch (Unilever, Milan, Italy; suspended in 4.5 mL/kg distilled water). Glycemia was determined 0, 30, 60, 120, and 240 min after starch administration. At each recording time, a small (0.05 mL) blood sample was collected from the tip of the tail of each rat and analyzed enzymatically by means of GL5 Analox (Analox Ltd, London, UK). Glycemia was expressed in mg/dL.

Experiment 2: Effect of *Salvia miltiorrhiza* extract on glucose-induced rise in glycemia

On the test day (occurring approximately 15 d after the start of the single-housing period), rats were divided into four groups of $n = 7-8$, matched for body weight, and treated acutely with 0, 50, 100, and 200 mg/kg *S. miltiorrhiza* extract. *S. miltiorrhiza* extract was dissolved as described above and administered i.g. (infusion volume: 4 mL/kg) 60 min before lights off.

At lights off, rats were treated i.g. with 2 g/kg glucose (ACEF, Fiorenzuola d'Arda, Italy; dissolved in 3.5 mL/kg distilled water). Glycemia was determined 0, 30, 60, and 90 min after glucose administration. At each recording time, a small (0.05 mL) blood sample was collected and analyzed as described above.

Statistical analysis

In both experiments, data on the effect of treatment with *S. miltiorrhiza* extract on glycemia over time were analyzed by 2-way (treatment; time) ANOVA with repeated measures on the factor "time," followed by the least significant difference (LSD) test for *post hoc* comparisons. Data on the effect of treatment with *S. miltiorrhiza* extract on the area under the curve of the time-course of glycemia were expressed as mg/dL and mg·min/dL, respectively, and analyzed by 1-way ANOVA, followed by the LSD test for *post hoc* comparisons.

RESULTS

Experiment 1: Effect of *Salvia miltiorrhiza* extract on starch-induced rise in glycemia

In vehicle-treated rats, glycemia peaked up to approximately 130 mg/dL at the 30- and 60-min recording times and then decreased progressively [Figure 1, top panel]. ANOVA revealed a significant effect of treatment with *S. miltiorrhiza* extract ($F(3,44) = 4.60, P < 0.01$) and a highly significant effect of time ($F(3,132) = 115.47, P < 0.0001$), but not a significant interaction ($F(9,132) = 1.35, P > 0.05$), on glycemia over the 240-min recording period. *Post hoc* analysis revealed that glycemia was significantly reduced, compared to vehicle, by (a) 100 mg/kg *S. miltiorrhiza* extract at the 60-min recording time, and (b) 200 mg/kg *S. miltiorrhiza* extract at the 30-, 60-, and 120-min recording times [Figure 1, top panel].

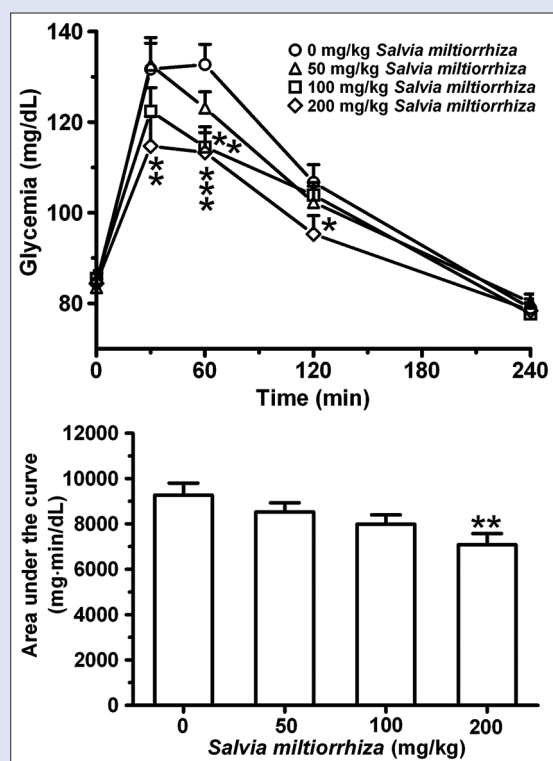


Figure 1: Effect of the acute, intragastric administration of different doses of a standardized extract of *Salvia miltiorrhiza* on time-course of glycemia (top panel) and area under the curve of the time-course of glycemia (bottom panel) in Wistar rats treated intragastric with a bolus of starch (3 g/kg) at time 0. Each point or bar is the mean \pm standard error mean of $n = 12$ rats. * $P < 0.05$, ** $P < 0.005$, and *** $P < 0.001$ in comparison to vehicle-treated rat group (least significant difference test)

Treatment with *S. miltiorrhiza* extract also reduced the area under the curve of the time-course of glycemia ($F(3,44) = 3.88, P < 0.05$) [Figure 1, bottom panel]. In the rat group treated with 200 mg/kg *S. miltiorrhiza* extract, this reduction (in comparison to vehicle-treated rat group) averaged approximately 25% and achieved statistical significance at *post hoc* test [Figure 1, bottom panel].

Experiment 2: Effect of *Salvia miltiorrhiza* extract on glucose-induced rise in glycemia

In vehicle-treated rats, glycemia reached an average value of approximately 130 mg/dL at the 30- and 60-min recording times, and decreased markedly at the subsequent 90-min recording time [Figure 2, top panel]. ANOVA revealed a significant effect of treatment with *S. miltiorrhiza* extract ($F(3,27) = 4.67, P < 0.01$), a highly significant effect of time ($F(2,54) = 48.94, P < 0.0001$), and a significant interaction ($F(6,54) = 2.44, P < 0.05$), on glycemia over the 90-min recording period. *Post hoc* analysis revealed that glycemia was significantly reduced, compared to vehicle, by (a) all three doses of *S. miltiorrhiza* extract at the 60-min recording time, and (b) 50 and 200 mg/kg *S. miltiorrhiza* extract at the 90-min recording time [Figure 2, top panel].

Treatment with *S. miltiorrhiza* extract also reduced the area under the curve of the time-course of glycemia ($F(3,27) = 5.88, P < 0.005$) [Figure 2, bottom panel]. Compared to vehicle-treated rat group, magnitude of the reducing effect of treatment with 50, 100, and 200 mg/kg *S. miltiorrhiza* extract on the area under the curve of the time-course of glycemia averaged approximately 30%, 30%, and 45%, respectively; this

reducing effect reached statistical significance at *post hoc* analysis at each dose of *S. miltiorrhiza* extract [Figure 2, bottom panel].

DISCUSSION

The results of this study indicate that acute administration of a standardized extract of roots from the Chinese medicinal plant, *S. miltiorrhiza*, effectively reduced the rise in glycemia produced in healthy, fasted rats by administration of a bolus of starch (Experiment 1) or glucose (Experiment 2). This effect of *S. miltiorrhiza* extract (a) tended to be dose-related, as evidenced more clearly by the sets of data on the curve of the time-course of glycemia, and (b) occurred at doses known to be devoid of behavioral toxicity in rats.^[7,8]

Data from Experiment 1 demonstrate the hypoglycemic properties of *S. miltiorrhiza* extract in a semi-naturalistic experimental condition, represented by the infusion of a bolus of starch. However, these data may not fully clarify the issue of efficacy of *S. miltiorrhiza* extract on glucose metabolism, as the active ingredient(s) of the extract may have acted in the lumen of the small intestine, inhibiting hydrolysis of complex starches or oligosaccharides, thus resulting in poor absorption of monosaccharides and a modest rise in glycemia. However, data from Experiment 2 demonstrate that the hypoglycemic effect of *S. miltiorrhiza* extract was likely secondary to an action on carbohydrate metabolism, as administration of the extract inhibited the rise in glycemia induced by infusion of a bolus of pure glucose (the final product of dietary carbohydrate catabolism) to an extent comparable to that observed following infusion of the starch bolus (Experiment 1).

The results of the present study are in close agreement with recent lines of experimental evidence demonstrating that chronic treatment with (a) a polyphenolic fraction of *S. miltiorrhiza* decreased fasting glycemia in rats made diabetic by treatment with streptozotocin (experimental model of hyperglycemia and diabetes)^[11] and (b) the active ingredient, tanshinone IIA, reduced the rise in glycemia produced in fasted mice by acute injection of a glucose bolus.^[12] However, divergent data have also been collected, depicting the inability of *S. miltiorrhiza* extracts to affect glycemia in rats;^[13,14] the mode of extract preparation, content of active ingredient(s), and tested dose-ranges of *S. miltiorrhiza* extracts may be fundamental in justifying these discrepancies. Preclinical data showing the *anti*-hyperglycemic effects of *S. miltiorrhiza* extracts^[11,12, present study] are however consistent with findings reported elsewhere, in traditional Chinese medicine, on the hypoglycemic effects of *S. miltiorrhiza* extracts or preparations given to humans affected by type-2 diabetes.^[1,2,9]

The present study did not specifically address the biochemical mechanism(s) of action and active ingredient(s) responsible for the hypoglycemic effect of the tested *S. miltiorrhiza* extract. However, data present in literature may be of help in advancing several hypotheses. *S. miltiorrhiza* extracts – as well as their active lipophilic constituents, tanshinone IIA, tanshinone I, cryptotanshinone, and 15,16-dihydro-tanshinone (DHTH) – have been found to exert insulin-sensitizing effects, enhancing phosphorylation, and downstream signaling at the insulin receptor.^[15] This potentiation of insulin activity is likely responsible for the observed increase in glucose uptake exerted by DHTH.^[16] Additionally, DHTH has been found to inhibit gluconeogenesis.^[16] Finally, a polyphenolic fraction from *S. miltiorrhiza* decreased blood insulin in diabetic rats.^[11] Similar data were collected in mice after chronic treatment with tanshinone IIA.^[12]

Notably, the beneficial effects of *S. miltiorrhiza* extracts apparently extend to the complications of diabetes; recent studies have indeed demonstrated the ability of *S. miltiorrhiza* extracts to decrease (a) urinary protein excretion (experimental model of diabetes-induced nephropathy)^[13] and (b) oxidative stress in the eye and aorta (key event of pathogenesis of diabetes complications)^[14] in streptozotocin-treated rats. When considering treatment for type-2 diabetes and associated and/or promoting disorders

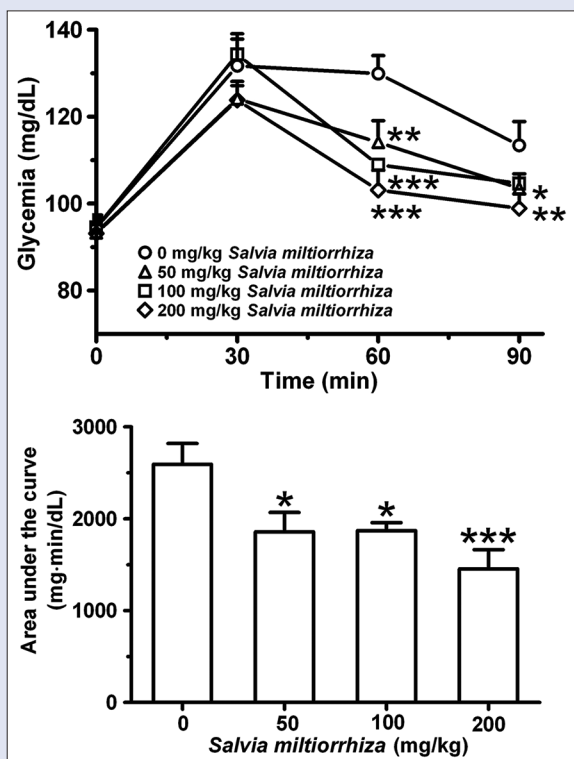


Figure 2: Effect of the acute, intragastric administration of different doses of a standardized extract of *Salvia miltiorrhiza* on time-course of glycemia (top panel) and area under the curve of the time-course of glycemia (bottom panel) in Wistar rats treated intragastric with a bolus of glucose (2 g/kg) at time 0. Each point or bar is the mean \pm standard error mean of $n = 7-8$ rats. * $P < 0.5$, ** $P < 0.005$, and *** $P < 0.0005$ in comparison to vehicle-treated rat group (least significant difference test)

such as hyperlipidemia and overweight, it should be taken into account that *S. miltiorrhiza* extracts and tanshinone IIA have been reported to regulate lipid metabolism, (a) decreasing plasma levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides in rats and mice,^[11,12,17] (b) increasing high-density lipoprotein cholesterol in rats and mice,^[12,17] and (c) reducing adipose mass and body weight in mice.^[12]

CONCLUSION

These results indicate an interesting and promising anti-diabetic profile for *S. miltiorrhiza* extracts, including the extract tested in this study. To this end, it may be of interest to note how several clinical trials in China have reported a good safety profile and small number of side effects when using products containing *S. miltiorrhiza*.^[6]

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Nil.

Conflicts of interest

There are no conflicts of interest.

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