Effect of Salvia rhytidea Benth. extract on serum glucose, gut alphasglucosidase in healthy and streptozotocin-induced diabetic rats

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ABSTRACT

Salvia rhytidea Benth. is one of the endemic species in Iran which has been used as anti-diabetic in traditional medicine. The aim of this study was to evaluate the hypoglycemic effect of Salvia rhytidea Benth. methanol extract on healthy and induced diabetic rats. Determination of alphasglucosidase activity also was the other objective. Aerial parts of the plant were extracted after authentication with methanol 80% by macerating method. The methanol extract was evaporated under reduced pressure to evaporate its methanol content then lyophilized and stored in screw cap vials. Plant LD₅₀ was determined 4.9 g/kg. Diabetes was induced by single i.p injection of a freshly prepared streptozotocin (STZ) solution (55 mg/kg in normal saline) to overnight fasted male wistar rats. Test animals received extract at doses of 0, 0.5, 1, 2 g/kg. Negative and positive control groups received normal saline and glibenclamid respectively. Serum glucose levels were determined at various points of time (0, 60, 120 and 180min after injection). Results showed that Salvia rhytidea extract suppressed the elevated blood glucose at doses of 0.5, 1 and 2 g/kg body weight for 180 min. At 0.5 g/kg body weight was found to be comparable to glibenclamide, a reference drug. Blood glucose lowering effect of extract in dose of 1 g/kg was higher than glibenclamide at dose of 20mg/kg. At dose of 0.5g/kg, plant extract exhibited similar activity with glibenclamide. Our studies indicates that the leaves of this plant possess significant antihyperglycemic potential.

Keywords: Salvia rhytidea Benth, Antidiabetic, Alphasglucosidase, Rat.

INTRODUCTION

Despite the advances in the synthetic medicinal chemistry in the past 100 years, there are still different diseases which have not been cured successfully. Treatment of these diseases is considered problematic due to the lack of effective and safe drugs capable of inducing sustained clinical, biochemical, and histological cure. In many developing countries, traditional medicine in particularly herbal medicine is sometimes the only affordable source of healthcare. As for the developed countries, the use of herbal medicine by the sufferers of chronic diseases is encouraged by the concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer gentler means of managing such diseases. Diabetes mellitus is one of the chronic diseases affecting 5–10% of the world population. It is estimated by the year 2010, the total number of people worldwide to reach 239 million. Diabetes is a major cause of disability and hospitalization. It can result in a range of complications that occur primarily in the arteries and capillaries. Diabetes patients, particularly those with type II diabetes are at considerable risk of excessive morbidity and mortality from cardiovascular, cerebrovascular, and peripheral vascular diseases leading to myocardial infarction, strokes, and amputations. The plant kingdom has become a target for the search by multinational drug and biologically active lead compounds. One of the member of this kingdom that recognized recently is Salvia rhytidea (persian sage). In the present study we investigated the hypoglycaemic effect of plant extract on healthy and streptozotocin-induced diabetic rats. Also inhibition of α-glucosidase is the one of the significant activity in diabetes treatment, so we examined whether a methanolic extract of this plant has an anti-diabetic effect through α-glucosidase inhibitory action.
MATERIALS AND METHODS

Plant material
The plant was gathered from Kerman province at the middle of spring and was identified as Salvia rhytidea Benth. by Professor Mirtajadini, Botanist, Department of Biology, Bahonar University of Kerman-Iran. A voucher specimen was deposited at the Herbarium center of faculty of Pharmacy (KF 1249). The aerial parts of the plant were cleaned, shade dried and ground with a blender and passed through sieve.

Extraction of methanolic plant material
The plant was extracted by warm maceration method with methanol 80% for 72h [10]. The filtered extract was replaced with fresh solvent every 24h. All extracts from three daily extraction was completely mixed, lyophilized and stored in screw cap vials [10, 11].

Animals
Male wistar rats, (weighing 200–300 g) obtained from the Bioterium of Neuroscience Research Center, Kerman Medical Scence University and were housed in clean cages, with free access to food and water for at least 1 week in an air conditioned room. Permission for the study was obtained from the country government.

Preliminary LD<sub>50</sub> test
The plant extract was administered (i.p.) to each group (n=6) at doses of 0.5, 1, 2 g/kg and mortality was determined during 72 hour after injection (Table 1) [12, 13].

Induction of diabetes
Male adult wistar rats were injected (i.p.) with streptozotocin (STZ) (Pharmacia and upjohn; USA) 55mg/kg in normal saline. Five days after injection, the rats with fasting blood glucose higher than 180mg/dl (Pharmacia and upjohn; USA) 55mg/kg in normal saline. Five days after injection, the rats with fasting blood glucose higher than 180mg/dl were used for the expriments. Animals had free access to food and water after injection. Five rats were used in each experiment [12]. The food were removed from cages 12 hour before testing [13].

Blood sampling
Before administration and 1, 2, 3 hour after administration of normal saline, glibenclamid at dose 20mg/kg and methanolic extract at doses of 0.5,1.2 g/kg, blood samples were taken from tail vein of animals.

Determination of the serum glucose concentration
Blood samples were collected, centrifuged, and the serum glucose levels were determined by the glucose oxidase method [14].

Table 2: Effect of injected of methanol extract of Salvia rhytidea on serum glucose levels in hyperglycemic rat

<table>
<thead>
<tr>
<th>Group/Time</th>
<th>0</th>
<th>60min</th>
<th>120min</th>
<th>180min</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>124±8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156±9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>153.6±8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152±7.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal saline</td>
<td>385±56.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>441.2±23.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>466.4±28.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>478±25.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamid</td>
<td>288±30.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>323±36.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>285±28.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>284±24.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract (0.5g/kg)</td>
<td>317±65.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>320±73.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303±81.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>278±91.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract (1g/kg)</td>
<td>433±59.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>392±31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>376±35.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>348±34.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract (2g/kg)</td>
<td>386±39.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>382±30.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>382±22.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>406±19.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Assay of alpha glucosidase inhibition in vitro
Enzyme inhibitory activity against α-glucosidase was observed spectrophotometrically (pH 6.8, 37 °C). At first, 850μl phosphate buffer with 50 mM p-nitrophenyl-α-d-glucopyranoside (PNPG) (in phosphate buffer), then extracts at doses of 0.5,10,20 and 100mg/ml were tested. Absorption increasing at 405 nm due to hydrolysis to PNPG by α-glucosidase was monitored with a spectrophotometer [15].

Statistical analysis
Data were analyzed by SPSS for Win.version 14 softwar. The homogenicy of variances was tested before using ANOVA. By heterogenicity of variances, differences between groups were considered to be significant at P=0.05 using ANOVA. At p<0.05 the post hoc test and Duncan test were used.

RESULTS

Determination of LD<sub>50</sub>
Mortality and LD<sub>50</sub> values of S. rhytidea methanolic extract was 4.9g/kg within 72 hour after administration (i.p.) (Table 1).

Table 1: Mortality and LD<sub>50</sub> values of Salvia rhytidea Benth. methanol extract within 72 h. after i.p. administration

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>0.5 hour</th>
<th>24 hour</th>
<th>48hour</th>
<th>72hour</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

Effect of Salvia rhytidea methanolic extract on serum glucose
Rats with fasting blood glucose level above 180 mg/dl were considered to be diabetic and used in the experiments. Changes in serum glucose level before and 1,2 and 3 hour after administration of normal saline, glibenclamid at dose of 20 mg/kg and methanolic extract at doses of 0.5,1 and 2 g/kg were determined. ANOVA test in heterogeneity of variances was used. Post hoc test and Duncan test were used to determined groups that had significant difference. The comparing results between extract receiving groups and glibenclamid receiving groups show that there was not significant difference between them but one hour after administration there was a significant difference between extract receiving groups at doses of 0/5g/kg and 1g/kg and group that receiving extract at dose of 2g/kg, but there was no significant difference at different doses 2 and 3 hour after extract administration (Table 2).
Inhibition of alphaglucosidase activity

Results of α-glucosidase inhibitory effect has given in Table 3. As shown, plant extract exhibited 50% α-glucosidase inhibition at dose of 100mg/ml.

Table 3: α-glucosidase inhibitory activity of Salvia rhytidea extract in different doses

<table>
<thead>
<tr>
<th>Extract concentration(mg/ml)</th>
<th>T1 (after 5 min.)</th>
<th>T2 (after 35 min.)</th>
<th>T2-T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.083</td>
<td>0.269</td>
<td>0.186</td>
</tr>
<tr>
<td>5</td>
<td>0.190</td>
<td>0.374</td>
<td>0.184</td>
</tr>
<tr>
<td>10</td>
<td>0.270</td>
<td>0.456</td>
<td>0.186</td>
</tr>
<tr>
<td>20</td>
<td>0.436</td>
<td>0.601</td>
<td>0.165</td>
</tr>
<tr>
<td>100</td>
<td>1.495</td>
<td>1.591</td>
<td>0.096</td>
</tr>
</tbody>
</table>

DISCUSSION

Salvia rhytidea Benth. (persian sage) is an endemic species of Salvia which is less known. In this study we investigated the hypoglycaemic effect of methanolic extract of Salvia rhytidea on streptozotocin-induced diabetic rats. As inhibition of α-glucosidase is the most significant activity in diabetes treatment, we examined whether a methanolic extract of this plant has an anti-diabetic effect through α-glucosidase inhibitory action. Determination of LD₅₀ exhibited no mortality up to 4.9g/kg, so the plant is safe enough for experiment. In this study, we have observed that methanolic extract of Salvia rhytidea decrease serum glucose level in streptozotocin-induced diabetic rats. The possible mechanism of action of could be correlated with the plasma insulin levels.

It is generally accepted that glibenclamide (a sulfonylurea family drug) causes reduction of blood glucose predominantly via stimulation of insulin release from pancreatic β-cells. Additionally, during long-term treatment, an insulin-independent blood glucose-decreasing mechanism may operate. The present study demonstrates that this extract in dose of 0.5g/kg has hypoglycemic action like glibenclamid while in dose of 1g/kg has a better efficacy than that of glibenclamid. Extract in dose of 2g/kg is not effective than that of extract in doses of 0.5 and 1g/kg but however a significant different was not seen between this group and glibenclamid receiving group. It is important to consider that 1 hour after administration the effect of this extract is not suitable but after 2 - 3 hours this effect is better. Thus we can introduce a new medicinal plant with a good hypoglycamic activity.

As inhibition of α-glucosidase is the most significant activity in diabetes treatment, we examined whether a methanolic extract of this plant has an anti-diabetic effect through α-glucosidase inhibitory action. So enzyme inhibitory activity against α-glucosidase was observed spectrophotometrically at pH 6.8 and 37 °C. The results show that methanolic extract of this plant inhibits the activity of α-glucosidase in vitro. The activities of α-glucosidase were inhibited 50% by 100 mg/ml of this extract. So plant metabolic extract showed α-glucosidase inhibitory effect.

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Conflict-of-Interest: There is no conflict of interest

REFERENCES


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