Original Paper

Antidiabetic and Antioxidant potential of Methanol extract of Salix caprea inflorescence

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Abstract

Salix caprea is widely used in folk medicine for rheumatoid arthritis, malaria, various hemorrhages, gout, neuralgia and intestinal diseases as an antipyretic, analgesic, anti-inflammatory, antibacterial, hemostatic, sedative, and antihelminthic agent. However, there are no critical reports available about anti diabetic potential of Salix caprea flowers. So anti diabetic effect of methanol extract of Salix Caprea flower (SCF) on alloxan-induced diabetic rats was studied. A single dose of 400mg/ml of the extract were orally administered as the treatment dose and the blood glucose levels (BGL) examined for 0, 7, 14, 21, 28 days intervals. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The data generated were analyzed using Spss reports. The % reduction of glucose levels calculated as 35.23% - 62.80% (standard drug, 10mg/ml), 30.60 - 46.69 (test extract, 400mg/ml) respectively. The extract exhibited significant (p<0.05 and p<0.01) reduction in the blood glucose levels of the albino rats along with some other biochemical variables. The extract compared favorably with the standard reference drug (Glibenclamide) which all gave their maximum BGL reduction at 20 Day duration.

Keywords: Salix caprea, Anti diabetic potential, Albino rats, Glibenclamide, Antioxidant;

Introduction

Diabetes is any disorder characterized by excessive urine excretion. The most common form of Diabetes is Diabetes mellitus, a chronic, progressive, systemic condition of impaired Carbohydrate metabolism [1]. Insulin unavailability may be due to degenerative changes in β-cells in the pancreatic islets, reduced effectiveness of the hormones owing to the formation of anti-insulin antibodies or inactive complexes, immune-mediated islet, Cytotoxicity or inappropriate secretion of hormones by neoplasm in other endocrine organs [2]. More than 1200 plants have been reported as hypoglycemic agents in various scientific and popular literatures [3,4]. Plant drugs are generally considered to be less toxic with lesser or rare side effects than those of synthetic ones [5].

Salix caprea L. is belonging to family Salicaceae and commonly known as goat willow. It is a folkloric medicinal plant used to treat rheumatoid arthritis, malaria, various hemorrhages, gout, neuralgia and intestinal diseases. It is also used as an antipyretic, analgesic, antibacterial, haemostatic, sedative and anthelmintic agent [6,7]. Plant has revealed the presence of many potent anti-oxidants such as luteolin, dihydrokaempferol and quercetin as its principle constituents along with (+)-catechin and isor-hamnetin as minor constituents [8,9]. Flavonoids present in Salix caprea wood were reported to have antifungal properties. Six identified flavonoids (dihydrokaempferide, naringenin, aromadendrin, taxifolin, prunin and (+)-catechin, naringenin was found to be the most effective one against both fungi and microbes [10]. Astralgins, Quercimeritrin and quercitin-3, 7-di-O-glucoside were found in pollen of Salix caprea [11].
While salicin, Saligenin, Gallocatechin, Rutin, cynaroside, quercitin and luteolin were reported in the leaves [12]. *Salix caprea* has been tested to determine their effects as feed additives to decrease ruminal methanogenesis *in vitro*. Significant antioxidant and anticancer activity of its alcoholic extracts has also been reported.

Besides this there is no report available about the anti diabetic potential of this unique and epidemic species, thus this study is conducted to evaluate anti diabetic potential of methanol extract of *Salix caprea* inflorescencce.

**Material and Methods**

**Extraction and Dose preparation**

The Flowers of *Salix caprea* were collected from the Survey of medicinal plant unit (SMPU) of Regional Research Institute of Unani Medicine (RRIUM), Srinagar, India. Where the identification and authentification of the plant material was done and a voucher specimen (1536) was deposited in the herbarium of the RRIUM Srinagar, India. Flowers were dried under shade and pulverized into powder in a grinding machine. The powdered material was extracted with methanol in Soxhlet apparatus. The extract was concentrated using vacuum rotary evaporation after which it was stored in a refrigerator at 4°C. The concentration and percentage yield of the extract were determined. The concentrate was dissolved in distilled water and administered orally in a dose of 400mg/kg/BW (as 1ml volume) as a single dose for 0, 7th, 14th, 21 and 28th day for both antidiabetic as well as safety evaluation.

**Experimental design and Assessment of extract on alloxan-induced diabetic animals**

Young adult Wistar albino rats obtained from the animal unit of the RRIUM, Srinagar were used for the study. They were supplied clean drinking water and standard feed (Grower mash pellets) was provided as the normal diet.

Single injection of alloxan monohydrate (150 mg/kg) intraperitoneally [13] were creating diabetes in rats. (Sigma, Co, St. Louis, USA). According to the weight for each animal individually. Alloxan was first weighed and then solubilized with 0.3 ml saline (154mM NaCl) just prior to injection. After 48 hrs of alloxan injection, rats with plasma glucose levels of >250 mg/dl were included in the study.

All the animals were randomly divided into the three groups with five animals in each group. Group A, B, and C were served as diabetic control, standard drug (glibenclamide, 10 mg/kg per day p.o) control, and treated fruit extracts control respectively. Blood samples were drawn at weekly intervals till the end of study (i.e. 4 weeks). Fasting blood glucose estimation was done on day 0, 7, 14, 21 and 28 of the study. On day 28, blood was collected from ventricles under mild ether anesthesia from overnight fasted rats and fasting blood sugar [14] was estimated. Serum was separated and evaluated for the estimation of various biochemical parameters such as cholesterol, triglycerides, urea, creatine, total protein, alkaline phosphatase as per reported method. Using biochemistry analysers (E. Merck, Mumbai) along with diagnostic kits (Sigma, Diagnostic, Pvt. Ltd). Further percentage decrease in blood glucose were monitored at 0, 7, 14, 21, 28 day after administration of the methanol extract at the dose level of 400mg/ml and were compared with the standard treated and diabetic controls.

**Determination of antioxidant activity**

The free radical scavenging activity of methanol extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in Methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of extract solution in water at different concentrations (50-250 μg/ml). Thirty minutes later the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

\[
\% \text{ scavenging activity} = \left(\frac{A_0 - At}{A_0}\right) \times 100
\]

Where A0 was the absorbance of the control (blank, without extract) and At was the absorbance in the reaction mixture indicated higher free radical scavenging activity. Radical scavenging presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

**Statistical Analysis**

Results were presented as mean ± standard deviation (S.D). Data were statistically analyzed using one-way ANOVA. Statistical significance was considered at P≤ 0.05.

**Results and discussions**

The experimental results of the effect of methanol extract of *Salix caprea* flowers in Alloxan induced diabetic rats showed that blood glucose levels decrease significantly with effect from day 7 (p<0.05) onwards till the end of day 28 (p<0.001), in case of standard and test extract treated group, the % reduction of glucose levels calculated as 35.23% - 62.80% (standard drug, 10mg/ml), 30.60 - 46.69 (test extract, 400mg/ml) respectively. The anti hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown in Fig. 1. Administration of alloxan (150 mg/kg, i.p.) led to 1.4-fold elevation of fasting blood glucose levels, which was maintained over a period of 4 weeks. Effect seems to reach maximum after 20 days of treatment and remains constant in last week. Control animals were found to
be stable in their body weight but diabetic rats showed significant reduction in body weight during 28 days. Alloxan caused body weight reduction, which is reversed by methanol extracts after 7 days of treatment.

Serum cholesterol, serum triglycerides, serum creatinine, serum urea, and serum alkaline phosphatase levels were decreased significantly by glibenclamide and the Methanol extract due to 28 days of treatment table 1

Antioxidant activity
The in vitro antioxidant assay performed on this plant reveals significant antioxidant potential compared with gallic acid as a standard. DPPH radicals are widely used in the model system to investigate the scavenging activity of several natural phytocompounds. The result of DPPH scavenging activity in this study indicates that this species were potentially active where Methanol extract shows %age inhibition of 66.74 at 250 µg/ml (Figure 2). The DPPH contains an odd electron, which is responsible for purple color and absorb wavelength of 517nm.

Table1: Effect of Methanolic extract of *Salix caprea* inflorescence on serum profile in alloxan (150 mg/kg, i.p.)-induced diabetic albino rats after 28 days of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic</th>
<th>Control (ST)</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRYGLY</td>
<td>140±8.90</td>
<td>95±5.40*</td>
<td>57±3.30*</td>
</tr>
<tr>
<td>CHO</td>
<td>130±2.23</td>
<td>82±4.52*</td>
<td>65±1.45*</td>
</tr>
<tr>
<td>UREA</td>
<td>81.5±6.68</td>
<td>49±2.34*</td>
<td>55±2.44*</td>
</tr>
<tr>
<td>CREA</td>
<td>1.30±0.31</td>
<td>0.67±0.20*</td>
<td>0.65±0.03*</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>5.61±1.06</td>
<td>7.40±0.78*</td>
<td>8.18±0.69*</td>
</tr>
<tr>
<td>ALP</td>
<td>118.30±4.02</td>
<td>62.20±6.34*</td>
<td>64.10±3.20*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of five animals each; Diabetic control (0.5% Tween 80 solution in normal saline). Alloxan single dose of 150 mg/kg in normal saline on day 0. *P < 0.01, extract treated groups were compared with the diabetic control.

Figure 2: Antioxidant potential of methanol extract (M).

Conclusion
In this study, several animal models were applied to evaluate the hypoglycemic potential of methanol extract of *Salix caprea* flowers. In light of the results, our study indicates that *Salix caprea* flowers extracts have good antidiabetic activity. Methanol extracts exhibited significant anti hyperglycemic activities in alloxan induced hyperglycemic rats without significant change in body weight. It can also improve the condition of DB as indicated by parameters like body weight and lipid profile along with serum creatinine, serum urea, and serum alkaline phosphatase. it could be speculated that the observed hypoglycemic activities of studied extract might be related to the presence of phenolics, and flavonoids contents and having the potential to impart beneficial therapeutic effect in diabetes. Results indicate that
the flowers of *Salix caprea* endowed with hypoglycemic activity presumably due to the antioxidant potential of the plant. To the best of our knowledge, antidiabetic potential of *Salix caprea* inflorescence has not been reported before and therefore our results can be evaluated as the first report about the evaluated parameters of this unique and endemic species. Isolation of secondary metabolites and their toxicological evaluations from this studied extract is warranted.

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**References**