ANTIDIABETIC ACTIVITY OF CLERODENDRUM PHILIPPINUM SCHAUER LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study has been undertaken to evaluate the antidiabetic activity of Clerodendrum philippinum Schauer leaves.

Methods: The fresh leaves were collected from Kuruan village of Jagpur district in the state of Odisha, India and extracted successively with n-hexane, methanol and water. The effect of extracts at the dose level of 400 mg/kg body weight was studied in normal, glucose loaded and streptozotocin-induced diabetic rats.

Results: The test extracts showed significant reduction of blood glucose level in normal, glucose loaded and streptozotocin-induced diabetic rats. Methanol extract demonstrated maximum blood glucose lowering potential as compared to other extracts.

Conclusion: The leaf of Clerodendrum philippinum Schauer is endowed with blood sugar lowering potential in both normal and diabetic rats.

Keywords: Clerodendrum philippinum Schauer, Antidiabetic activity, Streptozotocin.

INTRODUCTION

Clerodendrum philippinum Schauer (Synonym: Clerodendrum fragrans Willd.) belongs to family Verbenaceae, is a semi woody shrub distributed in southern Asia. It is commonly known as Chinese glory tree, Scent mali and Bramjamauli in the state of Odisha, India. It grows wild, spreads vegetative and is also grown as ornamental [1, 2]. Various species of Clerodendrum are used as folk and traditional medicines in various parts of the world like India, China, Japan, Thailand, Africa etc. and are reported to be used for the remedial purpose in inflammatory disorders, diabetes, cancers, malaria, fever, etc [3].

Leaves of C. Philistinism has been used as a traditional medicine for treatment of colic pain and exhibited anti-fungal activity [4]. The dried root is used as an anti-inflammatory [5] and for myalgia, tinea and rheumatoid arthritis [6]. The seed is used in constipation [7]. The water and ethanol extracts of leaf show anti-fungal [6] and ethanol extract possesses antibacterial activity [1]. The ethanol extracts of flower exhibits anti-anxiety and CNS depressant properties [9]. The leaf juice is used externally for scabies, cuts and burns [10]. The leaf juice mixed with the equal amount of ‘tushi’ (Ocimum sanctum) juice is used to reduce sugar content in blood by tribal and rural people in the state of Odisha, India [2].

The major chemical constituents reported from Clerodendrum philippinum are phenolics, flavonoids, terpenoids, steroids, etc. Flavonoids such as Cirsimarin and Sorbifolin were isolated from the leaf and stem [11]. Flavone, 5,7-B-Dihydroxy-4-Methoxy [12] and Kaempferol [13] were isolated from the dried leaf. Phenolic compounds, Acteoside, Leucosceptoside A, Isoacteoside, Methyl and Ethyl esters of Caffeic acid, Jinoside, etc. were reported from the whole plant [15].

Toubi et al. [14] isolated Bacoside, Derhamnosyl, Verbacaside, Iso-Verbacaside, and Calceolariside A from the leaves and also reported the presence of O-Iridoids and O-Flavonoids. Triterpenes (α-Amyrin and Clerodolone) and N-Tricostane were isolated from different parts of C. philippinum [15]. Steroids (Glerosterol, Daucosterol, β-Stosterol, Poriferasterol, Stigmasterol, etc.) were also reported from leaves of C. philippinum [15-18]. The present investigation deals with the evaluation of antidiabetic activity of various extracts of C. philippinum.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) and glibenclamide were procured from SIGMA-Aldrich, Mumbai. All other chemicals and reagents used were of analytical grade.

Plant material and extraction

The plants were collected from Kuruan village of Jagpur district in the state of Odisha, India. The taxonomic identity of the plant was confirmed by Dr. K. B. Satapathy, P. G. Department of Botany, Utkal University and the voucher specimen (SVN-534) was deposited at the departmental herbarium. The collected fresh leaves were washed, shade dried, powdered and extracted successively with n-hexane and methanol by using soxhlet apparatus. Then the marc was extracted with distilled water by the method of continuous hot extraction at 60 °C for 6 h [19]. Finally, the extracts were concentrated by evaporating the solvent using rotary evaporator. The yield of n-hexane, methanol and aqueous extracts were found to be 3.81%, 11.75% and 11.82% w/w respectively.

Qualitative phytochemical screening

The presence of phyto constituents in the extracts was determined by standard & prescribed chemical procedure [20-23].

Animals

Healthy adult Wistar albino rats of either sex (150-200 g body weight) procured from the animal house of School of Pharmaceutical Sciences (SPS), S’O’A University, Bhubaneswar were used for the study, and the experimental protocol was approved by the Institutional Animal Ethics Committee vide proposal no. 23/11, dated 24/01/2012 of SPS, S’O’A University, Bhubaneswar bearing Registration No. 1171/c/08/CP(CSEA).

Acute oral toxicity study

Healthy adult female Wistar albino rats starved overnight were divided into eight groups, each consisting of four rats and were orally fed with the test extracts in increasing dose levels of 500, 1000, 2000 and 4000 mg/kg body weight. The acute toxicity study was carried out according to OECD guidelines. The rats were observed continuously for 2 h under the following profiles [24].
(I) Behavioral profile: Alertness, restlessness, irritability, and fearfulness.

(II) Neurological profile: Spontaneous activities, reactivity, touch response, pain response and gait.

(III) Autonomic profile: Defecation and urination.

After a period of 24 h, 72 h and 14 days, the rats were observed for any lethality or death.

Induction of diabetes

Experimental diabetes was induced by single intra-peritoneal injection of 55 mg/kg of Streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5. After 5 days of STZ injection, rats with fasting blood glucose above 250 mg/dl were considered as diabetic.

Injection of 55 mg/kg of Streptozotocin (STZ), freshly dissolved in methanol (400 mg/kg); Group V: aqueous extract (400 mg/kg). The BGL was measured at 0, 1, 2, 4, 8 and 10 h following the administration of test substances, whereas 52.97% (P<0.001) fall of BGL respectively at 4 h following the administration of test substances.

Effect of extracts on normoglycemic rats

The effect of extracts on BGL was studied in normoglycemic rats [26], were divided into five groups of six rats each and fasted for 12 h with free access of water, and the treatments were made orally as: Group I: solvent control (Tween 40+distilled water); Group II: Glibenclamide (10 mg/kg); Group III: n-hexane extract (400 mg/kg); Group IV: methanol extract (400 mg/kg); Group V: aqueous extract (400 mg/kg). The BGL was measured at 0, 1, 2, 4, 8 and 10 h following the treatment.

Effect of extracts on glucose loaded hyperglycemic rats

Methanol and aqueous extracts showed 46.51% and 37.28% (P<0.001) fall of BGL respectively at 4 h following the administration of test substances, whereas 52.97% (P<0.001) with the standard. Methanol extract exhibited maximum reduction of blood glucose and better glucose tolerability among all the extracts when compared with the solvent control group at the end of 4 h (Table 3).

Induction of diabetes

The results are expressed as mean±SEM the statistical analysis is carried out using one-way ANOVA followed by Dunnett's t-test. Statistical P<0.01 is considered as significant.

RESULTS

Table 1: Preliminary phytochemical screening of Clerodendrum philippinum Schaur leaf extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Carbohydrates</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Phenolic compounds</th>
<th>Proteins</th>
<th>Saponins</th>
<th>Steroids</th>
<th>Triterpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*a* indicates present, *-* indicates absent.

Acute oral toxicity study

The gross observational results revealed that the extracts of C. philippinum leaves did not show any sign of toxicity and mortality up to 14 d of the study in the dose level of 4000 mg/kg. One-tenth of the observed safety dose was taken for experimental purpose considering the fact it may show the therapeutic effect as well as safe in longer duration of use, as per previously published literature [27, 28].

Effect of extracts on normoglycemic rats

The data represented in table 1 depicted the preliminary phytochemical investigation reports of the various extracts of C. philippinum indicates that the n-hexane extract was found to contain alkaloids, steroids, triterpenoids; whereas methanol extract showed the presence of flavonoids, lignans, glycosides, phenolic compounds, saponins, steroids, triterpenoids; and the aqueous extract showed the presence of flavonoids, glycosides, phenolic compounds, saponins.

Table 2: Effect of extracts of C. philippinum leaves on BGL in normal rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood Glucose Levels (mg/dl)</th>
<th>% decrease at 10 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>103.6±5.47</td>
<td>101.5±5.82</td>
</tr>
<tr>
<td>(Tween+Water)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilbenclamide (10 mg/kg)</td>
<td>101.8±5.16</td>
<td>91.5±3.29</td>
</tr>
<tr>
<td>n-Hexane Extract (400 mg/kg)</td>
<td>94.16±4.23</td>
<td>101.1±4.69</td>
</tr>
<tr>
<td>Methanol Extract (400 mg/kg)</td>
<td>99.6±4.49</td>
<td>98.6±3.01</td>
</tr>
<tr>
<td>Aqueous Extract (400 mg/kg)</td>
<td>102.6±3.92</td>
<td>97.8±3.48</td>
</tr>
<tr>
<td>F (4, 25)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed in mean±SEM of six rats. One Way ANOVA followed by Dunnet’s t-test. F-value denotes statistical significance at *P<0.05, **P<0.01 and f-value denotes statistical significance at *P<0.05, **P<0.01 and ***P<0.001 respectively, in comparison to the solvent control.
The experimental results of the present investigation conclude that the leaf extracts of *C. philippinum* is endowed with antidiabetic potential.

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**CONFLICT OF INTERESTS**

The authors declare that there are no conflicts of interest.

**REFERENCES**