ANTI DIABETIC EFFICACY OF ETHANOLIC EXTRACT OF PHRAGMITES VALLATORIA ON STZ-INDUCED DIABETIC RATS

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ABSTRACT

The aim of this study was to investigate the antidiabetic efficacy of ethanolic extract of Phragmites vallatoria leaf (EPVL), a member of Poaceae, on STZ-induced diabetic rats. When EPVL was given at a dose of 500 mg/kg body weight, it significantly decreased the levels of fasting blood glucose and HbA1c, but increased body weight and glycogen levels of muscle and liver in diabetic rats.

Keywords: Phragmites vallatoria, STZ, Diabetic rats, Blood glucose, HbA1c, Glycogen

INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia leading to disturbances in the metabolism of carbohydrates, lipids and proteins1. It is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced at target cells. Chronic hyperglycemia causes damage primarily to eyes, kidneys, nerves, heart and blood vessels2.

Currently the global prevalence of diabetes mellitus is estimated to be 150 million and this figure is expected to increase to over 300 million by 2025. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes.

Hypoglycemic and protective role of Anacardium occidentale was studied in streptozotocin-induced diabetic rats 3, 4. Aqueous extract of Boerhaavia diffusa was reported for its antihyperglycemic activity in STZ induced rats 5, 6. Similarly, plants like Bougainvillea spectabilis, Gossinia indica, Dioscorea dumetorum, Terminalia chebula10, Terminalia pallida11 and Muria koenigi12 have been used in the treatment of diabetes in traditional medicine. The Fruit, seeds and bark of Syzygium cumini and tea prepared from the leaves, have been used in treatment of diabetes throughout Asian countries13, 14. At present more than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because of their higher efficiency and multiple health benefits.

Phragmites vallatoria is a grass plant belongs to the family of Poaceae. It is found growing in moist fields in tropics of Asia, Africa and Australia. Our interactions with the local tribal community have revealed that leaves of this plant are used mainly for wound healing, arthritis, antimetics, fabrifuges, rheumatism and diabetes. It is interesting to note that so far no authentic reports have been found quoting the medicinal properties of Phragmites vallatoria and ours is the first scientific study on this plant (Fig.1). Our present study aimed to isolate the leaf extracts of this plant and validate its antidiabetic efficacy in STZ-induced diabetic rats. Our lab has got promising results on wound healing property of leaf extracts (yet to be published).

We focused on to investigate the role of ethanolic extract of Phragmites vallatoria leaf (EPVL) on levels of fasting blood glucose, liver and muscle glycogen, HbA1c and body weight in normal and STZ induced diabetic rats. The common observation is that diabetic subjects have less peripheral and skeletal muscle glycogen levels due to declined uptake of glucose but ethanolic extract of Phragmites vallatoria at a dose of 500mg/kg played a beneficial role in forming of glycogen in liver and skeletal muscles in STZ-induced diabetic rats.
MATERIAL & METHODS

Collection of plant material
The leaves of Phragmites vallatoria were obtained from chirala (Prakasam district, Andhra Pradesh, India), shade dried and powdered. Then powdered leaves were extracted with ethanol using soxhlet apparatus. Extracts were concentrated by rotary evaporator under vacuum. (Buchi).

Chemicals
Streptozotocin was purchased from Sigma- Aldrich St-Louis USA, all other chemical used in the experiments were of analytical grade.

Experimental Animals
The male healthy wistar albino rats weighing 150-160 g were obtained from the animal house of Sri Venkateswara agenesis, Bangalore and used in this study. The animals were caged and acclimatized for at least one week before the treatments started. They were provided with feed and water ad libitum.

Induction of Diabetes mellitus
After 12 hr fasting, STZ at a dose of 55 mg/kg body weight, dissolved in 0.1M citrate buffer (pH 4.5) was administrated intraperitonially 15.

Experimental design
24 male albino rats were divided into 4 groups with each group containing 6 rats.
Group 1 represented the control.
Group 2 represented the normal rats treated with EPVL extract of 500mg/kg body weight/day.
Group 3 represented the STZ-induced diabetic control rats
Group 4 represented STZ-induced diabetic rats treated with EPVL extract (500mg/kg body weight/day) for 9 weeks.

Sample collection and estimation of blood glucose
Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (Miles Inc, USA) and glucostix (Bayer diagnostic India Ltd., Baroda).

Table 1: HbA1c and Fasting blood glucose levels of Phragmites vallatoria leaf ethanolic extract in normal and diabetic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>EPVLN</th>
<th>Diabetic control</th>
<th>EPVLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>2.87 ± 0.10</td>
<td>2.66 ± 0.14</td>
<td>3.89 ± 0.20</td>
<td>2.71 ± 0.18</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>88.6 ± 2.15</td>
<td>83.6 ± 0.58</td>
<td>347.5 ± 20.26</td>
<td>115.5 ± 1.33</td>
</tr>
</tbody>
</table>

Data was expressed as a mean ± SEM; EPVLN: Normal rats treated with Phragmites vallatoria leaf ethanolic extract; EPVLD: Diabetic rats treated with Phragmites vallatoria leaf ethanolic extract

HbA1c estimation
Glycosylated hemoglobin was estimated by the method of Eross et al.10. To the erythrocytes (0.5ml) collected from whole EDTA blood, 0.125ml of distilled water and 0.125ml of carbon tetrachloride were added, mixed well and centrifuged. The supernatant hemolysate was separated and its hemoglobin concentration was adjusted to 10% with distilled water. To 2ml of hemolysate, 1ml of 0.3N oxalic acid was added in stoppered test tubes and heated at 100°C in a water bath for 60 min. After cooling the contents, 1ml of 4% TCA was added, shaken well and centrifuged. To 2ml of supernatant pipetted out into another set of test tubes, 0.5ml of 0.05M TBA was added and incubated at 37°C for 40 min. A blank with 2ml of distilled water was treated similarly.

Statistical analysis
Data was expressed as a mean ± SEM.

RESULTS AND DISCUSSIONS
In the present study ethanolic extract of Phragmites vallatoria leaf at a dose of 500mg/kg body weight/day was administered for 63 days. When blood samples were analyzed in all the groups, the levels of fasting blood glucose (FBG) and glycated hemoglobin (HBA1c) were significantly decreased in normal rats treated with EPVL when compared to controls (Table 1). Similarly, FBG and HBA1c levels were greatly decreased in EPVL treated diabetic rats than diabetic controls (Table 1).

Table 2: Body weight, skeletal muscle and liver glycogen levels of Phragmites vallatoria leaf ethanolic extract in normal and diabetic rats

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>EPVLN</th>
<th>Diabetic control</th>
<th>EPVLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (gm)</td>
<td>167.2 ± 2.10</td>
<td>168.5 ± 1.11</td>
<td>150.4 ± 1.28</td>
<td>164.1 ± 1.14</td>
</tr>
<tr>
<td>Skeletal muscle Glycogen (mg/g)</td>
<td>8.43±1.90</td>
<td>8.38±0.82</td>
<td>17.7±0.83</td>
<td>6.33±0.74</td>
</tr>
<tr>
<td>Liver glycogen (mg/g)</td>
<td>43.8±1.29</td>
<td>42.9±1.96</td>
<td>7.65±0.94</td>
<td>25.9±2.33</td>
</tr>
</tbody>
</table>

Data was expressed as a mean ± SEM; EPVLN: Normal Treated with Phragmites vallatoria leaf ethanolic extract; EPVLD: Diabetic Treated with Phragmites vallatoria leaf ethanolic extract
CONCLUSION

The present study suggested that *Phragmites vallatoria* leaf ethanolic extract showed a promising role in therapy to diabetic rats. Further studies are in progress to isolate the active principle(s) of the extract as well as to elucidate their exact mechanism(s) of action. Our present documented findings may suggest the use of *Phragmites vallatoria* leaf ethanolic extract to treat the diabetic patients.

REFERENCES

17. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars.