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Research Article

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

A.NAGA VAMSIKRISHNA[1], M.RAMGOPAL[3], B.VENKATA RAMAN[2*] AND M. BALAJI[3*]

[1] Department of Biochemistry, Achraya Nagarjuna University, Guntur, India, [2]Department of Biotechnology, K.L. University, Guntur, India, [3]Department of Biochemistry, Sri Venkateswara University, Tirupati, India. Email: drbvraman@yahoo.co.in, balaji.meriga@gmail.com

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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 HbA_1c, 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 proteins[1]. 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 vessels[2].

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 diffusea was reported for its antihyperglycemic activity in STZ induced rats [5, 6]. Similarly, plants like Bougainvillea spectabilis[7], Coccinia indica[8], Dioscorea dumetorum[9], Terminalia chebula[10], Terminalia pallida[11] and Muria koenigii [12] have been used in the treatment of diabetes in traditional medicine. The Fruit, seeds and bark of Syzyguim cumini and tea prepared from the leaves, have been used in treatment of diabetes throughout Asian countries[13, 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, HBA_1C 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.

Fig. 1: *phragmites vallatoria* plant

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).

HbA1c estimation

Glycosylated hemoglobin was estimated by the method of Eross et al[16]. 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 100ºC in a water bath for 60min. After cooling the contents, 1ml of 40% 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 37ºC for 40min. A blank with 2ml of distilled water was treated similarly. The resulting yellowish color was read in a spectrophotometer at 443nm. Concentration of HbA_{1c} was calculated on the assumption that 1% HbA_{1c} corresponds to an absorbency of 0.029 at 443nm (experimentally determined millimolar extinction co-efficient for TBA-5 hydroxymethyl furfural adduct is 26 at 443nm). Hemoglobin in RBC (g %) was estimated by cyanmethemoglobin method.

Glycogen estimation

Liver and muscle tissues were digested with 2 ml of KOH (30%) and boiled in water bath for 30 min with occasional shaking and then allowed to cool at room temperature. Saturated Na2SO4 solution was added to the mixture and stirred well. Glycogen was precipitated by adding 5 ml of ice cold ethanol to the sample mixture and centrifuged at 10,000 rpm for 10 min. One ml of HCl (1.2 N) was added to the supernatant (1: 1 v/v) and incubated at 90o C for 2 h, and then allowed to cool. DNS method (Miller) [17] was followed to determine hydrolyzed product of tissue glycogen.

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 glycosylated hemoglobin (HBA_1C) were significantly decreased in normal rats treated with EPVL when compared to controls (Table 1). Similarly, FBG and HBA_1C levels were greatly decreased in EPVL treated diabetic rats than diabetic controls (Table 1).

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

Parameter	Control	EPVLN	Diabetic control	EPVLD
HBA_1C (%)	2.87 ± 0.10	2.66 ± 0.14	3.89 ± 0.20	2.71 ± 0.18
FBG (mg/dl)	88.6 ± 2.15	83.6 ± 0.58	347.5 ± 20.26	115.5 ± 1.33

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

With regard to body weight, liver and muscle glycogen levels, there was no significant difference between control and EPVL treated rats. However, there was a significant increase in body weight and glycogen levels of diabetic rats treated with EPLV than diabetic controls. An increase of 3-4 folds in muscle and liver glycogen levels was observed in diabetic rats treated with EPVL when compared to diabetic controls (Table 2). Earlierly santosh kumar et al [18] have proved antidiabetic activity of certain herbal remedies. In diabetes, glycogen content of liver and skeletal muscles usually decrease due to improper regulation of lipid metabolism in diabetic rats. On the other hand the HbA_1c levels were decreased upon treatment with ethanolic extract compared with diabetic treated & untreated rats. HbA_1c is produced by glycosylation of haemoglobin and stimulates increased insulin secretion in STZ-induced diabetic rats. In the case of body weights a significant increase was observed in normal and diabetic treated rats due to the supplementation of *Phragmites vallatoria* leaf ethanolic extract pocessing polyphenolic content. HbA_1clevels were decreased in both normal rats treated with EPLV and diabetic rats treated with EPLV when compared with diabetic and diabetic untreated rats. This may be mainly due to the decreased FBG levels.

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

Parameter	Control	EPVLN	Diabetic control	EPVLD
Body Weight (gm)	167.2 ± 2.10	168.5 ± 1.11	150.4 ± 1.28	164.1 ± 1.14
Skeletal muscle Glycogen (mg/g)	8.43±1.90	8.38±0.82	1.77±0.83	6.33±0.74
Liver glycogen (mg/g)	43.8±1.29	42.9±1.96	7.65±0.94	25.9±2.33

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.

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