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Screening for anti-diabetic activity of the ethanolic extract of *Barleria cristata* seeds

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Abstract

The anti-diabetic drugs from plants in current clinical use and their similar mechanism of action of herbal components are preferred mainly due to lesser side effect and low cost. Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences[1]. Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plants which possess medicinal virtues. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. Nowadays, diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Barleria cristata L* (Acanthaceae) for the selected area for diabetes. Another important objective of this study is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness and add value to the resource. Acoholic extracts of dried seeds of *Barleria cristata* were subjected for hypoglycaemic activity in Wistar rats (150-200 g). Blood sugar level was determined using digital glucometer. The oral administration of seeds extracts at doses of 200 mg/ kg lead to a significant blood glucose reduction. This laid the foundation to study the active compounds of such anti-diabetic plants that are responsible for the hypoglycemic activities.

Key-Words: Alloxan-induced diabetes, hypoglycaemic activity, *Barleria cristata*; Anti-diabetic activity

Introduction

Diabetes mellitus is a multifactorial disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion, and impaired insulin action. Diabetes mellitus is characterised by hyperglycaemia, lipidaemia and oxidative stress and predisposes affected individuals to long-term complications affeccting the eyes, skin, kidneys, nerves and blood vessels. There are lots of chemical components available to control and treat diabetic patients, but total recovery from diabetes may not possible. Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences[1].

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However, many medicinal plants have been provided a potential source of anti-diabetic principles and are widely used for the treatment of diabetes mellitus in various traditional systems of medicine worldwide and many of them are known to be effective against diabetes. The hypoglycemic effects of pharmacologically active components of plants in diabetes patients has been reported by assessing the lowering affects on alpha amylase (both salivary and pancreatic) for development of diabetes[2]. The anti-diabetic drugs in current clinical use and their similar mechanism of action herbal components are preferred mainly due to lesser side effects and low cost. Medicinal plants used in traditional system found to contain alkaloids, flavonoids, glycosides, saponins, phenols, tannins. Most of them have been tested on porcine pancreatic α-amylase (PPA) and salivary amylase. We also started screening medicinal plants with anti-diabetic activity through not only HPA but also salivary amylase inhibitors [2]. It has been shown that activity of Human Pancreatic α-amylase (HPA) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is

therefore an important aspect in treatment of diabetes. Phytochemical analysis revealed the presence of alkaloids, proteins, tannins, cardiac glycosides, flavonoids, saponins and steroids as probable inhibitory compounds[4,5]. Increased protein glycation and the subsequent build-up of tissue advanced glycation endproducts (AGEs) contribute towards the pathogenesis of diabetic complications. Protein glycation is accompanied by generation of free radicals through autoxidation of glucose and glycated proteins. Glycation derived free radicals can damage proteins, lipids and nucleic acids and contribute towards oxidative stress in diabetes.

Material and Methods

Plant Material

The seeds of *Barleria cristata L.* (Acanthaceae) were collected from medak district of Andhra Pradesh and were authenticated when they were collected and chemically studied for chemical components by us for the first time. The specimens were used for the extraction and the extracts were screened for anti-diabetic activity.

Extraction Procedure

The seeds of *Barleria cristata* were dried under shade and grinded in electrical grinder to coarse powder. Powdered seeds (1.5 kg) were extracted for 24 hrs with alcohol (60-80°C) and dried under reduced pressure and tested on alloxanised hyperglycaemic animals [29]

Drugs

Alloxan monohydrate was purchased from Sigma chemicals (St Louis, USA). All other chemicals used for this study were of analytical grade.

Animals

Wistar rats (200-225 g) of either sex were employed in this study. The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photo period [12 h dark/12 h light] were used for the experiment. Commercial pellet diet [Chakan oil mill, Sangli, India] and water were provided ad libitum. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government.

Toxicity Studies

Acute and chronic toxicity studies were carried out[18]. Animals of either sex were fasted for 18 h and used. In acute toxicity studies, a dose of 250 mg/kg of dried extract were orally administered to 12 mice; additionally, 3 mice were kept as control. Then, they were observed for motor reflexes for 48 h. Since no mortality was observed and the behavioural pattern was unaffected, further studies were carried out [Tables 1 and 2]. In chronic toxicity studies, 22 animals were used. They were divided into two groups: 6 as control and 16 as test animals. In the test group, a dose of dried extract of *Barleria cristata* of 250 mg/kg was administered daily for a period of 15 days. The body weights were recorded for both the groups at an interval of 5 days. Finally, the haematological parameters were studied in both the groups [Tables 3 and 5].

Experimental Design

Diabetes was induced using alloxan monohydrate (100 mg/kg). Only alloxanised hyperglycaemic animals were used for further studies. Animals were fasted for 18 h before the experiment and divided into 5 groups (6 animals in each group). The first group (control) received normal saline and the second group received alloxan monohydrate alone. The three test groups received 200 mg/kg of different extracts before the dose of alloxan. All the animals were regularly observed for their general behaviour.

Effects on Blood Glucose Levels

Dried ethanolic extracts of *Barleria cristata* seeds (200 mg/kg) were suspended in 1% bentonite and subjected for hypoglycemic activity in Wistar rats (200-225 g). Diabetes was induced by the intravenous administration of alloxan (100 mg/kg)[19] after anaesthesia with ethyl ether. Forty-eight hours later, the blood (1 mL) was collected from the orbital sinus into tubes and immediately used for the determination of glucose. Only animals that presented with glycaemic levels equal to or above 200 mg/dL were submitted to treatments, which consisted of a daily administration of extracts of *Barleria cristata* seeds for 7 days. The oral treatments (by gavage) of all groups were carried out at the same time (in the morning) and under the same conditions. One hour after the last administration, the blood was collected again for blood glucose measurements using a glucometer.

Statistical Analysis

All the values were expressed as mean ± Standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's t test. P values < 0.01 were considered as significant.

Results and Discussion

The effect of oral administration of ethanolic extracts of *Barleria cristata* seeds are shown in Table 4. Experimental studies reveals that the ethanolic extracts from *Barleria cristata* seeds (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats.

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas [22, 23].

In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis [29, 30]. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells [31]. Experimental studies revels that the ethanolic extracts from *Barleria cristata* seeds (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. It also proves the traditional claim with regard to *Barleria cristata* seeds for its anti-diabetic activity.

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Table 1: Acute toxicity studies of extract for 48 hrs.

Group	Dose	Extract toxicity (48 hrs)	
		Motor reflex	Behaviour pattern
Test	200	Normal	Normal
Control	-	Normal	Normal

Table 2: Acute toxicity studies of extract for 72 hrs.

Group	Dose	Extract toxicity (72 hrs)	
		Motor reflex	Behaviour pattern
Test	200	Normal	Normal
Control	-	Normal	Normal

Table 3: Chronic toxicity studies

Time in days	Average body weight (g)	
	Test	Control
5	20.45	20.35
10	22.15	22.27
15	22.49	22.59

Table 4: Effect of ethanolic extract of *Barleria cristata* seeds

Group	Blood glucose level (mg %)	
	Before treatment	After treatment
Control	84.37±0.15	84.36±0.14
Alcoholic extract	83.26±0.13	80.04±0.26

Table 5: Haematological parameters

Parameters	Test	Control
Bleeding time (min)	4.78 ± 0.13	4.84 ± 0.09
Clotting time(sec)	35.44 ± 0.17	37.51 ± 0.18
Total WBC/mm^3	6321 ± 0.30	6430 ± 0.34
Total RBC/mm^3	7.51x10^5 ± 0.62	7.90x10^5 ± 0.41
Haemoglobin (mg/dl)	16.04 ± 0.02	16.2 ± 0.01