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EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *BARLERIA CRISTATA* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a major degenerative disease in the world today affecting at least 15 million people. The plant *Barleria cristata* is popular in Tamilnadu and for it's used in house hold remedies. It is traditionally known for its various properties and used to treat several ailments like anemia, toothache and cough. Root and leaves are used in the treatment of swelling and inflammation. The study was carried out to evaluate the hypolipidemic activity of 50% ethanolic extract *Barleria cristata* by inducing hyperglycemia with the help of alloxan in albino rats and various parameters such as serum glucose, liver glycogen, Glycosylated haemoglobin, lipid profiles were determined. Ethanolic extract showed significant ($p<0.05$) hypolipidemic activity by lowering the levels of total cholesterol, triglycerides, LDL and VLDL, and simultaneously increasing the levels of HDL in alloxan induced diabetic rats. The phytochemical constituents such as alkaloids, flavanoids and phenols present in the plant may be responsible for hypolipidemic activity and the results justify the use of *Barleria cristata* as a significant hypolipidemic agent in diabetic rats.

KEY WORDS: ***Diabetes mellitus, Hypolipidemic activity, Anti-hyperglycemic activity, Antioxidant, Lipid profile, Barleria cristata.***

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INTRODUCTION

Diabetes mellitus is a group of disorder characterized by interruption in carbohydrates, protein and fat metabolism due to complete or relative deficiency of insulin secretion and insulin action.[1] Diabetes mellitus is a major degenerative disease in the world today affecting at least 15 million people. Diabetes mellitus is associated with long term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others. It is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells. It is ranked third among the leading causes of death when its fatal complications are taken into account. Today in India alone there are more than 4.00 crore diabetics and the number is going to be around 9.00 crore by 2030. Over 7.20 lakh Indians die every year due to diabetes. People with diabetes are 2-4 times more likely to develop heart diseases.[2] In the present scenario, the demand for herbal products is growing exponentially throughout the the world and major pharmaceutical companies are currently conducting extensive research on plant material for potential medicinal value.[3] There has been great demand for plant products due to low cost, easy availability and lesser side effects. For this plant materials are continuously being scrutinized and explored for their effect as anti diabetic agents. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic and antihyperlipidemic remedies. Antihyperglycemic effect of these attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity is available in literature. The present study was performed to determine the anti lipidemic activity of ethanolic extract of *Barleria cristata* in alloxan induced diabetic rats.

MATERIALS AND METHODS

(i) Preparation of plant extract

Large number of *Barleria cristata* leaves were collected from Maruthamalai Hills, Coimbatore. Before the leaves were used, they were identified and certified by the Botanical Survey of India (BSI), Tamilnadu Agricultural University, Coimbatore. (No. BSI/SC/5/23/0708/Tech.-378).1kg of coarse powder of *Barleria cristata* leaves were chopped in 50% alcohol (i.e. 1.5 ml of alcohol and 1.5 ml of water) and cold macerated for 3 days. During the maceration period occasional stirring was done. After 3 days the suspension was filtered through a fine musline cloth. The residue was removed. The filtrate was taken in a round-bottomed glass flask and the sample was evaporated to dryness at a low temperature (<40°C) under reduced pressure in a rotary evaporator. The saponin contents showed the frothy appearance. Finally dark green coloured crystals of approximately 75g were obtained which was stored in a air-tight desicator at 0° to 4°C. When needed the residual extracts were dissolved in distilled water and used for the study.

(ii) Selection and maintenance of animals

Male albino rats of wistar strain weighing about 130 to 170 g obtained from PSG Institute of Medical Sciences and Research, Coimbatore, and used for the study (No. 158/1999/CPCSEA). The animal house was well ventilated and animals had 12 ±1 hour day and night schedule with temperature between 11 and 20 ± 2°C. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with rat pellet feed supplied by M/s Hindustan Lever Limited, Bangalore, India and filtered water *ad libitum*. Animals described as “Fasting” group were deprived of food for at least 16 hours but allowed free access to water.

(iii) Induction of Diabetes

Alloxan monohydrate induced Diabetes mellitus was produced in the normoglycemic male albino rats. Animals were allowed to fast for 24 hrs and were injected intraperitonially with freshly prepared alloxan monohydrate in sterile saline in a dose of 120mg/kg body weight . Blood glucose was measured 24hrs after the alloxan injection. It was confirmed that the given dose was sufficient for inducing diabetes in the animals. The animals were maintained in the diabetic state over a period of 21 days. Rats showing fasting blood glucose levels greater than 350mg/dl were selected for the study.

(iv) Treatment protocol

Group-I: Consist of normal rats treated with 10ml/Kg of normal saline, orally for 30 days.
Group-II: The rats were made diabetic by an intraperitoneal injection of single dose of (120 mg/kg body weight) alloxan monohydrate in normal saline.
Group-III: Diabetic rats received 500 mg/kg body weight of *Barleria cristata* extract for 30 days, orally.
Group-IV Normal rats received 500 mg/kg body weight of *Barleria cristata* extract orally for 30 days.

(v) Sample collection

After 30 days of treatment, the blood glucose level and body weight were measured. Then blood was collected retro-orbitally under light ether anesthesia using capillary tubes. Blood was collected in fresh vials containing EDTA as anticoagulant agents and plasma was separated in a T8 electric centrifuge at 2000 rpm for 2 minutes. Then animals were sacrificed by decapitation. Liver and pancreas were immediately dissected out, washed in ice-cold saline to remove the blood and liver was used for estimation of enzyme activity while pancreas was subjected to histopathological studies.

(vi) Estimation of biochemical parameters

The serum was separated and was analyzed for blood glucose [4], glycogen [5], total cholesterol [6],triglycerides [7], HDL cholesterol [8]and LDL cholesterol. [9]

(vii) Statistical analysis

The results were expressed as mean ± S.D for each parameter under four different groups. The significance of difference in the means of two groups was tested using Student's t-test at 5% levels. The result were considered significant at 5% level if P<0.05.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1
Preliminary phytochemical studies of 50% ethanolic extract of *Barleria cristata* – Qualitative screening

Tests	Observation
Alkaloids	+
Carbohydrates	+
Glycosides	+
Flavonoids	+
Phenols	+
Steroids	-
Saponins	+

Phytochemical analysis of the extract showed the presence of alkaloids, flavanoids, phenols and glycosides. (Table 1)

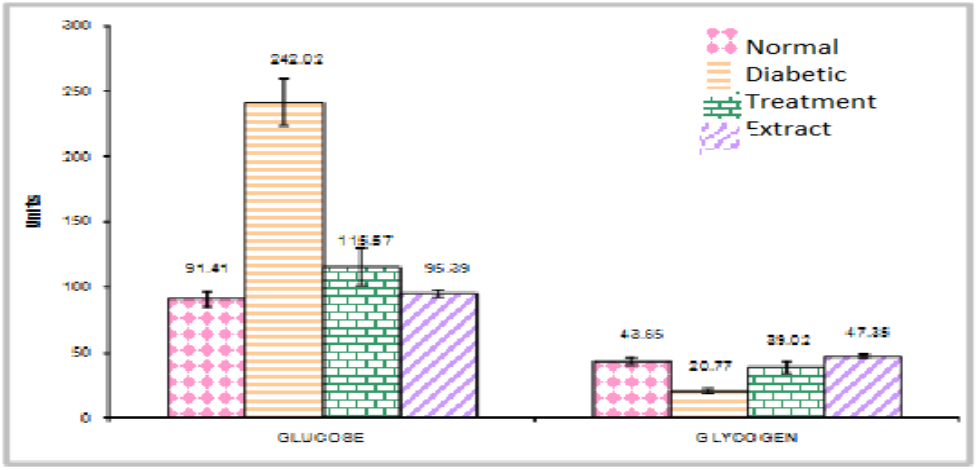
Serum glucose

Figure 1 shows the different levels of serum glucose in control and experimental rats. The Alloxan induced Diabetic rats, showed elevated blood glucose when compared with normal control rats. Administration of the *Barleria cristata* leaves extract was found to reduce the blood sugar level in alloxan induced diabetic rats. There was no significant (P<0.05) change in the blood glucose level in group I and group IV rats. The results of our study in accordance with earlier reports on anti-diabetic effect of *Hibiscus cannabinus* in streptozotocin induced diabetic rats.[10] Our results showed that extracts of *Barleria cristata* proved to be effective in lowering the blood glucose in alloxan induced diabetic rats to an extent of 50 percentage. Hepatic glycogen (Figure1) shows the various levels of Hepatic glycogen in control and experimental groups. Hepatic glycogen was checked for all rats in each group.

Liver glycogen

The liver glycogen level in diabetic rats was significantly (P<0.05) decreased when compared with that of normal rats. Glycogen synthesis in the liver is impaired in diabetes due to defective activation of glycogen synthase. In a previous study, extracts of *Azadirachta indica* enhanced the rate of hepatic glycogen in diabetic treated groups.[11]

Figure 1
Effect of Barleria cristata on serum glucose and liver glycogen



Units: Glucose mg/dl Glycogen mg/g of tissue

Cholesterol and Triglycerides

Table 2 shows the levels of cholesterol and Triglycerides in serum. In alloxan induced diabetic rats there was a significantly (P<0.05) increased in total cholesterol and triglycerides level in serum, when compared with the normal group I rats. Administration of *Barleria cristata* leaves extract was found to be effective in lowering the serum cholesterol and Triglycerides to the normal level. There was no significant change between the levels of serum cholesterol and triglyceride between group I and group IV rats. Vasanthamani *et al*., reported increased serum and cholesterol levels in their study of hypoglycemic and hypocholesterolemic effect of herbal powders. [12] The cholesterol and triglycerides are elevated in diabetic condition. Such an elevation represented the risk factor for coronary heart disease. Neither the standard drug nor did the extracts significantly affect the lipid profile and liver glycogen in

normal rats. In diabetic rats the plant / herbal extracts brought back the levels of lipid profile to the normal levels.

HDL, LDL and VLDL

Table 2 shows the levels of serum HDL, LDL and VLDL in experimental rats. There was a significant (P<0.05) increase in the levels of LDL and VLDL in diabetic rats when compared with the normal rats. It's well known that the level of glycemic control is the major determinant of serum level of very low density lipoprotein (VLDL) and triglycerides.[13] Also there are many reports, which proved that plants with hypoglycemic property also possess hypolipidemic effect. Excess of fatty acids in plasma produced by the alloxan induced diabetes promotes the linear conversion of some fatty acid into phospholipids and cholesterol .[14] The administration of ethanolic extract of *Barleria cristata* was found to revert back the lipid profile to normal levels. There was also significant (P < 0.05) decrease in the serum HDL levels in diabetic rats, when compared with the normal ones. On administration of the ethanolic extract the serum HDL level was reverted back near to normal levels.

Table 2

Levels of Total cholesterol, Triglycerides, HDL, LDL, VLDL in serum of control and experimental rats.

Groups	Total Cholesterol	HDL Cholesterol	Triglyceride	LDL Cholesterol	VLDL Cholesterol
Group I	159.27 ± 10.96	26.47 ± 0.46	24.27 ± 1.74	15.14 ± 0.43	11.77 ± 9.04
Group II	311.74 ±34.06^{a}	14.57 ± 0.04^{a}	72.82 ± 1.05^{a}	136.48 ± 0.60^{a}	36.37 ± 0.59^{a}
Group III	182.98 ± 9.74^{b}	18.60 ± 0.28^{b*}	42.66 ± 0.99^{b}	78.72 ± 0.09^{b*}	30.39 ± 0.28^{b*}
Group IV	158.35 ± 16.08$^{c\ NS}$	26.27 ± 0.15^{cNS}	23.92 ± 1.04$^{c\ NS}$	16.94 ± 0.19$^{c\ NS}$	13.32 ± 0.22$^{c\ NS}$
CD (0.05)	**1.6379**	**0.3696**	**1.456**	**0.5071**	**5.9795**

***Values are expressed by mean ± SD of six samples*Groups comparison: a – G1 vs G2, b – G2 vs G3, c – G1 vs G4**
Statistical Significance: * - Significant (P< 0.05); NS– not significant

CONCLUSION

In our study 50% ethanolic extract of *Barleria cristata* leaves exhibited significant antihyperglycemic activities in alloxan induced diabetic rats. The extract showed improvement in parameters like blood glucose, liver glycogen and lipid profile so the extract might be of value in Diabetes treatment.

CONFLICT OF INTEREST

No Conflict of interest to declare.

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