ANTIDIABETIC ACTIVITY OF EXTRACT OF *BERBERIS ARISTATA* ROOT IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

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Summary

Berberis aristata DC root is used in traditional medicine for a number of ailments including antibacterial, antiplatelet, anti-inflammatory, analgesic, antipyretic, antioxidant and hepatoprotective activities etc. The study was conducted to evaluate the antidiabetic activity of the ethanolic extract of root of *B. aristata* at two dose levels of 100 and 200mg/kg bw in sucrose challenged normal as well as in rats with streptozotocin induced diabetes. Oral application of extracts at doses of 100 and 200mg/kg bw, significantly ($p<0.05$) lowered the plasma glucose levels in normal as well as diabetic rats. Antihyperglycemic effect of extract at a dose of 200mg/kg bw was more pronounced and causes maximum fall of blood glucose level to 22.9% ($p<0.05$) and 29.4% ($p<0.01$) respectively with the two doses in normal and 30.3% ($p<0.01$) and 48.4% ($p<0.001$) in diabetic rats after 3 hrs and 6 hrs of treatment in normal and diabetic rat respectively. The findings of the present study suggest that the ethanolic extract *B. aristata* produced significant antihyperglycemic activity in streptozotocin induced diabetic rat which is comparable to metformin (a standard oral hypoglycaemic agent).

Keyword: *Berberis aristata,* antihyperglycemic, metformin, streptozotocin

Introduction

Diabetes mellitus is a complex and multifarious group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. The number of people affected with diabetes worldwide is projected to be 366 million by year 2030.[1] Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycaemia in diabetes. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems.[2] Alternative strategies to the current modern pharmacological therapy of diabetes are urgently needed[3], because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Plants used in traditional medicine to treat diabetes represent a valuable alternative for the control of this disease.[4] The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential.[5] Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research.[6]
Berberis aristata (Berberidaceae) is commonly known as daruharidra, garhwal and chitri. It is a shrub found in the northern mountainous region of Pakistan and India, as well as in the Nilgiri Hills of Southern India.[7] B. aristata extract has been used by the natives of Sikkim and Darjeeling as a folklore medicine for the treatment of diabetes. [8] The properties like cholegogue, hepato-stimulant and astringent are useful in treating anorexia, dysentery, hepatitis and liver disorders.[9] Antioxidant[10], antibacterial[11,12], anti-inflammatory[12], analgesic[12] and antipyretic[12] activities have been evaluated. Chemical analysis revealed the presence of alkaloids, amino acids, tannins, terpenes, resins, phenols and reducing sugars as major compounds. FTIR-spectral analysis of *Berberis aristata* root extract revealed the presence of berberine, as a major constituent, along with other chemical constituents. [12] The alkaloids found in bark and root of B. aristata are berberine, berbamine, aromoline, karachine, palmatine and oxycanthine. The alkaloid berberine possesses antibacterial and anti-inflammatory activities. [13] The study showed berberine significantly inhibited the progression of diabetes induced by alloxan. [14] Thus, we selected this plant to evaluate for its anti-diabetic action in streptozotocin (STZ) induced diabetic rat.

Materials and Methods

Plant Material

The roots of *B. aristata* were procured from Yucca Enterprises, Mumbai. The roots were authenticated by Dr. Suresh Baburaj, Director, Survey of Medicinal Plants and Collection Unit, Ooty. The voucher specimen was deposited at our department for future reference.

Preparation of Plant Extract

The roots of *B. aristata* were washed thoroughly with tap water, shade dried, cut into small pieces, and were crushed to moderately coarse powder. It was extracted using 95% ethanol in soxhlet apparatus for 6 hrs. The extract was concentrated using rotary evaporator at 40-45oC under reduced pressure.

Experimental Animals

Healthy adult male albino rats of Wistar strain weighing 150-200 g were obtained from Central Animal House, J.S.S. College of Pharmacy, Ooty, India. The animal house was well ventilated and animals had 12 ± 1 hour and day and night schedule with temperature between 15-20±5^{o}C. The animals were housed in standard polypropylene hygienic cages (three animals per cage). The animals were fed with rat pellet feed supplied by M/S. Hindustan Lever Ltd., Bangalore. The current work was carried our after approval by our institutional ethical committee.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). [15] Wistar rats (n =5) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the was administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2 - 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 500 and 1000 mg/kg body weight.

Hypoglycemic Activity in Normal Rats

The hypoglycaemic effect of *B. aristata* root in normal rats was assessed by improvement of glucose tolerance. Overnight fasting blood glucose of Wistar strain male albino rats was measured and the animals showing fasting blood glucose level from 60 to 80 mg/dl were selected and divided into four groups of five animals in each. The blood glucose measured at this time period was termed as baseline (0 min) blood glucose. Rats of experimental group were administered orally suspension of the ethanolic extract at 100 and 200 mg/kg dose prepared in 2.0% gum tragacanth. The standard drug metformin was given at 100 mg/kg dose level. Animals of control group received vehicle (2.0% gum tragacanth). An oral sucrose load of 10 g/kg body weight was given to rats of all groups exactly post 30 min administration of the test sample/vehicle. Blood glucose level was again measured at 30, 60, 90, 120 and 180 min post administration of sucrose. Food but not water was withheld from the cages during the course of experiment.

Hypoglycemic Activity in STZ-Induced Diabetic Rats

Induction of diabetes in rats

Overnight fasted male albino rats (Wistar strain) were made diabetic by intraperitoneal injection of streptozotocin at 60 mg/kg body weight dose prepared in 0.1M citrate buffer (pH 4.5). Fasting blood glucose level was measured after 48 hours and animals showing blood glucose level above 180 mg/dl were considered as diabetic. [16]

Experimental groups for effect of extract on STZ-induced diabetic

The diabetic rats with fasting blood glucose values (baseline at 0min) from 180 to 270 mg/dl were included in this study. Animals were divided into four groups of five animals each.

Group1: Control, received only vehicle (1% gum acacia)

Group2: Diabetic rats treated orally with suspension of the ethanolic extract at 150mg/kg b.w. dose .

Group3: Diabetic rats treated orally with suspension of the ethanolic extract at 250mg/kg b.w. dose .

Group4: Diabetic rats treated orally with suspension of metformin at 100 mg/kg b.w. dose.

An oral sucrose load of 2.5 g/kg was given to all groups 30 min post administration of the test sample/vehicle. Blood glucose levels of the animals of all groups were again measured at 1, 2, 3, 4, 5, and 6 hour after sucrose load. Food (not water) was removed from the cages during the experimental period.

Collection of blood samples and glucose determination

Blood samples were collected by end tail vein cutting method and blood glucose level was estimated by GOD-POD method. [17]

Statistical Analysis:

The data obtained were statically analyzed by one way ANOVA and expressed as mean ± S.E.M. followed by Dunett's t test using computerized Graph Pad InStat version 3.06, Graph pad software, U.S.A

Results

Acute Toxicity Studies

This study showed no mortality up to the dose of 1,000 mg/kg body weight. So, the extract from root of *B. aristata* safe for long term administration

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Effect of *B. aristata* on Normoglycemic Rats

The effects of *B. aristata* root (100 and 200mg/kg b.w.) and metformin (100 mg/kg b.w.) on sucrose challenge in normoglycemic rats. The fasting blood glucose levels (mean ± SEM) of the control, test and standard drug groups were 65.52 ± 2.45, 65.27± 3.50, 65.36 ± 2.19 and 65.48 ± 2.85 mg/dl (Table 1) respectively. Three hours after administration of sucrose, the mean blood glucose concentrations of the extract treated group (91.43 ± 5.44, 82.19 ± 2.36 mg/dl) and the metformin treated group (79.52 ± 3.21 mg/dl), were significantly lower ($p<0.05$, $p<0.01$ and $p<0.01$) than that of the control group treated with 2% gum tragacanth (105.22 ± 4.16 mg/dl). The decrease in the blood glucose level at 3 hrs in the test group compared with that of the control was 13.11 % and 21.87 % whereas the decrease in the metformin treated group was 24.41 %.

Effect of *B. aristata* on STZ-Induced Diabetic Rats

The effects of *B. aristata* root (100 and 200mg/kg b.w and metformin (100 mg/kg b.w.) on sucrose challenged STZ-induced diabetic rats. The fasting blood glucose levels (mean ± SEM) of control, test and standard drug groups were 248.34 ± 11.09, 240.88 ± 10.35, 245.24 ± 13.67 and 251.07 ± 10.23 mg/dl (Table 2) respectively. Six hours after administration of sucrose, the mean blood glucose concentrations of the *B. aristata* treated group (225.76 ± 12.44 and 189.34 ± 10.11 mg/dl) and the metformin treated group (180.3 ± 09.23mg/dl), were significantly lower ($p<0.01$, $p<0.001$ and $p<0.001$) than that of the control group treated with 2% gum tragacanth (352.93 ± 12.44 mg/dl). The decrease in the blood glucose level in the test group compared with that of the control was 30.04 % and 66.35 % whereas the decrease in the standard drug treated group (metformin) was 48.91 %.

Table 1: Effect of Berberis aristata extract and metformin on glucose tolerance of normoglycemic rats.

Group	Blood glucose concentration (mg/dl)						
	O min	30 min	60 min	90 min	120 min	180 min	% reduction compare to control at 3 hrs
Control	65.52 ± 2.45	106.34 ± 3.72	115.38 ± 2.90	118.73 ± 2.66	116.32 ± 4.77	105.22 ± 4.16	
BA (100 mg/kg)	65.27± 3.50	104.85 ± 4.21	109.23 ± 3.55	100.14 ± 3.11	92.3 ± 4.23	91.43 ± 5.44*	13.11*
BA (200 mg/ kg)	65.36 ± 2.19	97.12 ± 3.45	102.24 ± 1.85	94.63 ± 3.73	85.51 ± 2.99*	82.19 ± 2.36**	21.87**
Metformin (100 mg/kg)	65.48 ± 2.85	95.19 ± 2.95	99.65 ± 2.13	91.31± 3.60	83.84 ± 1.59*	79.52 ± 3.21**	24.41**

Value are mean ± SEM, n=5 *p<0.05 and **p<0.01 Vs Control

Table 2 : Effect of Berberis aristata extract and metformin on blood glucose level of STZ-Induced diabetic rats.

Group	Blood glucose concentration (mg/dl)		
	Fasting	6 hrs post sucrose load	% reduction compare to control
Control	248.34 ± 11.09	106.34 ± 3.72	
BA (100 mg/kg)	240.88 ± 10.35	225.76 ± 12.44**	30.04 **
BA (200 mg/	245.24 ± 13.67	189.34 ± 10.11***	66.35 ***
Metformin (100 mg/kg)	251.07 ± 10.23	352.93 ± 12.44***	48.91***

Value are mean ± SEM, n=5 **p<0.01 and ***p<0.001 Vs Control

Discussion

Results of the present study confirm that *Berberis aristata* shows clear hypoglycemic activity which is in accordance to the use in Sikkim and Darjeeling as a folklore medicine for the treatment of diabetes. The ethanolic extract of *B. aristata* root, significantly lowered blood glucose level in normal and diabetic rats at variable dose levels (100 and 200 mg/kg body weight).Results obtained from the present study are very much promising and comparable with metformin, a standard drug used to treat diabetes mellitus. It is reported the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimictic action or by preventing the death of cells and/or it may permit recovery of partially destroyed cells or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles. [18]
It will be difficult to predict the exact mechanism of action of these extracts, as the study has not been aimed at that angle. One of the actions of berberine is to enhance the intracellular calcium. [19] Rise in intracellular calcium enhances the degranulation and the release of insulin from the β-cells. Apart from this, a plant material namely, *Coptis teeta*, that contains berberine as one of the constituents, has been shown to inhibit phosphodiesterase enzyme to raise cyclic AMP levels. Rise in cyclic AMP level is also known to contribute to promotion of insulin release. These actions attributed to berberine, could be the causes for the hypoglycemic/ antidiabetic activity. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of *B. aristata* root.

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References

1. Veeramani C, Pushpavalli G, Pugalendi VK. Antihyperglycaemic effect of *Cardiospermum halicacabum* Linn. leaf extract on STZ-induced diabetic rats. J. Appl. Biomed 2008;6:19–26.
2. Kamaeswara BR, Giri R, Kesavulu MM, Apparao C. Effect of oral administration of bark of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. J. Ethnopharmacol. 2001;74:69–74.
3. WHO: WHO launches the first global strategy on traditional medicine, Press Release WHO/38, 2002.
4. Ueda H, Kaneda N, Kawanishi K, Alves SM, Moriyasu M. A new isoflavone glycoside from *Ceiba pentandra* (L). Gaertner. Chem. Pharm. Bull. 2002;50:403–404.
5. Aguilara FJA, Ramos RR, Gutierrez SP, et al. Study of the antihyperglycemic effect of plants used as antidiabeties. J. Ethanopharmacol.2008;61:101-110.
6. Daisy P, Eliza J.Hypoglycemic property of polyherbal formulation in streptozotocin induced diabetic rats. Biochem. Cell. Arch.2007;7:135-140.

7. Chopra RN, Glossary of Indian medicinal plants, CSIR New Delhi, 1956, 36.
8. Chhetri DR, Parajuli P, Subba GC.Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. Journal of Ethnopharmacology 2005;99:199-202.
9. Paranjpe P. Indian medicinal plants forgotten healer, Chaukhamba sanskrit pratishthan, 2001, 66-67.
10. Singha J, Kakka P. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. Journal of Ethnopharmacology 2009;123:22-26
11. Singha M, Srivastava S, Rawat AKS. Antimicrobial activities of Indian Berberis species. Fitoterapia 2007;78:574-576
12. Shahid M, Rahim T, Shahzad A et al.Ethanobotanical studies on *Berberis aristata* DC. root extracts. African Journal of Biotechnology 2009;8:556-563.
13. Mukherjee KP, Wahile A, Kumar V, Rai S, Saha BP. Marker profiling of botanicals used for hepatoprotection in Indian system of medicine. Drug Information Journal 2003;40:131–139.
14. Tang LQ, Wei W, Chen LM, Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J Ethnopharmacol 2006 May 2.
15. Ecobichon DJ.The basis of toxicology testing (RC press, New York) 1997;43-86.
16. Shukla P, Singh AB, Srivastava AK, Pratap R.Chalcone based aryloxypropanolamines as potential antihyperglycemic agents. Bioorganic & Medicinal Chemistry Letters 2007;17:799-802.
17. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-27.
18. Burcelain R, Eddouks M, Maury J, Kande J, Assan R, Girard J. Execessive glucose production rather than insulin resistance accounts for hypoglycaemia in recent-onset diabetic rats. Diabetologia 1995;38:283-290.
19. Xu SZ, Zhang Y, Ren JY. Effects of berberine of L- and T-type calcium channels in guinea pig ventricular myocytes. Zhongguo Yao Li Xue Bao 1997;18:515-518.