EFFECT OF *BERBERIS ARISTATA* ON TYPE I AND II DIABETES MELLITUS MODELS IN ALBINO RATS

Naresh Kumar Rameshwar, Rekha Raghuveer Shenoy, Arun Theerthahalli Sudheendra, Chamalamudi Mallikarjuna Rao*

Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal- 576 104, Karnataka, India

Summary

Aim: To explore the anti-diabetic potential of Berberis aristata in rodents
Materials and methods: Albino rats of Wistar strain were treated with the aqueous and ethanolic extracts of BA. Blood glucose level, liver glycogen level, thio- barbituric acid reactive substance (TBARS) and protein in pancreas were estimated.
Results: Both the extracts of BA produced significant hypoglycemic effect in Streptozotocin (type I) and Streptozotocin- Nicotinamide (type II) induced diabetic models. Significant ($p< 0.05$) reversal in liver glycogen depletion was observed. The oxidative stress caused by streptozotocin-induced diabetes was not significantly altered by the extracts. The extracts showed more hypoglycemic effect than glibenclamide in the Streptozotocin (STZ) - Nicotinamide induced diabetic model.
Conclusion: Berberis aristata can be a promising candidate for the treatment of type II diabetes mellitus.

Keywords: Streptozotocin; Oxidative stress; Wistar strain; Nicotinamide

Introduction

Diabetes mellitus (DM) is one of the common metabolic disorders with micro and macro-vascular complications that results in significant morbidity and mortality (1). It is considered as one of the five leading causes of death in the world (2). It has been noticed that certain cases of diabetes mellitus resistant to insulin, sulphonylureas, biguanides, etc responded well when treated with herbal preparations, alone or in combination with insulin or other oral hypoglycemic agents (3). The ancient Indian literature has prescribed various herbs and metals for the treatment of DM (4). There has been a lot of advancement with synthetic drugs for the treatment of type-II DM, still there seems to be unmet medical need to have more safe and effective treatment. We have made an attempt to study an Indian plant, Berberis aristata (BA) (family- Berberidaceae) which is an edible plant commonly used in Ayurvedic system of medicine (5). BA extract has been used by the natives of Sikkim and Darjeeling as a folklore medicine for the treatment of diabetes (6). However, the plant BA has not been studied in depth for its effect on insulin dependent diabetes mellitus (IDDM) and non- insulin dependent diabetes mellitus (NIDDM). Thus, we selected this plant to evaluate for its anti-diabetic action in STZ and STZ- Nicotinamide induced diabetic albino rats.

Methods

Materials

Streptozotocin was procured from Sigma Aldrich Ltd, USA. Nicotinamide was purchased from SD Fine Chemicals, Boisar. Anthrone was procured from Merck Laboratories Ltd, Mumbai. Ethanol was obtained from Qualigens Fine chemicals, Mumbai. Glucose estimation kit was procured from Ranbaxy Ltd. All other chemicals were of analytical grade.

Collection of plant and extraction

The stem of Berberis aristata was obtained from Yucca Enterprises, Mumbai. The stem was authenticated by comparing with a standard sample (provided by National Botanical Research Institute, Lucknow) by powder microscopy and TLC methods. A voucher specimen (#20035) was deposited at the department's drug archive. The stems were powdered to obtain a fine coarse form, sieved through # 40. The powder was soaked for 24 hrs with distilled water and boiled for 3 hrs. The extract so obtained was decanted through muslin cloth and concentrated to $1/6^{th}$ of the total volume to get the aqueous extract. The powder was extracted with 95% ethanol for 3 days. The extract obtained was distilled and vacuum dried to get the alcoholic extract (7,8)

Identification of phytochemical constituents

Chemical tests were carried out on the extracts for the qualitative determination of phytochemical constituents (9)

Acute toxicity studies

To determine the safe dose of the extracts, acute toxicity studies were conducted by an up and down staircase method (10) followed by Irwin's test to observe any possible behavioural changes (11). The doses selected were $1/ 10^{th}$ of the safe dose found in toxicity studies.

Animals

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines issued by Ministry of Environment and Forests, Govt. of India and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study. Albino rats of Wistar strain of either sex were selected for the study. The rats were housed in an air- conditioned facility at 22±2ºC with a 12 hour light and dark cycle. The animals were given free access to food and water.

Experimental design

The animals were divided into three main groups, i.e. normal, STZ and STZ- Nicotinamide induced diabetes. Each of these, were further split into four groups consisting of 10 rats. These groups received gum acacia, glibenclamide, alcoholic extract and aqueous extract of BA accordingly for a period of 20 days. Both the extracts (suspended in 2% w/v gum acacia) were administered orally at a dose of 500mg/kg body weight. Glibenclamide was also given in suspension form at a dose of 0.25mg/kg body weight. The control group received 2% w/v gum acacia.

Hypoglycemic study
For the determination of blood glucose levels, blood samples were drawn from the orbital sinus with a capillary from experimental animals after an overnight fast on day 0, 5, 10, 15 and 20 following 3 hours, after vehicle or extracts or glibenclamide administration. The FBS levels were estimated using glucose oxidase- peroxidase method

Liver glycogen, TBARS and protein estimation
On day 21, all the animals were sacrificed by cervical dislocation, livers and pancreas excised out for estimation. Liver glycogen was determined using Anthrone method (12) The pancreatic free radicals were estimated by determining TBARS in the pancreatic samples using lipid peroxidation method (13). Pancreatic protein was estimated (14)

Induction of insulin dependent diabetes mellitus
Ninety-day old animals, weighing between 150- 200 g, of either sex, and fasted overnight, were administered streptozotocin 50 mg/kg by intraperitoneal (i.p.) route. Fasting blood samples were withdrawn at 72 h and on day 7 after administering streptozotocin to confirm stable hyperglycemia (15).

Induction of non insulin dependent diabetes mellitus
Ninety-day old animals, weighing between 200- 250 g, of either sex, and fasted overnight, were administered nicotinamide 120 mg/ kg by i.p. route. After 30 minutes, streptozotocin 65 mg/ kg was given intraperitoneally. Fasting blood samples were withdrawn at 72 h to find out the blood glucose level, and on day 7 after administering streptozotocin to confirm stable hyperglycemia.
Blood was withdrawn on two occasions on day 0, i.e. before administration of drug/ vehicle and 3h after administration, where as on day- 5, 10 and 15 blood was withdrawn only 3 h after administration of drug/ vehicle **(**16).
On day 21, the liver glycogen and pancreatic free radicals were estimated

Statistical analysis
Results of biochemical estimations were recorded as mean± S.E. The total variation present in the data was analyzed by one- way ANOVA. Differences among the means were analyzed by Scheffe's test.

Results

Identification of chemical constituents
The extract were found to consist of alkaloids, carbohydrates, phenolic compounds and tannins, proteins, resin, gums and mucilage and flavonoids.

Studies in normal rats
In normal control animals, blood sugar level remained fairly constant. The alcoholic extract showed similar hypoglycemic effect to that of glibenclamide (Table 1). Liver glycogen level was not affected by any of the treatments (Table 2).Neither glibenclamide, nor did the extracts significantly alter the levels of pancreatic free radicals. The extracts significantly promoted the protein levels in pancreas (Table 2).

Table 1: Effect of the BA extracts on the fasting blood sugar levels in normal rats (non- diabetic).

		FBS mg/ dl (Mean ± SE)				
Treatment (*n*=10)	**Dose (mg/kg, p.o.)**	**Before treatment (0 hr)**	**5th day**	**10th day**	**15th day**	**20th day**
Control[a]	Vehicle	56.78 ± 3.26	63.15 ± 2.34	65.48 ± 3.66	66.67 ± 2.06	66.55 ± 1.47
Gb[b]	0.25	54.70 ± 4.36	47.05 ± 4.15	44.86 ± 4.45	48.55 ± 2.43	52.28 ± 6.87*
Aq.ext[c]	500	54.33 ± 2.76	53.15 ± 1.88	50.99 ± 2.83	51.01 ± 2.04	53.52 ± 2.39*
Alc.ext[d]	500	56.04 ± 3.01	48.01 ± 2.84	44.46 ± 3.52	48.05 ± 1.45	45.93 ± 1.19*
Allowance values by Scheffe's test	--	--	12.65	15.81	8.85	9.94

Control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. b-d; *p<0.05.

Table 2: Effect of the BA extracts on the liver glycogen, pancreatic free radicals and protein in normal (non- diabetic) rats.

Treatment(*n*=10)	**Dose (mg/kg, p.o.)**	**Liver glycogen mg/g (Mean ± SE)**	**Pancreatic free radicals conc. (µ moles/ g) (Mean ± SE)**	**Pancreatic protein µg/ g (Mean ± SE)**
Control[a]	Vehicle	8.07 ± 0.76	20.02 ± 1.60	43.63 ± 12.44
Gb[b]	0.25	8.31 ± 0.54	12.22 ± 3.01	48.01 ± 16.93
Aq.ext[c]	500	8.64 ± 1.01	14.14 ± 5.82	220.45 ± 28.73*,**
Alc.ext[d]	500	8.74 ± 1.03	15.79 ± 3.99	196.98 ± 27.86*,**
Allowance values by Scheffe's test	--	3.71	16.66	91.98

Control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. c-d; *p<0.05 and b vs. c-d; **p<0.05

Table 3: Effect of the BA extracts on the fasting blood sugar levels in STZ induced diabetic rats.

Treatment (*n*=10)	Dose (mg/kg,p.o.)	FBS mg/ dl (Mean ± SE)			
		After treatment			
		Before treatment (0 hr)	5th day	10th day	15th day
Diabetic control[a]	Vehicle	303.88 ± 3.93	314.74 ± 3.29	312.26 ± 5.27	317.24 ± 3.85
Gb[b]	0.25	282.3 ± 4.26	211.86 ± 8.55	138.69 ± 19.45	67.98 ± 8.54*
Aq. ext[c]	500	279.88 ± 5.95	185.19 ± 13.36	125.54 ± 13.08	94.98 ±13.04*
Alc. ext[d]	500	286.02 ± 3.10	208.77 ± 8.33	102.45 ± 7.89	80.65 ± 8.63*
Allowance values by Scheffe's test	--	18.00	43.82	52.59	40.74

Diabetic control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. b-d; *p<0.05.

Studies in STZ induced diabetic rats (type I DM)

Glibenclamide significantly reduced FBS levels (p< 0.05). Both the extracts showed significant (p< 0.05) hypoglycemic effect (Table 3) and reversal of depleted liver glycogen levels in the STZ induced diabetic rats (Table 4). Treatments with glibenclamide and extracts did not significantly alter the oxidative stress caused by STZ induction. All the treatment groups showed similar protein levels to that of the diabetic control (Table 4).

Table 4: Effect of the BA extracts on the liver glycogen, pancreatic free radicals and protein in STZ induced diabetic rats.

Treatment (*n*=10)	Dose (mg/kg, p.o.)	Liver glycogen (mg/ g) Mean ± SE	Pancreatic free radicals conc. (µ moles/ g) (Mean ± SE)	Pancreatic protein (µg/ g) (Mean ± SE)
Control[a]	Vehicle	8.07 ± 0.76	20.02 ± 1.60	43.63 ± 12.44
Diabetic control[b]	Vehicle	3.63 ± 0.23	35.98 ± 7.04	242.16 ± 73.95
Gb[c]	0.25	15.98 ± 1.68*,**	29.48 ± 3.32	312.14 ± 56.14
Aq. ext[d]	500	13.06 ± 0.76*,**	24.82 ± 7.94	373.3 ± 53.5
Alc. ext[e]	500	13.71 ± 1.54*,**	27.67 ± 7.68	464.16 ± 46.72
Allowance values by Scheffe's test	--	5.10	28.81	238.28

Control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. c-e; *p<0.05 and b vs. c-e; ** p<0.05

Studies in STZ- Nicotinamide induced diabetic rats (type II DM)

The hypoglycemic effects of both the extracts were found to be more significant than glibenclamide by day 10 (Table 5). Treatment with glibenclamide and the extracts for 10 days restored the glycogen levels (Table 6). None of the treatments, including glibenclamide, could reduce the free radical levels of pancreas. The same was the case with pancreatic protein (Table 6).

Table 5: Effect of the BA extracts on the fasting blood sugar levels in STZ and nicotinamide induced diabetic rats.

Treatment (*n*=10)	Dose (mg/kg, p.o.)	FBS mg/ dl (Mean ± SE)		
		After treatment		
		Before treatment (0 day)	5th day	10th day
Diabetic control[a]	Vehicle	228.16 ± 6.41	228.50 ± 9.96	231.83 ± 11.20
Gb[b]	0.25	152.11 ± 8.78	122.52 ± 19.68	123.95 ± 18.63*
Aq. ext[c]	500	178.85 ± 7.83	85.72 ± 11.13	51.93 ± 8.61*, **
Alc. ext[d]	500	175.26 ± 5.79	115.71 ± 15.64	79.82 ± 9.35*, **
Allowance values by Scheffe's test	--	31.58	64.46	56.38

Control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. c-d; *p<0.05 and b vs. c-d; ** p<0.05

Table 6: Effect of BA extracts on the liver glycogen, pancreatic free radicals and protein in STZ and nicotinamide induced diabetic rats.

Treatment (*n*=10)	Dose (mg/kg, p.o.)	Liver glycogen (mg/ g) Mean ± SE	Pancreatic free radicals conc. (µ moles/ g) (Mean ± SE)	Pancreatic protein (µg/ g) (Mean ± SE)
Normal control[a]	Vehicle	8.07 ± 0.76	20.02 ± 1.60	43.63 ± 12.44
Control[b]	Vehicle	4.23 ± 0.17*	37.53 ± 1.89	395.99 ± 71.31
Gb[c]	0.25	12.34 ± 0.32*, **	29.71 ± 2.95	245.28 ± 56.79
Aq.ext[d]	500	11.79 ± 0.43*, **	28.25 ± 2.43	345.47 ± 40.09
Alc. ext[e]	500	11.85 ± 0.89*, **	29.75 ± 2.49	236.7 ± 64.65
Allowance values by Scheffe's test	--	2.69	10.95	273.58

Control: vehicle (2% w/v gum acacia); Gb: Glibenclamide; *n*= number of animals. a vs. b-e; *p<0.05 and b vs. c-e; ** p<0.05

Discussion

It is evident from the results that the extracts of BA have got significant hypoglycemic activity in normal and diabetic animals with similar efficacy as glibenclamide. Besides the hypoglycemic actions, the extracts reversed the diabetes- induced changes in liver glycogen stores. In case of diabetes induced oxidative stress, the extracts and the standard drug caused an apparent reversal in oxidative stress.

Streptozotocin in adult rats theoretically induces IDDM. However, in the present study a sulfonylurea derivative, glibenclamide, produced hypoglycemic effect in this model. So it could be assumed that, insulin could have been released from the fraction of β- cells that were viable. Besides, glibenclamide could have also acted through other mechanisms, such as inhibition of gluconeogenesis, reduction of serum glucose concentration and potentiation of insulin action on target tissue. Thus, in this particular model, the extracts also could have acted like glibenclamide. Additionally, the extracts might be having one or many of the following mechanisms, as the extract showed more hypoglycemic potential than the standard drug, viz.Production of insulin (17), Decrease in absorption of intestinal glucose, Inhibition of gluconeogenesis, Promotion of peripheral utilization of glucose.

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 (18). 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 mechanism, which needs to be explored in future.

Acknowledgement

Authors are thankful to dept of Pharmacology, MCOPS for providing the necessary facilities for the project

References

1. Vats V., Yadav, S.P., Grover J.K. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin induced alteration in glycogen content and carbohydrate metabolism in rats. Journal of Ethnopharmacology 2004; 155-160.
2. Kumar G.P.S., Kumar S.D.A.P., Subramanian P.S. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. Journal of Health Science 2006; 52: 283-291.
3. Anturlikar S. D., Gopumadhavan S. Effect of D-400, a herbal formulation, on blood sugar of normal and alloxan- induced diabetic rats. Indian Journal of Physiology and Pharmacology 1995; 39: 95.
4. Shah V. Diabetes mellitus in Indian medicine. Chaukhamba Orientalia; 4th edition, Concept publishing Co 1995

5. Kirtikar K.R., Basu B.D. Indian medicinal plants. International book distributors, DehraDun 1999.
6. Chhetri D.R., Parajuli P., Subba G.C. Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. Journal of Ethnopharmacology 2005; 99: 199-202.
7. Kokate CK, Purohit AP, Gokhale SB. Phytochemical Screening In: *Practical Pharmacognosy*. New Delhi: Vallabh Prakashan, 1994; 107–13.
8. Harborne JB. Methods of Extraction and Isolation In: Phytochemical Methods. London: Chapman & Hall, 1998; 60–6
9. Trease G E, Evans W C, Pharmacognosy. Bailliere Tindall Press, London, 1983; 309-706
10. Ghosh M N. Fundamentals of experimental Pharmacology. Scientific book agency, Calcutta, 1984; 153.
11. Turner M A. Screening methods in Pharmacology. Academic Press 1965; New York, pp 26.
12. Morris, D. L. Determination of Glycogen in Liver. Science 1948; 187: 254.
13. Draper H.H.,Hadley M.,. Malondialdehyde determination as index of lipid peroxidation. Methods of enzymology 1990 ;186: 421- 431.
14 .Lowry, Rose Brough N.J., Farr A., Randall R. Protein determination with the folins reagent. J Biol Chem 1951; 133- 140.
15. Clare A., Williams R.C. The in vitro effects of insulin and the effects of the acute fasting on cardiac β- adrenoceptor responses in the short- term streptozotocin induced diabetic rats. Journal of Physiology and Pharmacology 1994; 46: 321- 326.
16. Pelligrino M. et al. Development of new model in adult rats: Administered streptozotocin and Nicotinamide. Diabetes 1998; 47: 224-229.
17. Ananthan R., Latha M., Pari L., Bhaskar C., Narmatha V. Modulatory effects of *Gymnema montanum* leaf extract on alloxan induced oxidative stress in Wistar rats. Nutrition 2004; 20: 280-285.
18 .Xu S.Z., Zhang Y., Ren J.Y. Effects of berberine of L- and T-type calcium channels in guinea pig ventricular myocytes. Zhongguo Yao Li Xue Bao 1997; 18: 515-518.

***Corresponding author**
Chamalamudi Mallikarjuna Rao, Dept of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India. E-mail: mallik.rao@manipal.edu Phone: +91 820 2922482 Fax: +91 820 2571998