International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 1, 2011

Research Article

HYPOGLYCEMIC EFFECT OF METHANOLIC EXTRACT OF *BERBERIS ARISTATA* DC STEM ON NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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Received: 04 Nov 2010, Revised and Accepted: 03 Dec 2010

ABSTRACT

Berberis aristata DC (Berberidaceae) is used in Indian traditional medicine for treating antibacterial, antiperiodic, antidiarrhoeal, ophthalmic, skin diseases and diabetes mellitus. Objective of this study is to induce experimental diabetes mellitus using streptozotocin in normal adult male wistar rats and to study the Antidiabetic activity of methanolic extract of *Berberis aristata* DC stem (MEBA) by comparison of blood glucose level and total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDLC) levels between normal and diabetic rats. Repeated oral administration of the MEBA (250 & 500 mg/kg) effectively reduced the blood glucose in diabetic rats. ($p < 0.05$) and also show significant reduction ($p < 0.05$) in the serum levels of Total cholesterol and Triglycerides and significant increase ($p< 0.05$) in HDL cholesterol level. Diabetes mellitus is common endocrine disorder. Hypoglycemic agents from natural and synthetic sources are available for treatment of this disease. Indian medicinal plants have been found to be useful to successfully manage diabetes. The stem of *Berberis aristata* DC was investigated in normal and streptozotocin induced diabetic rats. Significant hypoglycemic activity and hypolipidemic activity was exhibited by the methanoilc extract of *Berberis aristata* DC.

Keywords: *Berberis aristata* DC, Streptozotocin, Hypoglycemic, Hypolipidemic effect.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder old as mankind and its incidence considered to be high (4-5%) all over world[1]. The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 B.C. A multitude of herbs spices and other plant materials have been described for the treatment of diabetes throughout the world[2]. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies[3]. Few of the plants used for the treatment of diabetes have received scientific or medicinal scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention[4].

Berberis aristata DC (Berberidaceae) commonly called as 'Daruhaldi' in Hindi is indigenous to India. It is an erect spinous shrub, often found in small patches on the hill slopes. In India mainly found wild in sub –Himalayan tract[5]. The stem nearly cylindrical, surface rough and colour yellow[6]. The stem is used for diaphoretic, laxative and useful in rheumatism. Fruits, stem, bark and root of *Berberis aristata* DC have been used in ethno medicine and in many Ayurvedic preparation for several medicinal properties: alternative, antibacterial, antiperiodic, antidiarrhoeal, ophthalmic and in skin diseases[7,8].

There are no available reports on pharmacological action of *Berberis aristata* DC stem till date, therefore, the effect of methanolic extract of *Berberis aristata* DC stem on blood glucose in normal and streptozotocin induced diabetic rats has been investigated.

MATERIALS AND METHODS

Plant material

The Stem of *Berberis aristata* DC were purchased from Sanjivani Medicinal plant supplier, Nadiad, Gujarat, Authenticated by Dr. A. S. Reddy, Taxonomist, Bioscience Department, Sardar Patel University. Vallabh Vidyanagar,Gujarat, India.

Preparation of extracts

The Stem of *Berberis aristata* DC were dried in sun and made coarse powder. It was then passed through the 42 mesh sieve. A weighted quantity (200 gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus exhaustively. The extract was evaporated under pressure using rotary evaporator until all solvent has been removed to give an extract sample. Percentage yield of methanol extract was 4.2% w/w.

Animals

Male Albino Wistar rats of body weight 250 to 300 gm were selected for all the experiments. Animals were kept in our animal house at an ambient temperature of 25 ^{0}C and 45-55% relative humidity with a12 h dark: 12h light cycle. Animal were fed pellet diet (Pranav Agro industries, Vadodara, Gujarat) and water *ad libitum*. The experiments on animal were conducted in accordance with the international accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional Ethical Committee (Reg. No. IAEC/365/01/ab/CPCSEA).

Phytochemical screening

The Phytochemical examination of methanolic extract of *Berberis aristata* DC stem was performed by standard methods[9].

Acute toxicity study

Acute toxicity study of methanoilc extract of *Berberis aristata* DC stem was determined as per the OECD guideline No. 423 (Acute Toxic Class Method). It was observed that test extract was not lethal to the rats even at 2500 mg/kg dose. Hence, 1/10th (250 mg/kg) and 1/5th (500 mg/kg) of this dose were selected for further study[10].

Induction of experimental diabetes

A freshly prepared Streptozotocin (SISCO Research Laboratories Pvt. Ltd. India.) 45 mg/kg of body weight in 0.1M Citrate buffer PH 4.5 was injected intraperitoneally to overnight fasted rats[11]. Hyperglycemia was confirmed by the elevated blood glucose level determined at 48 hr after the dose. Animal that exhibited glycosuria after 48h was tested by urine test strips (Uristix, Bayer diagnostics Ltd, India) were considered as diabetic.

Experimental design

Hypoglycemic activity in normal rats

Initial testing is carried with the different doses of extract in healthy male rats fasted overnight.

The animals were divided into four groups and each group consisted of 6 rats.

1. Normal control (untreated rats)

2. Normal rats treated with *Berberis aristata* DC extract (250 mg/kg body weight)

3. Normal rats treated with *Berberis aristata* DC extract (500 mg/kg body weight)

4. Normal rats treated with Glibenclamide (0.25 mg/kg of body weight)

Hypoglycemic activity in diabetic rats

Eight week after injection of streptozotocin the rats were checked for fasting blood glucose levels[12, 13]. The animal showing fasting blood glucose more than 200 mg/dl were considered as diabetic.

The animals were divided into four groups and each group consisted of 6 rats.

1. Diabetic control (untreated rats)

3. Diabetic rats treated with *Berberis aristata* DC extract (250 mg/kg body weight)

4. Diabetic rats treated with *Berberis aristata* DC extract (500 mg/kg body weight)

4. Diabetic rats treated with Glibenclamide (0.25 mg/kg of body weight)

Blood collection and serum separation

The blood sample were collected from 8 h fasted animals from the retro-orbital plexus in capillary tubes (Micro Hemocrit capillary, Mucaps) and serum was separated within 30 min. after collection using centrifuge at 2000 rpm for 2 min. for estimation of Glucose and Lipid profile.

Estimation of blood glucose and serum lipid profile

It was estimated by glucose oxidase-peroxidase (GOD-POD) method; total cholesterol (TC), Triglyceride (TG) and HDL cholesterol (HDLC) were estimated by enzymatic methods by using diagnostic kit. (Beacon diagnostic Ltd. India)

Statistical analysis

All the data reported are expressed as mean ±S.E.M. Statistical analysis was performed by using one-way ANOVA followed by Turkey's multiple tests using 2.0 version of computer software. The values were considered statistically significant when *P* value <0.05 compared to respective control.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of preliminary Phytochemical screening of the methanolic extract of *Berberis aristata* DC revealed that presence of alkaloids, glycosides, carbohydrates, bitter principles and saponins.

Determination of blood glucose level (BGL) of normal rat

In normal animals significant reduction in blood glucose level at day 12 was observed as compare to normal control (p<0.05).

Table 1: Effect of MEBA on normal rats blood glucose level (BGL)

Groups	Drugs	Dose	Blood glucose concentration	
			Day 0	Day 12
Group I	Normal Control	2% Tween 80 w/v soln	72.5±0.2	74.3±0.2
Group II	Normal Control+MEBA	250mg/kg	72.0±0.4	67.3±0.3a
Group III	Normal Control+MEBA	500mg/kg	72.5±0.5	64.9±0.2a
Group IV	Normal Control+Standard	Glibenclamide 0.25mg/kg	72.6±0.4	60.9±0.2a

Each value represents mean ±S.E.M. n=6.
[a] Represent statistical significance vs. control (p<0.05).
One-way ANOVA followed by Tukey's multiple test.

Determination of blood glucose level (BGL) and lipid profile of diabetic rats.

There were observable changes in blood glucose level (BGL) and lipid profile of treated and untreated rats. Treatment of diabetic rats with the methanolic extract of *Berberis aristata* DC and Glibenclamide significantly decreased the BGL compared to untreated diabetic rats (p<0.05). Dose dependent reduction (p<0.05) in BGL, TC and TG also increase in HDLC level (p<0.05) was observed in streptozotocin induced diabetic rats treated with methanol extract of *Berberis aristata* DC.

Table 2: Effect of MEBA on diabetic rats blood glucose level (BGL) after a prolonged treatment

Groups	Drugs	Dose	Blood glucose concentration	
			Day 0	Day 12
Group I	Diabetic Control	2% Tween 80 w/v soln	210.1±1.6	236.0±1.4
Group II	Diabetic Control+MEBA	250mg/kg	189.6±1.7	108.0±0.7 [a]
Group III	Diabetic Control+MEBA	500mg/kg	198.2±2.4	89.7±0.7 [a]
Group IV	Diabetic Control+Standard	Glibenclamide 0.25mg/kg	201.3±2.8	102.5±0.5 [a]

Each value represents mean ±S.E.M., n=6.
[a] Represent statistical significance vs. control (p<0.05).
One-way ANOVA followed by Tukey's multiple test.

Table 3: Effect of MEBA on serum cholesterol levels of streptozotocin induced diabetic rats after a prolonged treatment.

Groups	Drugs	Dose	Total	Triglycerides	HDL
				Cholesterol	Cholesterol
Group I	Normal Control	2% Tween 80 w/v soln	58.9±0.7	91.1±0.9	54.6±0.4
Group II	Diabetic control	2% Tween 80 w/v soln	121.8± 1.0	178.7 ± 1.2	34.7±0.4
Group III	Diabetic control +MEBA	250 mg/kg	75.7± 1.1ab	102.6± 0.9ab	53.6±0.4a
Group IV	Diabetic control + MEBA	500 mg/kg	66.4± 0.6ab	87.4±0.9ab	55.9±0.5a
Group V	Diabetic control +Standard Glibenclamide	0.25 mg/kg	75.8 ± 0.9b	90.3 ± 1.56b	53.1±0.6b

Each value represents mean ±S.E.M. n=6.
aRepresent statistical significance vs. control (p<0.05).
bRepresent statistical significance vs. normal (p<0.05).
One-way ANOVA followed by Tukey's multiple test.

DISCUSSION AND CONCLUSION

According to Ayurvedic pharmacopeia of India *Berberis aristata* DC is used in diabetes. Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and co-morbidities such as obesity and hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes[14]. In order to establish scientific basis for the utility of this plant in the treatment of diabetes, it was decided to evaluate the hypoglycemic activity of methanolic extract of *Berberis aristata* DC on normal and diabetic rat by giving multiple doses in experimental design. The presence of alkaloids, glycosides, carbohydrates, bitter principles and Saponins has been implicated in the Antidiabetic activities of many plants[15].

Previous studies suggested that hyperglycemia and hyperlipidemia are the common characteristics of streptozotocin induced diabetes mellitus in experimental rats[16,17]. The maximum reduction in serum glucose levels was seen in methanolic extract of *Berberis aristata* DC at the dose of 500 mg/kg (Table 2) hence, we could say that methanolic extract of *Berberis aristata* DC had a beneficial effect on carbohydrate metabolism in diabetic rats.

In this study, we have also observed an increase in the concentration of TC and TG in streptozotocin induced diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus[18, 19]. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose[20]. Regarding the mechanism of action MEBA may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have decreased in serum cholesterol and triglycerides[21]. Most of the hypolipidemic drugs do not decrease serum TG level, but MEBA lowered it significantly since under diabetic condition, insulin activates the enzyme lipoprotein lipase and hydrolysis the triglycerides[22] and also MEBA reduces the serum TG of streptozotocin induced diabetic rats and may prevent the progression of CHD. The total lipid profile in serum (total cholesterol, triglycerides) of the streptozotocin induced diabetes rats treated with MEBA (250 or 500 mg/kg) showed significant ($p<0.05$) reduction, and improve the level of HDL cholesterol as compared to diabetic control rats (Table 3).

The strong antihyperglycemic effect of methanolic extract of *Berberis aristata* DC stem could indirectly be related to beneficial action against the abnormal high concentration of serum lipids observed in diabetes rats.

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