

ANTIHYPERGLYCEMIC EFFECTS OF *BERBERIS LYCEUM* ROYLE IN ALLOXAN INDUCED DIABETIC RATS

Muhammad Gulfraz, Ghulam Qadir, Fatima Nosheen, Zahida Parveen

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SUMMARY

Berberis lyceum is a medicinal shrub used in conventional therapy for a number of diseases including diabetes mellitus. The aim of the present study was to investigate the antihyperglycemic effects of aqueous and ethanol extracts of *Berberis lyceum* in alloxan induced diabetic and normal rats. Rats were administered 50 and 100 mg/kg of aqueous and ethanol root extracts and 20 mg/kg glibenclamide per os. The doses applied caused no acute toxicity or behavioral changes in study animals. Blood glucose was determined at 0, 1, 3 and 5 h (normal rats) and on days 0, 1, 3 and 5 (diabetic rats) after treatment. Various doses of extracts significantly ($p \leq 0.05$) reduced the level of hyperglycemia in both normal and alloxan induced diabetic rats. The effects of ethanol extracts on glucose tolerance tests were significant ($p \leq 0.05$) and the hypoglycemic effects were pronounced from 30 to 180 min after treatment.

Correspondence to: Muhammad Gulfraz, School of Pharmacy, Reading University, UK
E-mail: M.Gulfraz@Reading.ac.uk; gulfraz_satti@hotmail.com

INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate metabolism in which sugars in the body are not oxidized to produce energy due to the lack of pancreatic hormone (insulin). The accumulation of sugar leads to its appearance in the blood (hyperglycemia), then in urine. The symptoms that are associated with diabetes include fatigue, excessive thirst, frequent urination, blurred vision, mood changes and sexual problems. Diabetes that starts in childhood or adolescence (type 1) is usually more severe than that beginning in middle or old age (type 2) but may also develop in younger people (1). The pancreas retains some ability to produce insulin but it is inadequate for the body needs; alternatively, the body becomes resistant to the effects of insulin and patients may require treatment with oral hyperglycemic drugs or insulin (1-3).

Berberis lyceum (Berberidaceae) is an important traditional shrub, native to Pakistan and India but also found in other parts of the world. Inhabitants of these areas have been using *Berberis lyceum* for the treatment of diabetes, wounds, broken bones, ulcers and sore eyes. Roots are yellowish in color, rich in alkaloids (berberine, etc.) and other phytochemicals (4,5).

The aim of the present study was to demonstrate the antihyperglycemic effects of aqueous and ethanol root extracts of *Berberis lyceum* Royle in alloxan induced diabetic rats.

MATERIALS AND METHODS

All chemicals used in the study were of analytical grade and obtained from Sigma.

Plant material

Root samples of *Berberis lyceum* were collected from hilly areas of Kotli Sattian, Dsit Rawalpindi, Pakistan, during July and August 2006. Samples were collected into fine plastic bags, identified by a taxonomist and registered in voucher no. 117 for further references.

Preparation of plant extracts

Root samples were ground in a Waring blender and sifted through a wire screen (mesh size 2 mm x 2 mm). The roots were exhaustively extracted with ethanol (root to solvent ratio 1:5). The extracts were filtered and concentrated on a rotary evaporator.

Animals

Wistar rats of both sexes (170-200 g) were maintained under standard animal house conditions, fed commercial rat chow (Feed Mills, Islamabad) and allowed water *ad libitum*. Fasted animals were deprived of food for at least 16 h, but were allowed free access to water. All animals were carefully monitored and maintained in accordance with ethical recommendations of Pakistan Veterinary Science. Fasted animals received 65 mg/kg body weight of alloxan as a single dose by intravenous injection. Only diabetic rats were included in the experiment; their body weights and serum glucose levels were assessed on days 0 to 5. Insulin was administered by intraperitoneal route and all other treatments were given orally by gavage.

Acute toxicity and selection of doses

Acute toxicity studies were conducted for both extracts in order to select a suitable dose for evaluation of antidiabetic activity. The LD50 values of both extracts were calculated using the method described by Litchfield and Wilcoxon (6).

Effect of ethanol extracts of *Berberis lyceum* on serum glucose levels in normal fasted rats

Animal fasted overnight were randomly allocated into 3 groups of 6 rats (n=6): group 1 (vehicle) received 2% ethanol/H₂O mixture (0.5 mL); and groups 2 and 3 received ethanol extract of *Berberis lyceum* in a dose of 50 and 100 mg/kg, respectively. Serum glucose was measured at 0, 1, 3 and 5 h of treatment.

Effect of ethanol extracts of *Berberis lyceum* on serum glucose level in alloxan-induced diabetic rats

Diabetes was induced by a single intravenous injection of 60 mg/kg of alloxan monohydrate (dissolved in 0.9% NaCl) to overnight fasted rats. A serum glucose range of 400-500 mg/dL was used for the experiment. Hyperglycemia was confirmed in animals after 72 h of alloxan injection. Animals were divided into 5 groups of 6 animals (n=6): group 1 diabetic animals received insulin (0.5 IU); group 2 diabetic animals (vehicle) received 2% ethanol/ H₂O (0.5 mL); groups 3 and 4 diabetic animals received ethanol extracts in a dose of 50 and 100 mg/kg, respectively; and group 5 diabetic animals received glibenclamide (20 mg/kg). Serum glucose level was measured on days 0, 1, 2 and 5 following the treatment.

Effect of ethanol extracts of *Berberis lyceum* on glucose tolerance test

Fasted rats were divided into 5 groups of 6 animals (n=6) to each treatment: group 1 rats received glucose (3 g/kg) and insulin (0.5 IU); group 2 hyperglycemic rats received glucose (3 g/kg); groups 3 and 4 rats received glucose (3 g/kg) and ethanol extract (50 and 100 mg/kg, respectively); and group 5 hyperglycemic rats received glibenclamide (20 mg/kg). Blood

samples were collected at 0, 30, 60, 120 and 180 min after glucose loading and serum glucose was measured.

Effect of aqueous extracts of *Berberis lyceum* on serum glucose level (mg/dL) in alloxan diabetic rats

Animals were divided into 5 groups of 6 animals (n=6): group 1 diabetic rats received insulin (0.5 IU); group 2 diabetic animals (vehicle) received 0.5 mL of 2% ethanol/H₂O (0.5 mL); groups 3 and 4 animals received 50 and 100 mg/kg of aqueous root extracts, respectively; and group 5 animals received 20 mg/kg of glibenclamide. The level of glucose in experimental animals was quantified on days 0, 1, 3 and 5 following the treatment.

Effects of aqueous extracts of *Berberis lyceum* on serum glucose level (mg/dL) in oral glucose tolerance test

Fasted animals were divided into 5 groups of 6 animals (n=6): group 1 was given insulin (0.5 IU); group 2 received a dose of 3 g/kg of glucose; groups 3 and 4 received aqueous root extract in a dose of 50 and 100 mg/kg, respectively; and group 5 received 20 mg/kg of glibenclamide. Glucose level was quantified at 0, 30, 60, 120 and 180 min following the doses.

Determination of serum glucose concentration

Blood samples (100 µL) from the tail vein of anesthetized rats were collected and centrifuged. Serum was used to determine glycemia by the glucose oxidase method (7).

Statistical analysis

Data were analyzed by one-way ANOVA using the Newman-Keuls test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The doses of 50 and 100 mg/kg reduced hyperglycemia after 3 and 5 h of treatment (Table 1). A significant hypoglycemic activity was found at 5 h

with 50 and 100 mg/kg doses. The extract significantly reduced serum glucose level at 5 h as compared to zero time for each treatment.

After oral administration of 50 and 100 mg/kg of the ethanol extracts of *Berberis lyceum*, a significant reduction was observed in serum glucose level of alloxan diabetic rats. The dose of 50 mg/kg decreased the level of hyperglycemia on days 3 and 5, whereas the dose of 100 mg/kg reduced hyperglycemia throughout the study period (Table 2). Blood sugar level was determined before and after insulin (i.p.) or glibenclamide (oral gavage) treatment. Insulin (0.5 IU) reduced glycemia from day 1 to day 3 and significantly decreased glucose from day 3 to day 5 after treatments. Glibenclamide, a known oral hypoglycemic agent, reduced serum glucose level, however, on day 5 of treatment it reinforced diabetic condition in experimental animals, as also reported elsewhere (8,9).

At 30 min of initiating glucose tolerance test, serum glucose concentration was higher than at zero time but decreased significantly from 30 min to 120 min, then increased at 180 min after treatment (Table 3). Ethanol extracts significantly decreased the level of serum glucose at a dose of 50 and 100 mg/kg, and glibenclamide at a dose of 20 mg/kg from 30 min to 120 min but reinforced it at 180 min following treatment. Similar results have been reported by Ghosh and Suryawanshi using some plant extracts in diabetic rats (9).

DISCUSSION

In the present study, the hyperglycemic activity of ethanol root extract of *Berberis lyceum* was assessed in normal and alloxan induced diabetic rats. Oral administration of a single dose of ethanol extract of *Berberis lyceum* caused a significant decrease in serum glucose levels in normal rats. A dose of 50 mg/kg of ethanol or aqueous extract produced maximum glucose lowering effects in diabetic rats, whereas 100 mg/kg ethanol or aqueous extract showed a significant hypoglycemic effect throughout the study period (Tables 1-3). Ethanol extracts yielded slightly better results as compared to aqueous extract (Tables 4

Table 1. Effect of ethanol root extract of *Berberis lyceum* on serum glucose level (mg/dL) in normal fasted rats

Group	Treatment	0 h	1 h	3 h	5 h
Vehicle (2% ethanol/H ₂ O)	0.5 mL	124.02 ± 4.61	120.31 ± 5.53	116.82 ± 4.53	118.31 ± 0.12
Ethanol extract	50 mg/kg	132.62 ± 5.20	118.72 ± 5.83	104.8 ± 4.31*	101.21 ± 3.81*
Ethanol extract	100 mg/kg	115.64 ± 5.82	118.31 ± 4.62	87.32 ± 3.25*	91.24 ± 5.22*

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference as compared to zero time value; p<0.05

Table 2. Effect of different ethanol extracts of *Berberis lyceum* on serum glucose level (mg/dL) in alloxan diabetic rats

Group	Dose	Day 0	Day 1	Day 3	Day 5
Insulin	(0.5 IU)	409.63 ± 9.52	332.62 ± 6.20	188.20 ± 7.62	131.45 ± 6.42*
Vehicle (2% ethanol/H ₂ O)	0.5 mL	514.21 ± 5.31	517.62 ± 12.62	514.24 ± 5.71	494.32 ± 7.32
Ethanol extract	50 mg/kg	512.52 ± 7.92	478.01 ± 2.81	428.71 ± 9.50*	396.50 ± 8.20*
Ethanol extract	100 mg/kg	519.21 ± 12.51	471.30 ± 6.23*	384.20 ± 5.24*	351.20 ± 5.22
Glibenclamide	20 mg/kg	510.52 ± 4.22	492.42 ± 8.21	472.22 ± 4.21*	482.45 ± 1.24

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference from the corresponding zero time value; p<0.05

Table 3. Effect of different ethanol extracts of *Berberis lyceum* and insulin on serum glucose level (mg/dL) on oral glucose tolerance test

Group	Dose	0 min	30 min	60 min	120 min	180 min
Diabetic	Insulin, 0.5 IU	135.91 ± 5.32	123.42 ± 6.61	96.62 ± 4.41	82.23* ± 5.81	88.24 ± 4.15
Diabetic	Glucose, 3 g/kg	134.22 ± 2.52	148.63 ± 6.22	154.2 ± 5.13	161.35 ± 5.12	130.20 ± 4.35
Glucose fed-diabetic + Ethanol extracts	50 mg/kg	135.20 ± 1.45	121.01 ± 4.82	108.3 ± 2.52	92.51 ± 5.35*	93.35 ± 2.14*
Glucose fed-diabetic + Ethanol extracts	100 mg/kg	136.73 ± 5.45	118.75 ± 7.21	104.2 ± 4.75	88.22 ± 4.24*	91.23 ± 4.62
Glibenclamide	20 mg/kg	133.23 ± 3.84	156.12 ± 7.51	153.24 ± 7.84	121.43 ± 9.32*	123.21 ± 12.23

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference from the corresponding zero time value; p<0.05; *statistically significant difference from the corresponding hyperglycemic group; p<0.05

Table 4. Effect of aqueous extracts of *Berberis lyceum* on serum glucose level (mg/dL) in alloxan diabetic rats

Group	Dose	Day 0	Day 1	Day 3	Day 5
Insulin	(0.5 IU)	398.22 ± 9.71	338.23 ± 6.24	182.05 ± 3.60	130.4 ± 9.42
Vehicle (2% ethanol/H ₂ O)	0.5 mL	512.23 ± 5.33	511.62 ± 2.63	510.21 ± 5.71	494.3 ± 7.31
Aqueous extract	50 mg/kg	502.25 ± 6.32	368.01 ± 9.81*	473.71 ± 6.52	471.5 ± 8.23
Aqueous extract	100 mg/kg	503.62 ± 8.23	402.31 ± 7.22*	392.20 ± 5.24*	390.22 ± 5.23
Glibenclamide	20 mg/kg	492.2 ± 9.21	452.45 ± 7.23*	422.23 ± 4.23	432.47 ± 1.25

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference from the corresponding zero time value; p<0.05

Table 5. Effect of aqueous extracts of *Berberis lyceum* on serum glucose level (mg/dL) on oral glucose tolerance test

Group	Dose	0 min	30 min	60 min	120 min	180 min
Diabetic	Insulin (0.5 IU)	105.33 ± 5.22	87.41 ± 6.65	66.63 ± 5.43	62.24 ± 5.62	79.22 ± 5.52
Diabetic + glucose fed	Glucose, 3 g/kg	125.22 ± 2.72	153.62 ± 6.51	154.20 ± 4.91	161.3 ± 5.13	138.25 ± 2.53
Diabetic + aqueous extract	50 mg/kg	105.2 ± 4.81	102.01 ± 4.24	108.34 ± 8.52	97.35 ± 2.14*	98.32 ± 5.35
Diabetic + aqueous extract	100 mg/kg	106.51 ± 4.32	102.23 ± 5.74	99.33 ± 7.45	93.46 ± 2.43*	98.32 ± 4.82
Glibenclamide	20 mg/kg	104.74 ± 5.42	95.72 ± 6.31	92.21 ± 4.63	90.23 ± 4.65*	91.25 ± 4.24

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment; *statistically significant difference from the corresponding zero time value (normal rate) p<0.05; *statistically significant difference from the corresponding hyperglycemic group; p<0.05

and 5). In the oral glucose tolerance test, the *Berberis lyceum* extract showed significant reduction of serum glucose levels and these effects were dose dependent. Based on the hypoglycemic effects in normal and diabetic rats, it was observed that the hypoglycemic mechanism involved an insulin-like effect, most probably through the peripheral glucose consumption (10,11). The extract of *Berberis lyceum* displayed a significant hypoglycemic effect in normal rats; the main mechanism by which the extract brings hypoglycemic effects most probably involves stimulation of peripheral glucose consumption. Furthermore, the glycemia profile observed in the glibenclamide group indicates that the extract of *Berberis lyceum* acts on the liver or on peripheral glucose consumption (12).

The glibenclamide effects on glucose can be attributed to the enhanced activity of the β cells of the pancreas, resulting in secretion of a large amount of insulin (2,10). Oral administration of antidiabetic agents (insulin) is well known to produce hypoglycemia in both normal and alloxan induced rats (9). However, it was expected that the hypoglycemia principle in ethanol extracts of *Berberis lyceum* can act indirectly by stimulating the release of insulin into the bloodstream. Although alloxan treatment causes

permanent effects, these effects could be retrieved by the action of alcoholic plant extracts. These results revealed that some drugs may also be effective in insulin independent diabetes. The significant hypoglycemic effects of ethanol extracts of *Berberis lyceum* in diabetic rats indicate that this effect can be mediated by stimulation of glucose utilization by peripheral tissues.

It is concluded that the root extract reduced serum glucose level in normal and diabetic rats, however, the effects of 100 mg/kg ethanol extracts were more pronounced in alloxan diabetic rats. Furthermore, due to the presence of antihyperglycemic phytochemicals (berberine, etc.) the roots of *Berberis lyceum* have a potential to provide raw materials for pharmaceutical industries. Therefore, additional studies are needed for isolation and separation of bioactive compounds from the roots of this important medicinal plant.

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