

## A POTENTIAL ADJUNCT TO INSULIN: *BERBERIS LYCIUM ROYLE*

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### SUMMARY

The aim of the present study was to evaluate traditional antidiabetic folklore claim of *Berberis lycium Royle (BIR)*. Hypoglycemic effect of powdered root bark of BIR (250, 500 mg/kg body weight) was evaluated in both normal and diabetic rabbits. Gliclazide was used as positive control. Antidiabetic effects of different extracts were also determined. Hypoglycemic activity of water extract showing maximal effect was compared with and without different doses of insulin. Phytochemical tests were carried out on water extract. Both doses of crude drug significantly ( $P < 0.001$ ) reduced blood glucose levels in both normal and diabetic rabbits. Gliclazide also significantly reduced blood glucose levels in normal rabbits; however, it was unable to show such an effect in alloxanized rabbits. Water extract produced maximum, whereas chloroform extract was unable to show any hypoglycemic activity. All treatments with and without insulin significantly ( $P < 0.001$ ) reduced blood glucose levels in alloxanized rabbits. Water

extract was shown to test positive for possessing alkaloids, hydrolysable tannins, saponins, and cardiac glycosides. The result suggested that water extract of BIR could be exploited as an adjunct to insulin; the more so, these findings could explain the basis of extract to manage diabetes mellitus.

### INTRODUCTION

Traditional herbs and plants have been used for the treatment of various diseases (1). Plant drugs are considered less toxic and free from side effects when compared with synthetic ones (2). Many plant species have been reported to have antidiabetic activity (3,4). Berberidaceae are a famous family with manifold medicinal uses and included in British and Indian pharmacopeias (5). One of the species called *Berberis aristata* is reported to have hepatoprotective and curative effects in rats (6). The fruit of *Berberis vulgaris* from the same family has anticholinergic and antihistaminic effects (7). *Berberis lycium Royle (BIR)* is a thorny shrub belonging to the family Berberidaceae, and is commonly known as Ishkeen present in Himalayan region of India and Pakistan. In Pakistan, BIR is widely distributed in northern areas such as Gilgit, Baltistan, Ghizer, Astor, Diamer, Swat and Kashmir.

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*BIR* is a well known medicinal plant with edibility rating 3 (1-5) and medicinal rating 3 (1-5). *BIR* is reported to have antihyperlipidemic effect in broilers (8). Berbamine, an alkaloid separated from *BIR*, is also reported to have hypertensive action. *BIR* has been used in traditional medicines for treating various diseases including diabetes mellitus and local inhabitants of the Himalaya region use crude powder of *BIR* root bark to treat diabetes. The purpose of this study was to evaluate the antidiabetic activity of both crude powder and various extracts of *BIR* root bark to establish scientific evidence in support of the folklore claim. It was further aimed to determine its possible mechanism of action.

## MATERIALS AND METHODS

### Chemicals and drugs

Alloxan monohydrate was obtained from Sigma Chemical Co., and gliclazide from Servier Research and Pharmaceuticals, Lahore, Pakistan.

### Animals

Male rabbits (*Oryctolagus cuniculus*), weight range of 1.0-1.5 kg, were used in the study. All rabbits were housed at animal house of Department of Pharmacy, Bahauddin Zakariya University Multan in stainless cages under controlled conditions of humidity, temperature  $22 \pm 2$  °C and 12-h light-dark cycle. Animals were fed green fodder and water *ad libitum*. Animals received human care in accordance with the NIH guidelines and the study protocol was approved by the local ethics committee.

### Plant material

*BIR* root bark was collected from the Shikyote village, Gilgit District, Pakistan, by native researcher Alamgeer in April 2007. *BIR* root bark was identified and authenticated by Dr. Bukhari (renowned taxonomist), Department of Botany (Ex Director) Bahauddin Zakariya University, Multan, Pakistan. A voucher specimen was preserved at Department of Pharmacy, University of Sargodha, Pakistan, with

voucher no. (DOP# BIR 604) for future reference. The root bark was shade dried and powdered finely with a Chinese herbal grinder.

### Preparation of various extracts of *BIR*

Water, methanol, aqueous methanol (30:70), chloroform, and n-hexane extract of *BIR* were prepared using Soxhlet apparatus. The percentage yields (w/w) of these extracts were 15.7%, 13.5%, 12.4%, 6.1% and 2.1%, respectively. All extracts were dried with the help of a lyophilizer and rotary evaporator.

### Administration of drugs

Various extracts and gliclazide were administered orally, while insulin was given subcutaneously.

### Biochemical analysis

Blood glucose levels were measured by Optium Xceed Glucometer using glucose oxidized Optium kits (Abbott Laboratories, USA).

### Hypoglycemic activity in normal and diabetic rabbits

#### Normal rabbits

Rabbits were divided into four groups (n=5) of normal control, crude drug treated (CDT) and gliclazide treated animals; CDT group was subdivided into two groups, CDT1 and CDT2, that received 250 and 500 mg/kg body weight (b.w.) *BIR* root bark powder, respectively. Gliclazide treated group received 80 mg/kg b.w. gliclazide (9).

#### Diabetic rabbits

The same procedure of grouping was adopted for diabetic rabbits by adding another group of diabetic control animals. Diabetes was induced by using the method described by Akhtar *et al.* and Khosla *et al.* (9,10). Blood samples were taken at 0, 2, 4 and 6 h after drug administration and blood glucose levels were measured.

## Screening of different *BIR* extracts for hypoglycemic activity in diabetic rabbits

Diabetic rabbits were divided into two groups (n=5) of diabetic control and extract treated animals. The extract treated group was further divided into five subgroups (n=5): methanolic extract treated (MET), aqueous methanolic extract treated (AMET), chloroform extract treated (CET), water extract treated (WET) and n-hexane extract treated (HET), receiving 500 mg/kg b.w. of the respective extract. Blood samples were taken at 0, 2, 4 and 6 h of extract administration.

## Comparison of hypoglycemic activity of aqueous *BIR* extract with or without insulin

Alloxanized rabbits were divided into four groups (n=5). Group 1 received water extract alone, group 2 and group 3 received 2 and 3 units of insulin in combination with 500 mg/kg b.w. of water extract, respectively, whereas group 4 received 6 units of insulin alone.

## Phytochemical analysis of water *BIR* extract

Water extract of *BIR* with highest antidiabetic activity was subjected to phytochemical analysis for alkaloids, tannins, glycosides, saponin, reducing sugars, anthracene derivative and flavonoids. All tests were performed by the methods described by Inwukaeme *et al.* (11).

## Statistical analysis

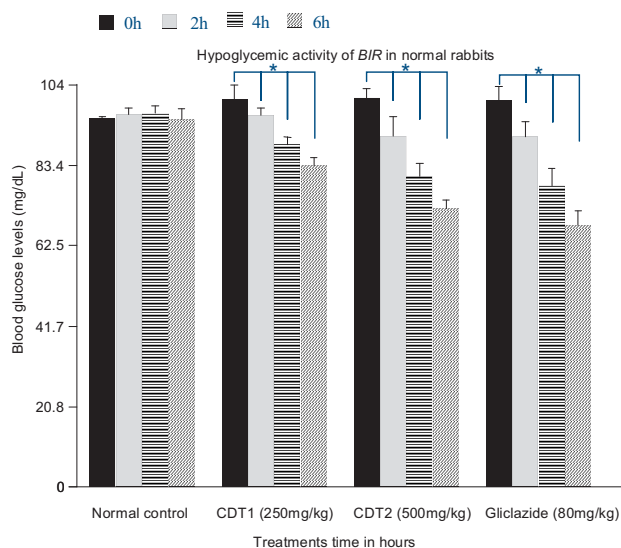
Data were expressed as mean  $\pm$  standard deviation (SD) and analyzed using analysis of variance (ANOVA) and paired t-test. The level of significance was set at  $P < 0.05$ .

## RESULTS

### Hypoglycemic activity of *BIR* in normal rabbits

Both doses of crude powder of *BIR* significantly ( $P < 0.001$ ) reduced blood glucose levels in the treated groups (CDT1 and CDT2). Gliclazide also reduced blood glucose level significantly ( $P < 0.001$ ) in the gliclazide treated group (Fig. 1).

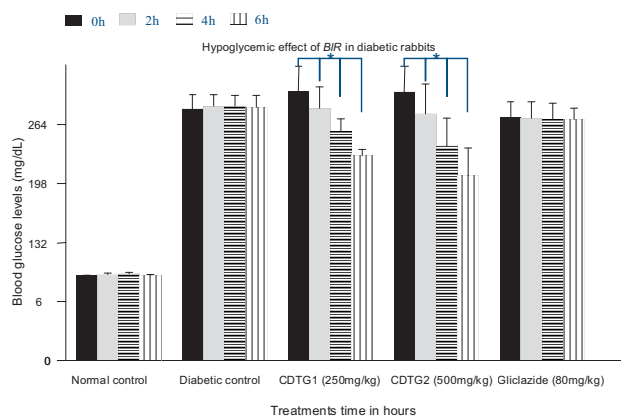
Figure 1. Effect of crude powder of *BIR* on blood glucose level in normal rabbits at 0, 2, 4 and 6 hours. CDT1: crude drug treated 1; CDT2: crude drug treated 2; gliclazide treated; values of five animals are given as mean  $\pm$  SD; \* $P < 0.001$  compared to normal control rabbits; n=5 rabbits in each group.



### Hypoglycemic activity of *BIR* in diabetic rabbits

Crude powder of *BIR* significantly ( $P < 0.001$ ) reduced blood glucose levels in both crude drug treated groups (CDT1 and CDT2), whereas gliclazide was unable to significantly reduce blood glucose levels in diabetic rabbits (Fig. 2).

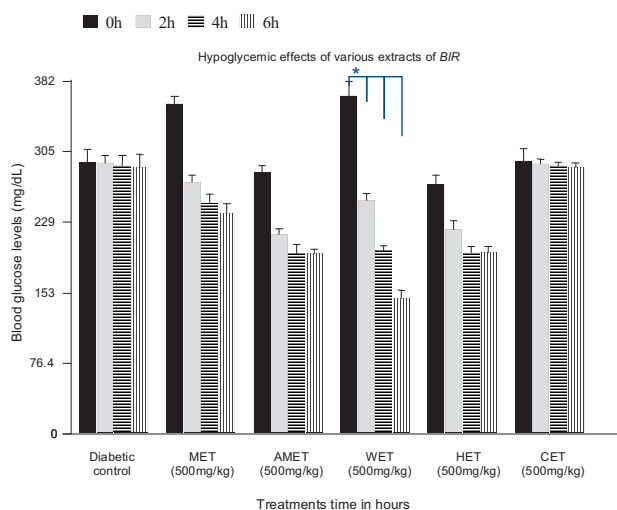
Figure 2. Effect of crude powder of *BIR* on blood glucose level in diabetic rabbits at 0, 2, 4 and 6 hours. CDT1: crude drug treated 1; CDT2: crude drug treated 2; gliclazide treated; values of five animals are given as mean  $\pm$  SD; \* $P < 0.001$  compared to normal control rabbits; n=5 rabbits in each group.



### Screening of different *BIR* extracts for hypoglycemic activity in diabetic rabbits

Water extract of *BIR* showed maximum hypoglycemic activity ( $P < 0.001$ ) for up to six hours. Other extracts except for chloroform also produced significant decrease in blood glucose level for up to 4 hours (Fig. 3).

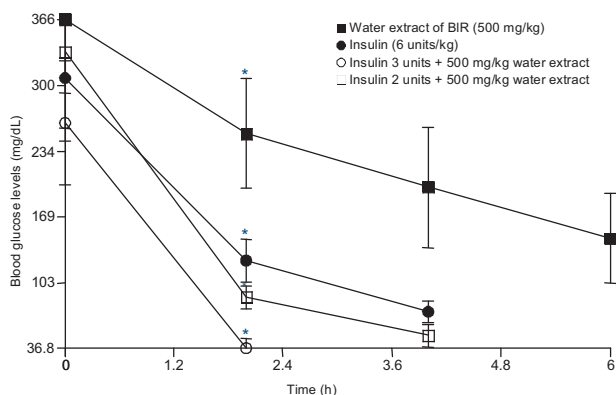
Figure 3. Effect of various extracts of *BIR* on blood glucose levels in diabetic rabbits at 0, 2, 4 and 6 hours. Diabetic control; MET: methanolic extract treated; AMET: aqueous methanolic extract treated; WET: water extract treated; HET: hexane extract treated; CET: chloroform extract treated; values of five animals are given as mean  $\pm$  SD;  $*P < 0.001$  compared to diabetic control rabbits;  $n = 5$  rabbits in each group.



### Comparison of hypoglycemic activity of water extract of *BIR* with or without insulin in diabetic rabbits

Both 6 units of insulin alone and 2 units of insulin plus 500 mg/kg b.w. of water extract significantly ( $P < 0.001$ ) reduced blood glucose levels in diabetic rabbits within 2 hours of drug administration. Three units of insulin plus 500 mg/kg b.w. of water extract produced severe hypoglycemic effect in diabetic rabbits after two hours of drug treatment (Fig. 4).

Figure 4. Hypoglycemic effect of water extract of *BIR* in the presence and absence of different doses of insulin in alloxanized rabbits. Values of five animals are given as mean  $\pm$  SD;  $*P < 0.001$  compared to 0 h;  $n = 5$  rabbits in each group.



### Phytochemical analysis of water extract of *BIR*

Alkaloids, hydrolysable tannins, cardioactive glycosides and saponins were found to be present, whereas reducing sugars, anthracene derivative and flavonoids were undetectable in water extract of *BIR*.

### DISCUSSION

Various antidiabetic plants and herbs are found through traditional use but their introduction into modern therapy needs testing of the compounds by modern research methodology. The results obtained in the present study showed powdered root bark of *BIR* to significantly ( $P < 0.001$ ) reduce blood glucose levels in both normal and diabetic rabbits. Gliclazide also significantly decreased ( $P < 0.001$ ) blood glucose levels in normal rabbits, however, it was unable to produce any significant change in blood glucose levels of diabetic rabbits. These results are in line with a previous study (9). Various extracts of *BIR* root bark were administered orally to compare hypoglycemic activity in alloxanized rabbits. Orally administered 500 mg/kg of water extract showed maximum antidiabetic activity for up to 6 h, whereas the same doses of methanolic, aqueous methanolic and n-hexane extracts reduced blood glucose levels for up to 4 h; however, chloroform extract was unable to produce any significant antidiabetic activity in diabetic rabbits. It indicates that antidiabetic compound(s)

is/are extractable maximally in water. Therefore, the antidiabetic activity of water extract was compared with insulin. Insulin in a dose of 6 units/kg b.w. significantly reduced blood glucose levels within two hours of subcutaneous injection. Insulin (3 units), when co-administered with 500 mg/kg b.w. of water extract given orally produced severe hypoglycemia in diabetic rabbits after 2 h. Insulin (2 units) with 500 mg/kg b.w. of water extract significantly ( $P < 0.001$ ) reduced blood glucose levels, which were comparable with 6 units of insulin alone in diabetic rabbits within 2 h of drug administration. These results suggested that *BIR* root bark contained some biological principle(s) that possess insulin protective or insulin-like activity. Recently, ethanolic extract of *BIR* root has been shown to have antidiabetic activity. Furthermore, this activity was comparable with the antidiabetic activity of berberine (12). These results are in line with our finding (13). Alkaloids and tannins have been reported to possess hypoglycemic activity (14,15). Berberine, a tetra isoquinoline alkaloid, is common in the family Berberidaceae. Berberine has

shown hypoglycemic activity in streptozotocin-nicotinamide induced type 2 diabetic rats by an extrapancreatic mechanism (16). Hydrolysable tannins have been reported to stimulate glucose utilization and inhibit adipocyte differentiation (15). In our study, alkaloids and hydrolysable tannins were detected in water extract of *BIR* root bark. It is suggested that either alkaloids or hydrolysable tannins, or both may possibly have such antidiabetic activity; however, further characterization of such active constituents would enable us to develop new therapy for diabetic patients. This study indicated that water extract of *BIR* may be exploited as an adjunct to insulin in diabetics. Furthermore, these findings provided experimental evidence that water extract of *BIR* could be used to manage diabetes. The study also indicated that antidiabetic constituent(s) were extracted by various organic solvents but to maximal extent in water. Furthermore, these constituent(s) are present not only in the root but also in the root bark. The study further verified the folklore claim of Himalayan natives.

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