Assessment of antidiabetic potential of Berberis lycium Royle root bark extract in experimental animal model

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Abstract

Background: Various medicines are in practice for the treatment of diabetes mellitus but phyto-based medicines got paramount importance in this regard. The current investigation focused on inspection of antidiabetic potential of Berberis lycium Royle root bark extract. Methods: α-amylase and α-glucosidase inhibition assay were performed for the evaluation of in-vitro antidiabetic activity. In-vivo antidiabetic potential was estimated in alloxan-induced swiss albino mice. Diabetic mice were treated with aqueous root bark extract (200 mg/kg b.w.) for 28 days. At the end of 28 days treatment period mice were sacrificed for biochemical and histopathological analysis of pancreas, liver and kidney. Results: Blood glucose level and all other biochemical parameters were significantly normalized when treated with B. lycium Royle root bark extract. Results of both in-vitro and in-vivo activity showed that B. lycium Royle has antidiabetic effect. Conclusion: B. lycium Royle root bark extract presented efficient antidiabetic activity in the diabetic mice model and therefore could have potential for development of drugs in the future. Keywords: Diabetes, alloxan, medicinal plants, Berberis lycium Royle

Assessment of antidiabetic potential of *Berberis lycium* Royle root bark extract in experimental animal model

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Introduction

Diabetes is generally caused by impairment of the pancreatic β cells. This in turn leads to declined formation of insulin or enhanced confrontation to the function of insulin in the peripheral tissues (Frode and Medeiros, 2008). It is stimulated by both environmental factors as well hereditary which cause elevated blood sugar level (Wu et al., 2014). It is a big problem all over the world. Scientists are trying to solve this problem. Available therapies have side effects and do not cure the syndrome completely. This is the need of time to search better alternate for the treatment of diabetes. Phyto-based materials can prove themselves as a good antidiabetic candidate.

Numerous plants have been confirmed for their antidiabetic activities in animal models which recommend that the world is probing for new phyto-based anti-diabetes mediators with fewer side effects (Khan et al., 2012). Flora is a spring of medications. Various phyto-based bioactive molecules have been assessed for

their proficiency in controlling several ailments. These medications have therapeutic potential with no side effects.

Berberis lycium Royle is a plant that has been used to treat arthritis, back pain, diarrhea, jaundice, piles, internal injuries, scabies, throat pain, pimples, cracked bones, fever and sun blindness (Shabbir et al., 2012). In current research *B. lycium* Royle was used as antidiabetic agent.

Methods:

Collection of medicinal plant

Berberis lycium Royle plant was collected from Chinari, District Jhelum valley, Azad Kashmir, Pakistan. Root bark was separated and washed with distilled water. Shade dried root bark was crushed into fine powder.

Preparation of plant extract

Ten gram of powder was boiled with 100 ml distilled water. After cooling extract (BLR extract) was filtered using filter paper and concentrated using rotary evaporator. Plants extract was dried in vacuum oven at 40°C and finally used for evaluation of antidiabetic activity.

Phytochemical Screening

The aqueous extract of BLR root bark was screened out for the presence of different phytochemicals.

Chemicals and Drugs: Glibenclamide was purchased from a local drug store and alloxan (Sigma Aldrich, USA) from chemical center Anarkali, Lahore.

In Vitro antidiabetic activity

α-amylase inhibition assay was completed using the DNSA method (Miller, 1959) and α glucosidase inhibition activity method of Kim et al., (2005) with some modification.

In vivo antidiabetic activity

Animal Management

Mice of various groups were kept in polypropylene cages. Normal laboratory pellet feed as well as drinking water *ad libitum* was provided to mice. Animal house temperature was retained at 22 ± 3 °C. 12 h light/12 h dark cycle was retained. Oral feeding needle (18-gauge) was used for oral dosing.

Animal Selection and Grouping

Twenty male Swiss albino mice, with average age 8 weeks and 35-40 g body mass were taken from GC University Lahore. All experimental animals were housed together for seven days before the commencement of the experiment. Mice were arbitrarily categorized into four groups, control group, diabetic group, BLR extract (200 mg/kg b.w) treated group and glibenclamide (10 mg/kg b.w) treated group.

Induction of Experimental Diabetes

Single intraperitoneal dose of alloxan (150 mg/kg) in 0.9% saline was used to prompt the diabetes in mice. After that fasting blood glucose was determined and the mice with blood glucose level over 200 mg/dL were indicated as diabetic. Complete research plan is given in figure 1.

Body mass measurement

Body mass of mice was measured via digital weighing machine. The mass of the mice was checked from the start till the completion of the experiment at 7-day interims (Days 1, 7, 14, 21, and 28).

Biochemical Determinations

After 28 days treatment blood was collected directly from heart and mice were sacrificed for different biochemical analysis and histology. Blood glucose (at 7^{th}, 14^{th}, 21^{st} and 28^{th} day), lipid profile (Cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C), LFT (ASAT, ALAT, ALP, bilirubin and total protein), RFT (Albumin, creatinine and urea), antioxidant activity (SOD, CAT and LPO) and hematological parameters were analyzed. Histopathological studies were also carried out. See (**figure 1)** for complete research plan.

Arithmetical analysis

GraphPad prism (version 5.0) was used for arithmetical studies. All values were expressed as mean±standard error of mean. Statistical difference among different groups was calculated by one way ANOVA with Bonferroni test. Values were measured statistically substantial at p[?] 0.05.

Results

Phytochemicals present:

Alkaloids, steroids, tannins, saponins, glycosides, terpenides, proteins, free amino acids, carbohydrates, phenols and flavonoids were present in BLR extract.

In vitro antidiabetic activity:

The BLR extract displayed antidiabetic action with affection to inhibition of ∝-amylase and ∝-glucosidase proximate to that perceived using the ordinary antidiabetic drug acarbose. The concentration of acarbose was used in identical to concentration of root bark extract. i.e 50, 100, 150, 200, 250 (µg/mL) **(figure 2a and 2b).**

In vivo antidiabetic activity:

Blood glucose levels and changes in body mass:

Alloxan induced diabetes amplified blood glucose level and declined body mass and insulin. These changes were significantly reversed in BLR extract treated group as equated to control group **(figure 3a, figure 3b, figure 3c)** .

Effect on lipid profile

Significant upsurge in total cholesterol, triglycerides, LDL-C, VLDL-C and decline in HDL-C level of diabetic group (group II) as equaled to control (group I) was seen. BLR extract and glibenclamide treatment reversed all these changes significantly **(figure 4).**

Effect on liver function markers:

The effect of BLR extract on serum levels liver function markers was estimated. Levels of ALAT, ASAT, ALP and bilirubin were high in group II than in group I while that of total protein was lesser in group II than group I. Treatment with BLR extract and glibenclamide significantly demolished all these changes **(figure 5).**

Effect on renal function markers:

Serum urea, uric acid, creatinine and albumin level was measured to evaluate the effect of BLR-extract. Level of urea, uric acid and creatinine was increased and that of albumin was decreased in group II as compared

to group I. BLR extract and glibenclamide treatment reversed all these changes to significant level except that of albumin in group III **(figure 6).**

Effect on antioxidant activity:

Antioxidant action of the liver supernatant was assessed by decisive SOD, CAT activity and lipid peroxidation. The activity of CAT and SOD was declined and that of LPO amplified significantly in group II as paralleled to group I. BLR extract and glibenclamide treatment significantly restored CAT and LPO level to normal range **(figure 7).**

Hematology:

Changes in WBCs, RBCs, Hb, hematocrit, MCV, platelets, neutrophils, eosinophils, monocytes and lymphocytes were evaluated. Induction of alloxan increased in WBCs, hematocrit, platelets and neutrophils and decreased in RBCs, Hb, MCV and lymphocytes significantly. Treatment with BLR extract and glibenclamide revesed most of these changes significantly. All other changes were non-significant **(figure 8a and 8b**

Histopathology:

In Histopathological studies, pancreas, kidney and liver sections of treated, untreated and normal control mice were examined.

Histopathology of pancreas:

Diabetic mice showed distortion in islet of Langerhans as compared to control group. The mice treated with BLR extract showed worthy recovery and regeneration of islet tissue of the pancreas **(Figure 9a)** .

Histopathology of liver:

The hepatic section of control group exhibited normal architecture with intact central vein (CV), slit-like sinusoids and noticeable nuclei. In diabetic mice, the hepatic section showed distressed CV with apoptotic nuclei. BLR extract treated mice showed significant recovery of the CV**(Figure 9b)** .

Histopathology of kidney:

Normal architecture is perceived in renal section of control group. The renal section of diabetic mice exhibited distorted glomerular and distended urinary space with necrosis. The renal section of BLR extract treated mice showed significant regeneration and revival in morphology**(Figure 9c)** .

Discussion

In the present research experiments were designed to evaluate the antidiabetic potential of aqueous extract of root bark of *Berberis lycium* Royle. Antidiabetic effect of *B. lycium* Royle root bark was compared with glibenclamide. Diabetes was induced by alloxan in animals and were treated with *B. lycium* Royle root bark extract and AgNPs for 28 days. The plasma glucose level was estimated at 0, 7^{th}, 14^{th}, $21^{st}$and 28^{th} day of the research work to assess the potency of the extract in clearing plasma glucose in mice induced with diabetes. Upsurge in glucose level was seen in alloxan induced diabetic mice but after treatment with*B. lycium* Royle root bark extract glucose level was normalized. The decline in glucose level after treatment might be brought by the pancreatic β- cells stimulation to yield insulin or by prevention of glucose absorption from the intestine or it may heighten the glucose uptake in peripheral tissues (Gayathri and Kannabiran, 2009).

Reduction in body mass was seen in alloxanized mice. Severe loss in body weight is due to loss or degradation of structural proteins (Rajkumar and Govindarajulu, 1991). The *B. lycium* Royle root bark extract prevented the loss in body mass in alloxan induced diabetic mice and from this it is evident that the *B. lycium* root bark extract possess a significant beneficial effect on body mass. This may be due to the protein sparing

effect of *B. lycium* Royle which avert protein ruin that upshots in improvement of body mass. Insulin paucity in diabetes makes the cells famish for glucose, so the cells use proteins as a substitute energy source which eventually fallouts in body mass reduction. Copious studies have shown a link between hyperglycemia and dwindled body mass of diabetic animals. It was observed that alloxan induced diabetes is associated with the reduction in the body mass of animals (Sharma, 2009).

Hyperlipidemia like secondary hitches are linked to diabetes mellitus. It is pigeon-holed by escalation in serum total cholesterol, triglycerides, LDL and VLDL. Potential of the *B. lycium* Royle root bark extract to lower the diabetic impediments by gaging the lipid profile was evaluated. Upsurge in total plasma cholesterol, triglycerides, LDL-C and VLDL-C and decline in HDL-C was seen in alloxan induced diabetic mice. Mice of therapeutic groups treated with extract have shown significant reduction in above serum parameters except HDL-C which was increased after treatment.

The relationship between hepatic ailment and diabetes mellitus is well known. In recent times, new understandings of this link came from the recognition that diabetes itself may be a motive of hepatic ailment. Hepatic damage leads to its faulty function and it is characterized by the augmented concentrations of hepatic enzymes ASAT and ALAT in the blood. Measurement of ASAT, ALAT and ALP have clinical and toxicological prominence as fluctuations in their activities are symptomatic of tissue damage (Somnath et al., 2001). An augmentation in ASAT activity is due to hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in ALAT activity (Rao et al., 1989). When cell membranes get impaired, the enzymes ASAT and ALAT, located in the cytosol, leak into the blood stream thus causing damage to hepatic tissues (Shyamala et al., 2003). It is signposted that *B. lycium*Royle comprehends various bioactive compounds that preserve the integrity of hepatic cells which preclude the leakage of ASAT and ALATin serum. In current study level of these enzymes increased in alloxanized mice but these hepatic markers were sustained in their normal range in diabetic treated mice.

Serum catalase is a major factor of hepatic antioxidants and catalyzes the reduction of H_2O_2 and shields the tissue from highly reactive OH*. Reduction in serum catalase activity might be the result from inactivation by O_2^{*-} and glycation of the enzyme (Rajasekaran *et al.,* 2005). In current study catalase and SOD level decreased significantly in diabetic mice and these changes were reversed in *B. lycium* Royle root bark extract treated mice. LPO level was increased in diabetic mice which was not significantly normalized by *B. lycium* Royle root bark extract. LPO upsurge is a significant findings in diabetes mellitus which is a marker of raised oxidative stress in diabetes mellitus (Sen *et al* ., 2005).

Intensification in urea, uric acid and creatinine levels are markers of renal dysfunction in the diabetic groups (Alarcon-Aguilara *et al* ., 1998). In present study upsurge in these markers were seen in diabetic group. Upon treatment with aqueous extract of *B. lycium*Royle the renal markers were significantly declined, this explains the shielding effect afforded by aqueous extract of *B. lycium* Royle on kidneys in diabetes. Protein level in alloxanized mice was decreased, this effect was reversed on treatment. Discount of protein level is an indicator of diabetic condition (Surana *et al.* , 2008). Declined serum protein level in alloxanized mice is supposed to be due to improved protein catabolism and gluconeogenesis in diabetes (Palanivel*et al.* , 2001). This implies that *B. lycium* Royle enhance glucose utilization and prevent protein degradation that normalized the altered protein level in *B. lycium* Royle treated groups.

Alloxan induced diabetic mice exhibited a substantial spread in total WBCs count. This upsurge in total WBCs count may be due to the improved hemopoitic activity as a result of the hemolysis of RBCs in diabetic mice. In diabetic mice group number of lymphocytes were dropped. This might be as a retort to stressful situation after alloxan injection, these results are in agreement with previous study (Palanivel *et al.* , 2001) or it may be due to the production of antibodies against different antigens, since lymphocytes are accountable for attaining the defense mechanism in the body (El Feki *et al* ., 1997). The rise in number of neutrophils may be due the participation of these cells in the phagocytosis against diverse antigens. The upsurge in neutrophils also may be due to drop in its phagocytic action in hyperglycemia and due to upsurge in hemopoitic activity after releasing the granules of neutrophil by exocytosis to lyses the antigens extracellularly (Ganong, 2003). *B. lycium* Royle root bark extract showed an improvement in percentage of

lymphocytes when compared to diabetic group. In treated mice groups WBCs count, neutrophils percentage and lymphocytes turned back to normal value. This indicates that phyto-treatment affects the defense mechanism to impede the inflammation resultant of alloxan treatment. This is in line with previous studies (Kollar & Roan, 1980; Shakoori *et al* ., 1992). Proteins glycation prompted by diabetic hyperglycemia can have modificational impacts on membrane proteins of RBCs and hemoglobulin (Hb) (Koga *et al* ., 1980). Haemoglobin (Hb) values in diabetic mice displayed substantial decline in agreement with prior hematological studies reporting anemia as a pathophysiological problem of diabetes mellitus (Akindele *et al* ., 2012). This discount is possibly because of blood osmoregulation flaw and abnormal synthesis of Hb (Akindele *et al* ., 2012). Platelets count were also measured, substantial variations in diabetic mice was perceived as compared to control group which was normalized after treatment with*B. lycium* Royle extract. No significant variation was seen in other hematological parameters. Histopathological studies showed damage in pancreas, liver and kidney in diabetic group and effect was reversed in *B. lycium* Royle extract treated groups (figure 9 a, b,c).

Conclusion

Diabetes is a deadly ailment worldwide and it should be treated effectively. Many plant based materials are effective in this regard. The existing research was done to evaluate the antidiabetic activities of *B. lycium* Royle root bark extract. It was observed that drug glibenclamide and *B. lycium* Royle root bark extract both showed antidiabetic activities. *B. lycium* Royle root bark extract showed effectiveness in normalizing blood sugar, lipid profile, LFT, RFT, antioxidants and some hematological parameters. Additionally, significant restoration and renewal of histo-morphology of pancreas, liver and kidney was also perceived. Outcomes of this study validated that the *B. lycium* Royle root bark extract have potential to shrink the ailments of diabetes.

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Competing interest

The authors have no competing interests.

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Figure 1: Research plan for antidiabetic activity.

Figure 2: (a) ∝-amylase inhibition assay and **(b)** ∝ glucosidase inhibition activity

Figure 3: (a) Changes in blood glucose level**, (b)**Changes in body mass**, (c)** Changes in blood insulin level.

Key: [a]depicts variance between group I and II. [b]depicts variance between group II and III.[c]depicts variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons: aaa, bbb, ccc=p [?] 0.001.

Figure 4: Analysis of lipid profile.

Key: [a]depicts variance between group I and II.[b]depicts variance between group II and III.[c]depicts variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons: cc= p [?] 0.01; aaa, bbb, ccc=p [?] 0.001.

Figure 5: Analysis of liver function markers.

Key: [a]depicts variance between group I and II.[b]depicts variance between group II and III.[c]depicts variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons: b, c= p [?] 0.05, bb= p [?] 0.01, aaa, bbb, ccc = p [?] 0.001.

Figure 6: Analysis of renal function markers.

Key: [a]depicts variance between group I and II.[b]depicts variance between group II and III.[c]depicts variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons:

b = p [?] 0.05, cc= p [?] 0.01, aaa, bbb, ccc = p [?] 0.001.

Figure 7: Analysis of liver antioxidants.

Key: [a]depicts variance between group I and II.[b]depicts variance between group II and III.[c]depicts variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons: b = p [?] 0.05, aaa, bbb, ccc = p [?] 0.001.

Figure 8: Hematology.

Key: [a]depicts variance between group I and II.[b]depicts variance between group II and III.[c]depicts

variance between group II and IV. Each bar signifies the mean value of five replicates and SEM. Arithmetical icons**:** a, c**=** p [?] 0.05, aa, bb,cc= p [?] 0.01, aaa, bbb, ccc = p [?] 0.001.

Figure 9 (a): Histopathology of pancreas. IL: Islets of Langerhans; Ac: Acini; Pd: Pancreatic duct; Int. LD: Intralobular duct**. (b):** Histopathology of liver. CV: Central vein.

(c): Histopathology of kidney. gl: Glomerulus; *: damage in glomerulus