

GLYCEMIC CONTROL EFFICACY OF *BERBERIS LYCIUM* IN DIABETIC PATIENTS

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Abstract

The rising global prevalence of type 2 diabetes mellitus (T2DM) has intensified interest in plant-derived therapeutic agents as complementary strategies for glycemic management. The present study investigated the efficacy of *Berberis lycium* in improving glycemic control and evaluated its potential as a nutraceutical intervention for patients with T2DM. Root extracts of *Berberis lycium* were analyzed for phytochemical composition and antioxidant activity using total phenolic content estimation and DPPH radical scavenging assays. A randomized 90-day clinical intervention trial was conducted involving patients diagnosed with T2DM, who were allocated into control and experimental groups. Participants in the experimental group received 500 mg *Berberis lycium* capsules daily in addition to conventional antidiabetic therapy and a prescribed therapeutic diet, whereas the control group received placebo capsules alongside standard medication and dietary management. Anthropometric and biochemical parameters, including body mass index (BMI), fasting blood glucose (FBG), oral glucose tolerance test (OGTT), glycated hemoglobin (HbA1c), and total cholesterol levels, were assessed at baseline, 45 days, and 90 days. The findings demonstrated considerable antioxidant potential in *Berberis lycium* root extracts and revealed significant improvements in BMI, FBG, OGTT, and total cholesterol levels among subjects receiving the supplementation. Although HbA1c levels exhibited a declining trend, the observed changes were not statistically significant. In conclusion, *Berberis lycium* supplementation may represent a safe, cost-effective, and supportive herbal approach for enhancing glycemic regulation and metabolic health in individuals with T2DM.

INTRODUCTION

Berberis lycium Royle is a member of the Berberidaceae family and is recognized for its dual importance as both food source and a medicinal

plant. The genus *Berberis* consists of around 500 species, of which 77 species are found in India. The majority of these species are native to central and southern Europe, with some also distributed

in the northern parts of Pakistan (Qaseem et al., 2019). It is found in regions of Pakistan such as Gilgit Baltistan, Hunza, upper Hunza Ghizer, Swat, and Astore. Its common names are Kashmal, Ishkeen and Sumbal in the area of its gathering. Within India, barberry flourishes in sub-tropical and temperate areas, specifically within the outer northern-western Himalayas, occupying elevations ranging from 850 to 3500 meters above sea level. *Berberis lycium* is adaptable to different ecological conditions and can breed in sandy, silty, or loamy soils (Parra et al., 2018).

The plant of *Berberis lycium* is effortless grown shrub 2 to 3 m in altitude, which is upright or suberect and semideciduous with dimorphic shoot (1-2 mm long). The spines are organized at intervals on stem and the color of branches is pale white to gray. Leaves of this plant are 2.5 to 7.5 by 8.18 mm, hardly elongate and leathery tough in shape with a limited big spinous teeth that are organized one after the other on the branch. The plant has flowers that are bisexual but fertilization occurs through insects too. The flowers bloom from May to June which has a size larger than the leaf mostly pale yellow in color and are cup shaped arranged in racemes (Irshad et al., 2013).

Berries that are ovoid or obvoid-subglobose are fruits of *Berberis lycium* plant that have bright red color and on ripening they attain purplish color. The fruit is juicy having pulp or juice in plum purple color that is slightly acidic in nature. On average estimation, the fruit is 7mm long, 4mm in diameter and 227mg in weight. The seeds of the fruit are 2-5 in number and yellow to pink in color. Berries of *Berberis lycium* are used to treat anti-inflammatory, stomachache and hypoglycemic agents (Purvika et al., 2013).

The root of the plant has a hard composition with a diameter spanning 3-8 cm. It branches out, gradually tapering and occasionally exhibiting longitudinal splits. The wood of the root is smooth and displays a bright yellow coloration, while the root bark can reach a thickness of up to 3 mm, showcasing external fissures alongside internal smoothness. The roots of plants *Berberis lycium*, serve as a significant source of a remarkable molecule called "berberine." This compound has

found application in various herbal formulations marketed for the treatment of diverse diseases. Berberine has captured significant attention due to its potent medicinal properties and is the subject of ongoing research and exploration. Berberine is present in root extract by 80 % of its dry weight. Berberine, palmatine and berbamine are the key alkaloids present in this specie (Gaur et al., 2018).

Diabetes mellitus (DM) is a metabolic disorder described by a decrease in insulin activity or insulin excretion, leading to abnormal glucose metabolism. As the disease progresses, it can give rise to several pathological changes, containing complications such as nephropathy, retinopathy and cardiovascular disorders. DM is commonly classified into two subtypes: type I DM and type II DM. The management approaches for these two types differ, type II diabetes mellitus is often cured with oral hypoglycemic medications (Padhi & Nayak, 2020).

Insulin resistance, a hallmark of type II DM, is often associated with impaired glucose tolerance. When insulin resistance is present, the body's cells grow into less responsive to the activities of insulin, leads to small glucose uptake. Furthermore, associated injury to the pancreatic islet beta cells can promote insulin deficiency, damaging the regulation of glucose consumption via numerous tissues, including skeletal muscle, liver, and adipose tissue so glucose cannot be taken up by cells that leads to its accumulation in blood. Multiple factors contribute to the progression of insulin resistance which may results in type II Diabetes mellitus, including genetic predisposition, environmental factors, dietary patterns, physical inactivity, and obesity (Oguntibeju, 2019).

19.4 million adults living in Pakistan with diabetes which requires effective control of elevated blood sugar levels. *Berberis lycium*, a natural remedy with reputed hypoglycemic effects, lacks sufficient scientific evidence to validate its efficacy and safety for type 2 diabetic patients. Therefore, there is a requirement for further investigation to assess its low glycemic index potential. This research has subsequent objectives: to study physico-chemical

characteristics of *Berberis lycium* and nutritional Management of diabetic patient in response to *Berberis lycium* administration

MATERIALS AND METHODS

Sample Selection and Preparation

Berberis lycium roots had berberine, the active ingredient, flavonoids, and antioxidants, therefore they were used to treat type 2 diabetes. The Quaid-e-Azam University botanical garden in Islamabad provided Berberis lycium Royal root samples. Weight, date, and sampling locations were recorded, and plant part physical attributes were examined. Wet, dried, and powdered samples were preserved for further investigation.

Total Phenolic Compounds

Total phenolic compounds were calculated using

Radical Scavenging Activity DPPH

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay Spectrophotometer wavelength 517nm as described by Nazie et al., (2021).

$$\text{Radical Scavenging Activity \%} = \frac{A_0 - A_1}{A_0} \times 100$$

The absorbance when the sample is absent from the DPPH solution is A0. A1 is the sample solution's absorbance following the DPPH reaction.

CLINICAL TRIAL

Study Design

The powder of *Berberis lycium* was filled manually in capsules. Each capsule was filled with 500mg serving size of herbal powder. These capsules were stored in suitable environment for further usage. Study was conducted at Holy Family Hospital Rawalpindi which includes type 2 diabetics both male and female having maximum 35 years of age and above. Patients diagnosed with type 2 diabetes receive treatment for minimum 3 months.

the Folin-Ciocalteu FC reagent and Berberis lycium extract's total phenolic content (TPC) was measured, using the method previously reported in (Ghareeb et al. 2018). A portion of the plant extract was combined with a stock solution of gallic acid, the Folin-Ciocalteu reagent, sodium carbonate, and distilled water, as well as a dry methanolic extract that has been prepared. We will measure the absorbance at 765 nm following a 30-minute incubation period. The standard curve expressed as gallic acid equivalent (mg GAE)/g was obtained of plant extract as mentioned in (Landry et al., 2021).

Patients with type-1 diabetes, patients of other diseases and non-diabetics. It used in which study participants were divided in 2 groups
T1: Experimental group
T2: controlled group

Screening Tool for Diabetes Mellitus

Data were collected in first visit and then follow up visits of the patients in following days;
D1 were the 1st day
D2 were the 45th day and
D3 were the 90th day of follow-up plan

Table 1: Treatment plan following groups and days

Treatments	1 st	45 th	90 th
T1(Placebo)	Recorded readings		
T2 (BL) 5mg	Recorded readings		

Anthropometric measurements

Body Mass Index (BMI), Waist Circumference and Body Fat Percentages of each participant were estimated before and after intervention. For

measurement of weight calibrated balance beam and for height stadiometer was used. Body mass index BMI was considered using formula defined by World Health Organization (WHO, 2018).

$$BMI = \frac{\text{Weight in kg}}{\text{Height in m}^2}$$

Waist circumference was measured to assess abdominal obesity by using a measuring tape placed in horizontal position around the body. All the readings were recorded (Ostchega et al., 2019).

Biochemical tests

Biochemical tests used for assessment of diabetic patients for research was Fasting Blood Glucose (FBG), Oral Glucose Tolerance Test (OGTT), Glycated Hemoglobin (HbA1c) and Lipid Profile

Table 2: Model dietary guidelines for both diabetic patient groups

Mediterranean diet + Exercise	
Plant-based food products	whole grains, legumes, nuts, and seeds; also fruits and vegetables. These foods are a good source of fiber, antioxidants, vitamins, and minerals.
Healthy fats	olive oil
Lean protein sources	fish, poultry, and legumes
Moderate dairy and yogurt	Skimmed milk
Herbs and spices	No added salts and excessive sugars

Intervention

The treatment group involves patients introduced to *Berberis lycium* 5mg capsules with their medications and prescribed model diet plan. Control group received placebo with medications previously prescribed and model diet plan.

respectively among the five extracts. Whereas chloroform extracts possess poor number of polyphenols. Wide variation in TPC was observed amongst the extracts as show in Table 3 based on the values of TPC, the extracts were divided into two categories a) extracts with high TPC i.e > 8 mg GAE/g dry wt: acetone, methanol and ethyl acetate extracts (b) extracts with low TPC i.e < 8mg GAE/g dry wt. aqueous and chloroform extracts. The result showed that the total phenolic content was observed in roots powder of *Berberis lycium* with the mean value of 15.12±0.22 mg GAE/g.

Statistical Analysis

Data obtained during the study was analyzed by one way and two-way ANOVA and Least Significant Difference (LSD) was used with Minitab software (Steel et al., 1997).

RESULTS AND DISCUSSIONS

Total Phenolic Compounds

Table 3 represented the yield, the total phenolic contents of roots powder of *Berberis lycium* extracts. Acetone and methanol extracts were found to process maximum phenolic contents with the acetone and methanol solvents extracts with the mean value of (15.12±0.22) mg GAE/g dry wet and 13.45±0.25 mg GAE/g dry wt.

Radical Scavenging Activity DPPH

In terms of DPPH (1, 1-Diphenyl -2-picrylhydrazyl) radical scavenging activity, among the five extracts of *Berberis lycium*, acetone possess good radical scavenging activity with 1C₅₀ value 329ug/ml followed by methanolic extracts (1C₅₀ value 503ug/ml), and aqueous extracts showed poor radical scavenging activity. Antioxidant activity of roots powder of *Berberis lycium* black was

determined by the method of DPPH. The results indicated that DPPH method showed roots

berberis lycium possessed with the mean value of 17 ± 0.33 % antioxidant activity.

Table 3: Phytochemical composition (%) of the roots powder of Berberis lycium

Extracts with different solvents	Extraction yield (%)	Total phenolic content (mg GAE/G dry wt.)	DPPH Assay (IC ₅₀ in ug/ml)
Methanol extract	16.86%	13.45±0.25	503
Aqueous Extract (Aq)	4.19%	3.15±0.05	6535

BIOCHEMICAL TESTS

In this study, there were 2 groups of people which were randomly selected: Control group denoted by T0, and experimental group denoted by T1. Each group comprised of 30 subjects. The control group was on a normal diet while the experimental group was on low calorie diet with consumption of capsule of Berberis lycium powder once a day. This study was conducted for 90 days to check the effect on different parameters of subjects. The results of different parameters are given below:

Body Mass Index (BMI)

BMI data was analyzed at 0, 45 and 90 days for both the control (T0) and experimental (T2) groups. Table 4 revealed that all the groups*days means at days 0, 45 and 90 had small changes in BMI. In 90 days, the control group's BMI decreased from 32.65 ± 0.03 Kg/m² to 25.61 ± 0.01 Kg/m². According to the experimental group (T2), the mean BMI at days 0, 45, and 90 was 26.87 ± 0.03 Kg/m², 26.97 ± 0.01 Kg/m², and 25.19 ± 0.02 Kg/m² respectively. Patients' BMIs dropped significantly in the experimental group after 90 days. Due to weight loss, the BMI of experimental group patients reduced from 26.87^b

± 0.03 Kg/m² to $26.97^b \pm 0.01$ Kg/m² on the 45th day and from 26.97 to 25.19 on the 90th day of the study. Due to daily Berberis lycium capsule administration for 90 days, individuals lost weight and BMI dropped. It was concluded that merely dietary guidelines did not alter patients' weight and BMI, but dietary guidelines plus Berberis lycium capsule decreased weight and normalized BMI. BMI is computed by dividing weight (kg) by height (m²). Its unit is Kg/m². A healthy BMI is 18.5–24.9 Kg/m², while 25 or above is overweight. Using analysis of variance, the p-value for interaction term groups*days is 0.051. This p value is very significant at < 0.05. The result of LSD showed a significant difference from the first day to day 90 between the experimental group (T2). It was seen that there was significant change in experimental group BMI, and no significant change in control group BMI, as shown in Table 4. Haidari et al. (2021) reported that low calorie diets and Berberis lycium extract was effective in decreasing BMI. It also helps us in our study. According to Lean (2019), the management strategies for weight loss result in an increase in metabolism, such as glycemic index and lipid profile, which in turn reduces mortality rate.

Table 4: Effect of Berberis lycium capsule on BMI of diabetic patients

	0 Day	45 Day	90 Day	Total Mean
T0	32.65 ^a ±0.03	27.41 ^b ±0.03	25.61 ^b ±0.01	27.41 ^a
T2	26.87 ^b ±0.03	26.97 ^b ±0.01	25.19 ^b ±0.02	26.87 ^a
Total Mean	29.76 ^a	27.19 ^b	25.40 ^b	

T0 = Control Group; T2 = Experimental Group

Fasting Blood Glucose (FBG)

The data collected from the control group (T0) and experimental group (T2) at 0, 45 and 90 days were examined for FBG. Table 5 showed that the control group (T0) means at day 0, day 45 and day 90 had minimal change in FBG, which decreased from 162.13 a ± 0.01 mg/dL to 121.13 c ± 0.01 mg/dL after 90 days. The FBG for patients in the experimental group on day 0, 45, 90 were 167.34 a ± 0.02 mg/dL, 123.67 c ± 0.03 mg/dL, and 103.55 d ± 0.02 mg/dL respectively where the p-

value for the interaction term groups*days was 0.001 using analysis of variance. This p value is very significant (< 0.05). Fabrication of BGs was performed using LSD. The results of the LSD showed significantly different means between day 0 and day 90 for the experimental group (T2). Table 5 shows that the change in FBG was higher in the experimental group compared to the control group. Low calorie diets and Berberis lycium extract had a positive effect on reducing FBG (Haidari et al. 2021).

Table 5: Effect of *Berberis lycium* capsule on FBG of diabetic patients

	0 Day	45 Day	90 Day	Total Mean
T0	162.13 ^a ±0.01	143.57 ^b ±0.01	121.13 ^c ±0.01	143.57 ^a
T2	167.34 ^a ±0.02	123.67 ^c ±0.03	103.55 ^d ±0.02	123.34 ^b
Total Mean	164.73 ^a	133.62 ^b	112.34 ^c	

T0 = Control Group; T2 = Experimental Group

Glycosylated Hemoglobin (HbA1c)

Hb1Ac was analyzed for both control group (T0) and experimental group (T2) at 0, 45 and 90 days. The results revealed no significant difference (p>0.05) between all the groups. Table 6 demonstrated that the Hb1Ac levels of the patients slightly changed only at days 0, 45 and 90 for the control group. Hb1Ac decreases in the control group from 11.12^a ± 0.02 to 7.35^b ± 0.02 after 90 days. In the experimental group (T2), Hb1Ac levels at days 0, 45, and 90 were 7.78^b ± 0.01, 6.88^b ± 0.02 and 6.37^b ± 0.03. The experimental group showed a non-significant decrease in patients' Hb1Ac after 90 days. The study found that Hb1Ac fell in the experimental group from 7.78^b ± 0.01 to 6.88^b ± 0.02 on the 45th day and from 6.88 to 6.37^b ± 0.03 on the 90th day. This significant change was observed

after taking Berberis lycium pills daily for 90 days. Therefore, patients' Hb1Ac was not affected using dietary guidelines, but it was decreased or normalized when dietary guidelines and Berberis lycium capsule were used together. The p-value of the interaction term groups*days by analysis of variance is 0.07. This p value is found to be greater than 0.05 and thus doesn't show significance. Hb1Ac was compared between the treatments using LSD. The pairwise difference between Day 0 and Day 90 of the experimental group (T2) indicated in the LSD results was non-significant. Table 6 indicated that there was a slight change in the level of Hb1Ac in the control group and enhanced in the experimental group. Haidari et al. (2021) reported that both low-calorie diets and Berberis lycium extract resulted in a decrease of Hb1Ac.

Table 6: Effect of *Berberis lycium* capsule on Hb1Ac of diabetic patients

	0 Day	45 Day	90 Day	Total Mean
T0	11.12 ^a ±0.02	7.95 ^b ±0.01	7.35 ^b ±0.02	7.95 ^a
T2	7.78 ^b ±0.01	6.88 ^b ±0.02	6.37 ^b ±0.03	6.88 ^b
Total Mean	9.45 ^a	7.41 ^b	6.86 ^b	

T0 = Control Group; T2 = Experimental Group

Oral Glucose Tolerance Test (OGTT)

The data of the OGTT, from the control group (T0) and experimental group (T2) at 0, 45, and 90 days was analyzed. Table 7 revealed that the patients in control group (T0) experienced slight change in OGTT, whereas the OGTT patients of experimental group (T2) experienced significant change. It was a substantial change that was seen with the use of Berberis lycium capsules for 90 days. Therefore, dietary advice alone did not affect the patients' OGTT, while the dietary guidelines combined with Berberis lycium capsule resulted in a decrease in the OGTT. The OGTT Factorial Analysis of Variance table in Appendix shows a 0.001 p value for interaction term groups*days.

This p value is less than 0.05, very significant results. The 2 treatments compared using LSD. For LSD, there were significant differences between pairs of experimental group days (T2) days 0, day 45 and day 45, day 90. Table 7 demonstrated that there was an experimental group (OGTT) that was significantly different, but there was no significant difference in the control group. In a study by Haidari et al. (2021), they were able to find that low calorie diets and capsules containing Berberis lycium lowered OGTT. This also helps us in our study. Weight loss management strategies increase metabolic markers, according to Lean (2019).

Table 7: Effect of *Berberis lycium* capsule on OGTT of diabetic patients

	0 Day	45 Day	90 Day	Total Mean
T0	219.37 ^b ±0.01	189.38 ^c ±0.03	164.78 ^e ±0.03	189.38 ^a
T2	238.35 ^a ±0.02	180.13 ^d ±0.03	126.97 ^f ±0.02	180.13 ^b
Total Mean	228.86 ^a	184.75 ^b	145.87 ^c	

T0 = Control Group; T2 = Experimental Group

Total cholesterol (TC)

Results from statistical analysis of TC data for control (T0) and experimental (T2) showed significant differences (p<0.05) between all groups at 0, 45 and 90 days. As compared with the control group (T0) patients, the experimental group (T2) patients showed a significant change in total cholesterol (TC) as given in Table 8. TC levels in the experimental group fell from 228.73 b ± 0.02 mg/dL to 185.13 d ± 0.02 mg/dL on the 45th day and from 185.13 d to 161.53 e ± 0.02 on the 90th day. This huge change was achieved after taking Berberis lycium capsules for 90 days every day. Therefore, no improvement in patients' overall cholesterol level could be achieved with dietary advice, whereas Berberis lycium capsules did. Cholesterol is a fat-like substance that is essential to produce bile acids, to produce hormones, and for the body. Maintaining cholesterol levels below 200mg/dL decreases the risk of heart disease. The

Factorial Analysis of Variance in Appendix has a p value of 0.001 for the interaction term groups*days. The results were highly significant as this p-value was < 0.05. The days 0 and 45 and days 45 and 90 were significantly different for the experimental group (T2) and the results of LSD are presented below as shown in Table 8, the mean of experimental group TC was significantly different from the mean of control group TC. The low-calorie diet and addition of Berberis lycium capsules significantly decreased patients' total cholesterol (Haidari et al., 2021). It helps with our learning as well. Lean (2019) reported that the use of weight reduction management strategies causes an improvement in metabolic parameters like glycemic index and lipid profile, which reduces the mortality rate. The ideal TC level is <200mg/dL, as 200-239mg/dL is borderline, and 240mg/dL indicates higher risk.

Table 8: Effect of *Berberis lycium* capsule on total cholesterol of diabetic patients

	0 Day	45 Day	90 Day	Total Mean
T0	244.31 ^a ±0.03	225.13 ^b ±0.01	201.27 ^c ±0.03	225.13 ^a
T2	228.73 ^b ±0.02	185.13 ^d ±0.02	161.53 ^e ±0.02	185.13 ^b
Total Mean	236.52 ^a	205.13 ^b	181.40 ^c	

T0 = Control Group; T2 = Experimental Group

CONCLUSION

Diabetes mellitus is a chronic metabolic condition that is characterized by inadequate insulin activity and abnormal glucose utilization and may result in complications like nephropathy, retinopathy, and cardiovascular disease. The conventional treatments and nutraceuticals for type 2 diabetes may have unwanted side effects. *Berberis lycium* is known for its potent antioxidant and anti-inflammatory effects, which are attributed to its bioactive compounds, including berberine.

The present study was designed to assess the antidiabetic effect of *Berberis lycium* powder on diabetic patients of Rawalpindi and Islamabad about its physicochemical properties. The study was divided into two parts: proximate analysis of *Berberis lycium* and 90-day clinical intervention trial. Subjects selected were split into control and treatment (T1) groups. The treatment group was given 500 mg *Berberis lycium* powder per day before breakfast, in addition to the usual medication and diet; the control group was given a normal diet and placebo treatment. Food frequency questionnaires and blood tests (FBS, HbA1c, cholesterol) were used to perform anthropometric, biochemical and dietary assessments on days 1, 45, and 90.

The results showed a significant decrease in body weight, BMI, waist circumference, fasting blood glucose, oral glucose tolerance and cholesterol levels when compared to the control group. The results indicate that *Berberis lycium* could be used as a safe, low-cost and effective herbal agent for the treatment of diabetes.

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All authors contributed equally to this research article.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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