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Research Article

Anti-hyperlipidemic Effect of *Berberis lycium* Royle Root Bark Extract in Alloxanized Swiss Albino Mice

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Abstract | Hyperlipidemia is a condition in which blood is overloaded with lipids. This condition is challenging for living beings. There are many ways to solve this problem but herbal medicines take on paramount importance in this regard. The purpose of the current research was to inspect the effects of an aqueous extract of *Berberis lycium* Royle root bark on lipid profiles of Swiss albino mice in which diabetic phenotype was induced by alloxan. A single injection of Alloxan (150 mg/kg) was applied intravenously to induce diabetic phenotype. Alloxan induction raised the overall level of triglycerides, low density- lipids (LDLs), and cholesterol and reduced the level of high density lipids (HDLs). Oral administration of 200 mg/kg aqueous extract of *B. lycium* Royle root bark for 28 days rescued all these changes. This study shows that the root bark extract of *B. lycium* Royle has antihyperlipidemic properties.

Novelty Statement | This study reports that root bark extract of *Berberis lycium* Royle has very strong antihyperlipidemic properties.

Introduction

Hyperlipidemia is a situation in which there is an overload of lipids, mostly cholesterol as well as triglycerides in the blood. There are many causes of hyperlipidemia, one of which is diabetes. Abnormal conditions of insulin production and insulin action are responsible for a metabolic disorder known as diabetes mellitus which results in the disturbance of lipid metabolism. Diabetes mellitus results in dysfunction, long term damage, and failure of numerous organs (Joshi *et al.*, 2015).

Medicinal plants are a potential source of antidiabetic drugs. Many of the synthetic drugs that have been created have been either directly or indirectly derived initially from plant sources (Swaroopa *et al.*, 2017).

Natural products play a vital part in current drug development programs. Herbal preparations have different properties, including production, low costs, fewer side effects and ease of availability that make them more suitable components in existing therapies (Singab *et al.*, 2014).

Berberis lycium Royle, which is native to the Himalayas, region is broadly used in folk medicines

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and as a food source. Plants also have been utilized to isolate a wide variety of nutritionally and medicinally important phytochemical constituents, including lipids, carbohydrates, proteins, alkaloids, tannins, vitamins, cardio-active glycosides, anthocyanins, cellulose, fiber content, phytic acid, β carotene, saponins, and phytate phosphorous. Plants contain numerous minerals, which are very important for the treatment of several disorders and contribute to a wide range of biological processes. These minerals include potassium, lead, sulphur, copper, sodium, phosphorus, zinc, calcium, manganese and iron. Traditionally, the *Berberis lycium* has been used to cure piles, diarrhea, gingivitis, diabetes, backache, intestinal colic, bone fractures, pustules, internal wounds, ophthalmia, fever, sun blindness, throat pain, menorrhagia, jaundice, rheumatism, scabies, and as diaphoretic expectorant and diuretic. *B. lycium* is reported to have anticoccidial, hepatoprotective, antifungal, antibacterial, antimutagenic, wound healing properties, pesticidal, and antidiabetic, supporting its traditional uses (Shabbir *et al.*, 2012). In this research, this plant is used as antihyperlipidemic in alloxan-treated swiss albino mice.

Materials and Methods

Ethical statement

All experimentations were carried out in agreement with the University guidelines and International laws for the maintenance of experimental animals as mentioned in (Ali *et al.*, 2019, 2020a, b, c; Hussain *et al.*, 2020; Ara *et al.*, 2020; Khan *et al.*, 2019; Mumtaz *et al.*, 2019; Mughal *et al.*, 2019; Dar *et al.*, 2019). The Committee of the Faculty of Science, Government College University, Lahore, Pakistan has approved all procedures used in the experiments included in this study.

Collection of medicinal plants

Berberis lycium Royle plants were collected from Chinari, District Jhelum valley, Azad Kashmir, Pakistan. An Ethnobotanist from the Department of Botany, Faculty of Science, University of Azad Jammu and Kashmir Muzaffarabad, Pakistan identified the plants. The dust from the plants was removed by rinsing them with water. These plants were placed in the shade to dry, after that they were ground into a fine powder. Shade dried plant parts were crushed into a fine powder.

Preparation of plant extract

Ten- grams of shade dried plant material was boiled in 100 ml of distilled water. This solution was filtered through filter paper (Whatman No. 1) and the solution was concentrated with a rotary evaporator to obtain the extracts. The plant extract was dried in a vacuum oven (Vacucell, Einrichtungen GmbH) at 40°C for 24 hours.

Chemicals and drugs

Alloxan was purchased from Sigma Aldrich, (St Louis, MO, USA). Glibenclamide was purchased from Sigma-Aldrich (Chemie, Steinheim, Germany).

Doses

BLR-Extract: 200 mg/kg b.w; Glibenclamide: 10 mg/ kg b.w.

Animal selection and grouping

Twenty-four male Swiss albino mice, with average age eight weeks and 36.6±1.5 g body weight were obtained from the GC University, Lahore. Before starting the experimental procedure, all mice were housed together for seven days. Then the experimental animals were randomly assigned to one of four different groups. The first group (four animals) did not receive any treatment and this group was designated as the normal control group. A single intraperitoneal injection of alloxan (one injection contained 150 mg/kg) was given to 20 animals to induce diabetes. Animals that showed glucose levels that were higher than 150 mg/dL were further categorized into three additional groups:

Diabetic control group (6 animals); Diabetic+BLR extract group (6 animals) were diabetic mice that received a daily oral dose of BLR extract of 200 mg/kg for 28 consecutive days; Diabetic + glibenclamide group (6 animals) were diabetic phenotypic mice that received an oral dose of glibenclamide 10 mg/kg g for 28 consecutive days. The diagrammatic scheme of the experimental design is shown in Figure 1.
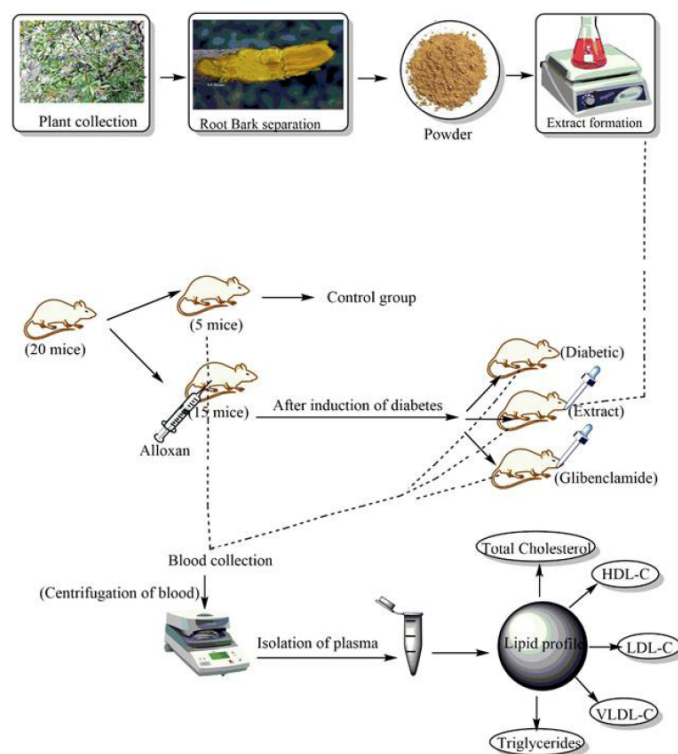

Figure 1: Diagrammatic summary of work done.

Animal management

Four polypropylene cages were used to house mice assigned to the different groups. Mice belonging to one group were housed in the same cage. Four or six mice were housed in each cage. Pure drinking water and Standard laboratory pelleted feed were given to animals *ad libitum*. The experimental room temperature was set at 22±3°C. A 12 h dark/ 12 h light cycle was maintained and 70% was the upper limit for humidity in the room. The oral dose of glibenclamide was administered using a gavage oral feeding tube.

Body weight measurement

The body weight of the mice in all groups was measured using a digital balance. The body weight of the mice was monitored from the beginning until the end of the study at seven-day intervals.

Induction of experimental diabetic phenotype

A single intraperitoneal dose of alloxan was administered to induce diabetic phenotype in the experimental animals, using a dose of 150 mg/kg in 0.9% saline. Freshly prepared alloxan was injected into twenty mice. Subsequently, the blood glucose levels of fasting mice were assessed and the diabetic group consisted of mice that exhibited a blood glucose level higher than 150 mg/dL.

Assessment of glucose levels

The tail vein of the mice was used to collect the blood samples. Fasting blood glucose was estimated with a One Touch Basic Glucometer after a 6- hour fasting period on the 0, 7th, 14th, 21st and 28th days.
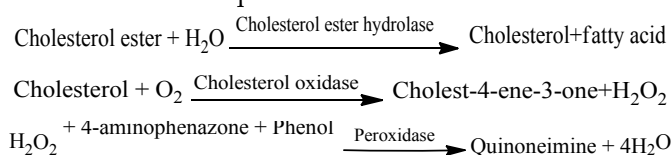
Analysis of lipid profiles
  Extraction of lipids

An approach proposed by Friedewald *et al.* (1972) was employed to perform serum lipids extraction.

  Estimation of total cholesterol

The enzymatic colorimetric method proposed by Allain *et al.* (1974) enzymatic colorimetric method was used to determine cholesterol level.

The reaction sequence was as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol+fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-ene-3-one+}H_2O_2$$

$$H_2O_2 \xrightarrow[\text{+ 4-aminophenazone + Phenol}]{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedures

Samples of mice serum, standards and the reagent blank were preincubated for 5 minutes at 37 °C. Samples (10 μL) or standard (10 μL) and reagent blank (1,000 μL) were pipetted into cuvettes and mixed carefully by inversion. The cuvettes were put into the holder and a
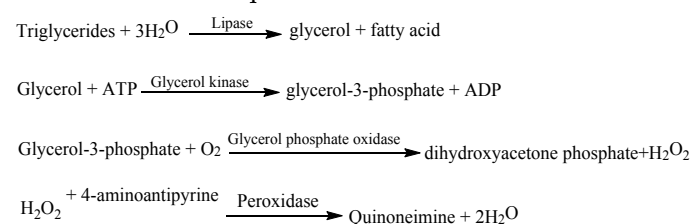
stopwatch was started to assess the time. The absorbance of the sample, standard and the reagent blank was measured at 400 nm wavelength within 60 minutes. As the final point, the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated.

TC concentration (mg/dl)= ΔAsample / ΔA standard × $C_{standard}$

Estimation of triglycerides

The enzymatic colorimetric method published by Hearne and Fraser (1981) enzymatic colorimetric method was used to determine triglyceride (TG) levels.

The reaction sequence was as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol + fatty acid}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{dihydroxyacetone phosphate+}H_2O_2$$

$$H_2O_2 \xrightarrow[\text{+ 4-aminoantipyrine}]{\text{Peroxidase}} \text{Quinoneimine} + 2H_2O$$

To examine serum of the mice, the samples and the standard reagents are prepared for use on an automated analyzer. The enzymatic test was carried out at a wavelength (540 nm), a 1 cm optical path, at 37°C and the measurements were compared against a reagent blank.

Procedure

The samples, standards and blank were preincubated for 5 minutes at 37 °C. The reagent blank (1,000 μL) and samples (10 μL) or standards (10 μL) were placed into cuvettes and mixed gently by inversion. The cuvettes were inserted into the cell holder and a stopwatch was used to assess the time. The absorbance of the samples, standards and blank was measured at 540 nm. Finally, the absorbance of the sample (ΔA sample) and the standards.
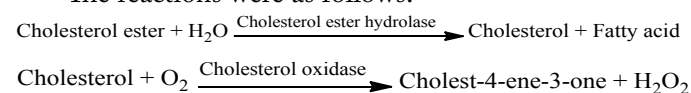
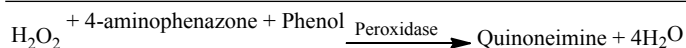(ΔA standard) against the reagent blank were calculated.

TG level concentration (mg/dl) = ΔA sample/ ΔA standard×$C_{standard}$

Estimation of high density lipoprotein (HDL) cholesterol

The VLDL and LDL from serum were precipitated by phosphotungstate in the presence of magnesium ions. After elimination by centrifugation, the clear supernatant comprising the high density lipoproteins (HDL)-fraction and the cholesterol content was confirmed enzymatically according to methods mentioned in Seigler and Wu (1981).

The reactions were as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol + Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-ene-3-one + }H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

For this research, mouse serum was sampled and standard reagents were used on an automated analyzer. The enzymatic assay was carried out at 593 nm wavelength, 1 cm optical path, 37 ˚C temperature and measurements done against the reagent blank.

Procedure for precipitation

100 µL of reagent and 10µL of the samples were pipetted into a centrifuge tube, mixed well, allowed to stand for 5 minutes at 37˚C and centrifuged at 3000 rpm for 20 minutes. The supernatant (sample) was collected for HDL test.

Procedure for determination of HDL

The reagent blank, samples and calibrator were preincubated for 5 minutes at 37˚C. The reagent blank (10 µL distilled water and 750 µL enzymes) and samples (10 µL samples and 750 µL enzymes) or calibrator (10 µL calibrator and 750 µL enzymes) were pipetted into cuvettes and mixed gently by inversion. The cuvettes were inserted into the cell holder and a stopwatch was used to assess the time. The absorbance of the samples, standards and the reagent blank was measured at 593 nm after 5 minutes. Finally, the absorbance of the samples (ΔA sample) and the calibrator (ΔA standard) against the reagent blank were calculated.

HDL concentration (mg̸dl)= ΔAsample / ΔAcalibratorx Ccalibrator

Estimation of low density lipoprotein (LDL) cholesterol

Indirect method TC, TG and HDL are measured and LDL cholesterol is calculated from the primary measurements by use of the empirical Freidewald Formula equation:

Concentration of LDL-c (mg/dl) = [Total cholesterol] - [Triglycerides/5]- [HDL]

OR

LDL cholesterol = Total cholesterol - (HDL cholesterol + VLDL cholesterol)

Estimation of very low density lipoprotein (VLDL) cholesterol

VLDL = Triglycerides/5

Statistical data analysis

Data were presented as mean±standard error of the mean. Shapiro-Wilk test was used to check the distribution of the data. Parametric tests were used on normally distributed data and then examined statistically via one-way ANOVA, followed by "Dunnett's Multiple Comparison Test", to find any substantial variances among the group means. GraphPad Prism version 5.0 for Windows (Graph Pad Software, San Diego, CA, USA) was used for analyses. Values of $P \leq 0.05$ were considered as significant.

Results and Discussion

Effect on body weight

Alloxan caused a gradual decrease in body weight. When the alloxan-treated mice were administered the extract and glibenclamide, the alloxan-induced changes in body weight were rescued (Table 1).

Effect on glucose level

Alloxan caused a gradual increase in blood glucose levels. When the alloxan-treated mice were administered the extract and glibenclamide, the alloxan-induced phenotypes in blood glucose were rescued (Table 2).

Effect on lipid profile
Total cholesterol

In this study, intraperitoneal administration of a single dose of alloxan (150 mg /kg body weight) caused a highly significant increase in the overall cholesterol level of the alloxan-treated group (185.6±5) as compared to control (91.76±3). When extract and glibenclamide were given to alloxan-treated mice they caused a significant decrease in total cholesterol level (alloxan-treated+extract: 116.14±2; alloxan-treated+ glibenclamide: 99.43±4) as compared to the alloxan-treated group (185.6±5) (Table 3).

Triglycerides

Intraperitoneal administration of a single dose of alloxan (150mg /kg body weight) caused a highly significant increase in the triglycerides level of the alloxan-treated groups (174.62±6) as compared to control (90.16±3). When extract and glibenclamide were given to the alloxan-treated mice they caused a significant decrease in the triglycerides level (alloxan-treated +extract: 130.98±3; alloxan-treated+ glibenclamide: 111.68±8) as compared to the alloxan-treated group (174.62±6) (Table 3).

HDL-C

Intraperitoneal administration of a single dose of alloxan (150mg /kg body weight) caused a highly significant decrease in the HDL-C levels in the alloxan-treated group (23.38±3) as compared to controls (45.96±3). When the extract and glibenclamide were given to alloxan-treated mice they caused no significant change in HDL-C level (alloxan-treated + extract: 42.54±0.8; alloxan-treated+ glibenclamide: 41.16±1.8) as compared to the alloxan-treated group (45.96±3) (Table 3).

LDL-C

Intraperitoneal administration of a single dose of alloxan (150mg /kg body weight) caused a highly significant increase in the LDL-C levels in alloxan-treated (127.296±6) mice as compared to controls (27.708±4).

When the extract and glibenclamide were given to alloxan-treated mice they caused a significant decrease in the LDL-C level (alloxan-treated + extract: 62.964±5; alloxan-treated + glibenclamide: 51.014±4) as compared to the alloxan-treated group (127.296±6) (Table 3).

VLDL-C

Intraperitoneal administration of a single dose of alloxan (150 mg/kg body weight) caused a highly significant increase in the VLDL-C level of the alloxan-treated group (34.924±1.1) as compared to control (18.032±0.5). When the extract and glibenclamide were given to the alloxan-treated mice they caused a significant decrease in VLDL-C level (alloxan-treated +extract: 26.196±0.6; alloxan-treated + glibenclamide: 22.336±1.6) as compared to the alloxan-treated group (34.924±1.1) (Table 3).

The present study showed that an aqueous extract of *B. lyceum* Royle root bark rescued the changes made by alloxan in Swiss albino mice. The present study revealed that *B. lycium* Royle root bark extract has an antihyperlipidemic effect in alloxan-treated Swiss albino mice.

The current results revealed a highly significant increase in plasma glucose, cholesterol, LDL and triglycerides in the alloxan-treated mice while a reduction in body weight and HDL level was seen. The same results were seen in Wistar rats, in which diabetes was induced by alloxan-induction (Luka and Tijjani, 2013; Bako *et al.*, 2014). The level of TG, cholesterol and LDL in the plasma was decreased after treatment with the plant extract, while the level of HDL was increased. Similar results were reported in alloxan-induced diabetic rabbits (Wojtowicz *et al.*, 2007). These results are in agreement with the current research. *B. lycium* Royel root bark powder significantly lessened the total LDL, triglycerides and cholesterol of treated mice as compared to alloxan-treated ones.

Table 1: The measurement of body weight.

Group	Treatment	Body weight (Mean ± SEM g)					
		Before induction of diabetes	Day 0 after treatment	Day 7 after treatment	Day 14 after treatment	Day 21 after treatment	Day 28 after treatment
I	Control	38.6±0.43	38.6± 0.43	39.2± 0.62	39.3± 0.22	39.6± 0.37	39.8± 0.73
II	Diabetic	38.2±0.31	36± 0.32a	34± 0.47aa	32± 0.7aaa	28.7± 0.17 aaa	25.7±0.37aaa
III	Diabetic+ Extract	38.4±0.34	36.4± 0.34	34.9± 0.39	35.7± 0.17bbb	36.8± 0.34bbb	37± 0.13bbb
IV	Diabetic+ glibenclamide	38.8±0.17	37.8± 0.17	35± 0.27	36.4± 0.43ccc	38.2± 0.6ccc	38.6± 0.43ccc

Keys: (a) specifies the significant difference among control and diabetic group; (b) indicates the significance difference between diabetic group and Diabetic + extract treated group and (c) presents the significance difference between diabetic group and Diabetic + glibenclamide treated group. Each value in table signifies the mean value of six replicates and SEM. Numerical icon: aaa, bbb, ccc= p ≤ 0.001.

Table 2: Effect on blood glucose level.

Group	Treatment	Blood glucose level (Mean ± SEM mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Control	117 ±4.04	116.25±3.52	121.75±4	123±3.09	120.5±2.19
II	Diabetic	322.5±26.44aaa	327.5±25.52aaa	355±39.42aaa	342.75±37.19 aaa	333.75±33.5 aaa
III	Diabetic+Extract	297.25±3.9	256.5±9.6	186.75±2.4bb	154.5±3.96 bbb	128±2.7bbb
IV	Diabetic+glibenclamide	292±15.32	252.25±16	174.75±3.5cc	129.25±3.66 ccc	115.25±6.16 ccc

Keys: (a) specifies the significant difference among control and diabetic group; (b) indicates the significance difference between diabetic group and Diabetic + extract treated group and (c) presents the significance difference between diabetic group and Diabetic + glibenclamide treated group. Each value in table signifies the mean value of six replicates and SEM. Numerical icons: bb, cc = p ≤ 0.01, aaa, bbb, ccc= p ≤ 0.001.

Table 3: Effect on lipid profile.

Group	Treatment	Lipids levels (Mean ± SEM mg/dl)				
		Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol	VLDL cholesterol
I	Control	91.76±3	90.16±3	45.96±3	27.708±4	18.032±0.5
II	Diabetic	185.6±5aaa	174.62±6aaa	23.38±3aaa	127.296±6 aaa	34.924±1.1aaa
III	Diabetic + Extract	116.14±2bbb	130.98±3bbb	26.98±5bbb	62.964±5bbb	26.196±0.6bbb
V	Diabetic + glibenclamide	99.43±4ccc	111.68±8ccc	26.08±3ccc	51.014±4ccc	22.336±1.6ccc

Keys: (a) specifies the significant difference among control and diabetic group; (b) indicates the significance difference between diabetic group and Diabetic + extract treated group and (c) presents the significance difference between diabetic group and Diabetic + glibenclamide treated group. Each value in table signifies the mean value of six replicates and SEM. Numerical icon: aaa, bbb, ccc= p ≤ 0.001.

Oral administration of 200 mg/kg and 600mg/kg extract fruit of Berberis lycium for six weeks resulted in a significant reduction in total cholesterol, triglyceride, and LDLs levels. Berberis lycium treatment increased the levels of HDLs. Oral administration of 200 mg/kg and 600mg/kg extract fruit of Berberis lycium for six weeks resulted in a significant reduction in total cholesterol, triglyceride, and LDLs levels. Berberis lycium treatment increased the levels of HDLs.

Oral administration of *Berberis lycium* fruit extract (200 mg/kg and 600mg/kg) for six weeks caused a significant elevation in the levels of HDLs while, a significant decline in LDLs, triglyceride and total cholesterol were found in the serum of diabetic phenotypic mice (Furman *et al.*, 2020). Belwal *et al.* (2020) also have reported that the ethanolic as well as the aqueous extract *of Berberis aristata* showed better antidiabetic activity through eliciting decreased total cholesterol and elevated HDL-C levels, in diabetic and hyperglycemic patients leaf extracts of B. *lycium* lessened the lipid profile (Hussain *et al.*, 2017). Many plant extracts lower the serum lipids especially total triglycerides, cholesterol, and level of low density lipoprotein, which are the therapeutic properties to combat atherosclerosis which is one of the main problems of diabetes (Luka and Tijjani, 2013).

Different atheromatous problems *e.g.*, ischemic heart diseases are persistently present in diabetic patients (Assmann *et al.*, 1997). In diabetic patients, the atheromatous disease is due to the decrease in the level of high-density protein (Rang and Urban, 1995). It was discovered that treatment with *B. lycium* Royle root extract caused a decrease in the levels of LDL and an increase in level of HDL that can save diabetic patients from athermanous disease.

Berberis lycium Royle possesses berberine, berbamine, chinabine, karakoramine, palmatine balauchistanamine, gilgitine, jhelumine, punjabine, sindamine, chinabine acetic acid, maleic acid, ascorbic acid (Khare, 2004). Its rook bark contains active components such as isoquinoline alkaloid that is responsible for its antihyperlipidemic effects in alloxan induced diabetic phenotypic mice (Saeed, 1976; Birdsall and Kelly, 1997; Chand *et al.*, 2007). Critical valuation exposed that pharmacological studies are deficient in the documentation of active ingredients responsible for pharmacological actions. Therefore, a further investigation aimed towards the identification and isolation of active constituents in future studies is suggested.

Conclusions and Recommendations

Current research proved that *B. lycium* Royle root bark extract contained antihyperlipidemic potential in alloxan-treated mice. It significantly increased the level of HDL while decreased the raised level of total LDL, triglycerides, cholesterol, in alloxan-induced diabetic mice.

Acknowledgment

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