

***Berberis lycium* Royle. extracts attenuate inflammation and modulates hyperglycemia in alloxan induced diabetic rats**

Maryam Zaib¹, Ali Sharif^{2*}, Bushra Akhtar³, Humaira Majeed Khan²,
Muhammad Furqan Akhtar⁴, Waseem Hassan⁵, Faisal Razzaq¹,
Sajid Nawaz¹ and Naeem Qaisar⁶

¹Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan

²Institute of Pharmacy, Faculty of Pharmaceutical and Allied Health Sciences, Lahore College for Women University, Lahore

³Department of Pharmacy, University of Agriculture, Faisalabad, Pakistan

⁴Ripah Institute of Pharmaceutical Sciences, Ripah International University, Lahore, Pakistan

⁵Department of Pharmacy, COMSATS University, Islamabad, Lahore Campus, Lahore, Pakistan

⁶College of Pharmacy, University of Sargodha, Sargodha, Pakistan

Abstract: *Berberis lycium* Royle (Berberidaceae) is traditionally used for the treatment of diabetes mellitus. Present study was conducted to determine the antioxidant, antidiabetic and anti-inflammatory effects of aqueous and methanolic whole plant extracts. Total phenolic contents were determined by Folin-ciocalteu method whereas antioxidant activity was determined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) method. *In vitro* anti-diabetic activity was determined using alpha amylase assay. Acute hypoglycemic activity was investigated on normoglycemic rats. Sub-acute anti-diabetic effects were investigated in alloxan induced diabetic rats for 14 days. Methanolic extract exhibited 183.5±1 mg/g Gallic acid equivalent (GAE) phenolic contents. The methanolic extract exhibited an IC₅₀ of 242µg/mL and 37.26 mg/mL in antioxidant and alpha amylase inhibitory assays respectively. Administration of methanolic extract in normoglycemic rats exhibited significant anti-hyperglycemic effect at 90 and 120 min. Methanolic extract (500 mg/kg extract) significantly reduced blood glucose at day 14. Methanolic extract (500 mg/kg) significantly reduced the concentration of tumor necrosis factor (TNF-α) and interleukin (IL-6) along with reduction in total cholesterol and triglyceride levels in diabetic rats. Administration of methanol extract also improved the hepatic markers. The study suggested that the methanolic extract possessed antidiabetic effect that might be attributed to its alpha amylase, antioxidant and anti-inflammatory potential.

Keywords: Diabetes, antioxidant, *Berberis lycium*, alpha amylase, anti-diabetic.

INTRODUCTION

Diabetes mellitus (DM) is a major metabolic disorder that affects the human at all stages of life. It is related to insufficient production of insulin or decreased action of insulin that leads to hyperglycemia. It was estimated by World Health Organization (WHO) that more than 180 million people worldwide suffer from diabetes. It was attributed to 1.1 million deaths in 2005 (World Health Organization, 2019).

Diabetes has strong co-relation with the induction of oxidative stress resulting in free radicals and decreased antioxidant status (Donath *et al.*, 2019). Since the time of evolution, plants are considered as a source of health and play a vital role in the health care system (Kiran *et al.*, 2018). Traditional plants and herbs are used for treating various diseases all around the world. The structural activity relationship (SAR) of the compounds present in different plant parts helps in improving the efficacy, reducing the side effects and toxicity of the drugs (Sharif *et al.*, 2017).

Berberis lycium Royle. a plant known as barberry is

considered an important source of food as well as medicines (Mahmood *et al.*, 2011). *Berberis lycium* Royle. is a member of Berberidaceae family. The genus *Berberis* consists of approximately 500 species of which 77 species are found in India. Most species of the genus are from central and southern Europe and northern regions of Pakistan (Qaseem *et al.*, 2019).

Several alkaloids are present in the roots of the plant. The most important one is berberine, which is used in preparing drugs for various diseases like diarrhea, eye troubles, dysentery and cholera (Khan *et al.*, 2016). *Berberis lycium* Royle. possesses anti-diabetic and antioxidant activity and reported for treating diabetes in traditional medicinal system. Pharmacological studies on the plant revealed that it reduces the glucose level as well as have insulin like action (Shabbir *et al.*, 2012). Since diabetes is an inflammatory condition we evaluated whether alloxan induced diabetes can be prevented by inhibiting action of *Berberis lycium* Royle. methanolic extract on the proinflammatory cytokines along with quantification of polyphenols using high pressure liquid chromatography (HPLC) and investigating the effect of methanolic extract of *Berberis lycium* Royle. on different biochemical markers.

*Corresponding author: e-mail: alisharif.pharmacist@gmail.com

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate 1,1- diphenyl-2-picryl-hydrazil (DPPH) was purchased from Sigma Aldrich chemical co. Glibenclamide (standard drug) gift sample from Servier pharmaceuticals. Rat ELISA kits (K1052) and K4145 were purchased by the manufacturer Bio vision, Inc, California

Plant material

Fresh samples of *Berberis lycium* Royle. were collected during the months of July and August (2016) from areas of Kashmir. Sample was identified by Dr. Zaheer ud din, Government College University, Lahore, Pakistan and the specimen has been preserved in herbarium (voucher no. 3364) for record.

Preparation of plant extracts

The stem part of the plant was air dried in the shade, broken into pieces, crushed into fine powder and sifted through a wire screen (mesh size 2 mm x 2 mm). Extracts were prepared using sequential extraction with n-hexane, n-butanol, methanol and water. All the extracts were dried using rotary evaporator. The percentage (%) yield of both the extracts were calculated using following formula (Abbas *et al.*, 2018).

$$\text{Percentage yield (\%)} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Phytochemical analysis

Qualitative phytochemical analysis of the crude extract of *Berberis lycium* Royle. stem part revealed the presence of different phytoconstituents. Alkaloids were confirmed by Mayer's, Wagner's and Hager's test. Glycosides were determined using Bontrager's test. Flavonoids were determined using chloride and gelatin test. Phenols were determined using lead acetate and alkaline reagent tests. Foam test was used to confirm the presence of saponins. (Kumar., 2015)

Total phenolic (TP) contents

Spectrophotometric determination of the TP contents was performed by using Folin-Ciocalteu method (Abbas *et al.*, 2018). Gallic acid was used as standard. The absorbance was measured at 760 nm using UV-spectrophotometer. The concentration of the total phenols was measured in the form of Gallic acid equivalent (mg of GA/g of extract). (Zafar *et al.*, 2020).

Antioxidant assay

The free radical scavenging potential of the extracts of *Berberis lycium* Royle. along with standard ascorbic acid was measured by determining their capacity to scavenge free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Bulut *et al.*, 2019). Absorbance was measured at 517 nm and antioxidant potential was expressed as inhibitory

concentration IC₅₀ (Bonesi *et al.*, 2013). Percentage (%) inhibition was calculated by using formula. (Adjimani and Asare, 2015).

$$\text{Radical scavenging activity \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

In vitro antidiabetic assay

Alpha-amylase inhibitory activity of the plant extracts was measured using different concentrations of methanolic and aqueous extracts of plant. Acarbose was used as standard. Absorbance of standard and extracts was measured at 540 nm (Fatima *et al.*, 2019). Percentage (%) inhibition was calculated using following formula.

$$\% \text{ inhibition} = \frac{\text{Abs540 (control)} - \text{Abs549 (extract)}}{\text{Abs540 (control)}} \times 100$$

Experimental animals

Wistar rats of either sex (180-200g) were housed in stainless steel cages under normal conditions of temperature 22±2°C, provided with commercial rat chow and free access to water ad libitum. All the protocols were performed according to institutional research and ethical recommendations of Faculty of Pharmacy, The University of Lahore No. IREC-2016-33A.

Oral Glucose Tolerance Test (OGTT) in normal rats

Experiment was performed in normal rats at three dose levels of both methanolic and aqueous extracts (125, 250 and 500 mg/kg) during 120 min time interval. Rats were divided into five groups (n=6). Group I control group, received distilled water. Group II received glibenclamide (10mg/kg), group III, IV and V received (125, 250 and 500 mg/kg) of plant extracts. Experiment was performed on fasting rats. Oral glucose (2 g/kg) was administered after 30 min of treatment with *Berberis lycium* Royle methanolic and aqueous extracts alongwith standard (glibenclamide). Blood samples were collected during 120 min dosing interval and blood glucose was measured by using glucometer (Derosa *et al.*, 2019).

Induction of diabetes by alloxan

Single intravenous injection (dissolved in 0.9% NaCl) containing 28,500mg of alloxan monohydrate (150mg/kg) was injected intravenously to induce diabetes in overnight fasted rats. Animals were monitored and serum glucose range of 200-300mg/dL was considered as diabetic.

Anti-hyperglycemic effect in diabetic rats

Experiment was performed in diabetic rats at three dose levels (125, 250 and 500mg/kg) of methanolic and aqueous extracts for 14 days. Rats were divided into six groups (n=6). Group I control group, received distilled water. Group II received glibenclamide (10 mg/kg) while group III served as disease control. Group IV, V and VI received (125, 250 and 500 mg/kg) of both methanolic

Table 1: %age inhibition of DPPH analysis at different concentration of methanol and aqueous extracts of *Berberis lycium* and their IC₅₀ values

S. no	Concentration $\mu\text{g/ml}$	%age Inhibition (methanol)	IC ₅₀ $\mu\text{g/ml}$	%age Inhibition (aqueous)	IC ₅₀ $\mu\text{g/ml}$
1	1000	76.9	242	32.6	1533
2	500	76.0		23.8	
3	250	74.8		18.7	
4	125	74.1		14.8	
5	62.5	72.9		9.9	

Table 2: Effect of *Berberis lycium* Royle. methanolic extract on lipid and liver profile in diabetic rats

S. no	Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	ALP (mg/dl)	ALT (mg/dl)	AST (mg/dl)	TB (mg/dl)	TP (mg/dl)
1	Diabetic control	120 \pm 5.1	130 \pm 4.3	38 \pm 1.4	178 \pm 4.1	49 \pm 2.8	42 \pm 2.1	1.4 \pm 0.8	0.39 \pm 0.4
2	Glibenclamide treated	89 \pm 2.8***	96 \pm 4.8***	41** \pm 1.8	102 \pm 4***	26 \pm 3.2***	23 \pm 2.8***	0.82 \pm 0.4	1.6*** \pm 1.2
3	Plant extract (125mg/kg)	118 \pm 4.2	126 \pm 3.0	37 \pm 2.1	172 \pm 4.6	45 \pm 2.9	41 \pm 3.4	1.36 \pm 0.5	0.52 \pm 1.4
4	Plant extract (250mg/kg)	110 \pm 3.7	120 \pm 5.6	47*** \pm 2.3	145 \pm 3.6***	37 \pm 1.9***	31 \pm 2.2***	1.3 \pm 1.0	1.05*** \pm 0.9
5	Plant extract (500mg/kg)	106.6 \pm 7** *	86.2 \pm 7.4** *	50.6*** \pm 4.9	114 \pm 3.4***	29 \pm 2***	25 \pm 2.6***	0.9 \pm 0.7	1.49*** \pm 1.1

Each value is expressed by taking mean \pm S.D and analyzed by one-way ANOVA.

*** indicates $p < 0.001$ compared to diabetic control

Table 3: Quantification of polyphenols in methanolic extract of *Berberis lycium* Royle

S. No.	Compound	Retention Time (min)	Amount (ppm)
1	Quercetin	2.91	1.25
2	Gallic Acid	3.72	13.57
3	Caffeic Acid	12.94	4.94
4	Ferulic Acid	22.34	3.05
5	p-coumeric acid	19.21	4.44

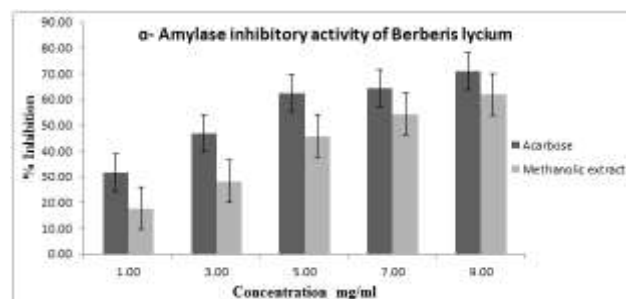
and aqueous plant extracts. Experiment was performed on fasting rats. Blood samples were collected at day 0, 3, 6, 9 and 14. Blood glucose was measured by using glucometer (Derosa *et al.*, 2019).

Proinflammatory Mediators

All the animals were slaughtered at the end of experiment (14 days). Blood samples were collected. Levels of proinflammatory cytokines including TNF- α and IL-6 were measured using Rat ELISA kits (K1052) and K4145 according to the standard protocols provided by the manufacturer Bio vision, Inc, California (Gundala *et al.*, 2018).

Biochemical markers

Blood samples were collected at the end of 14 days and serum was separated and stored at -20°C. Sample was then submitted to UOL Diagnostic and Research laboratory for the analysis of different physiological parameters like high density lipoproteins (HDL), triglycerides (TG), total cholesterol (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) by using (Zafar *et al.*, 2020).

**Fig. 1:** *In vitro* alpha amylase inhibitory activity at different concentrations of methanolic extract of *Berberis lycium* Royle. and Acarbose.

Quantification of polyphenols

Methanolic extract of *Berberis lycium* Royle. was subjected to analyze through HPLC, Shimadzu LC-20AT system, Shimadzu Japan for the quantification of phenolic compounds. The column used for HPLC analysis was C18 (15x0.46 cm, particle size 5 μm). Solution of methanol and 0.2% phosphoric acid solution in distilled water (65: 35) was used as mobile phase. The injection volume was 20 μL and the flow rate was 1ml/ min. Compounds were eluted on the basis of isocratic elution

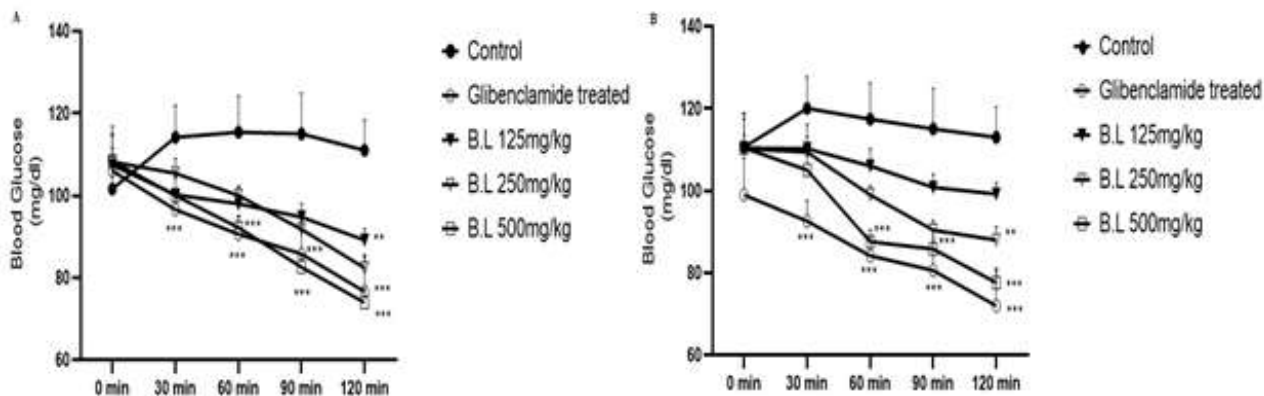


Fig. 2: Effect of Glibenclamide and different concentrations of (A) methanolic and (B) aqueous extracts of *Berberis lycium* Royle. in normal rats.

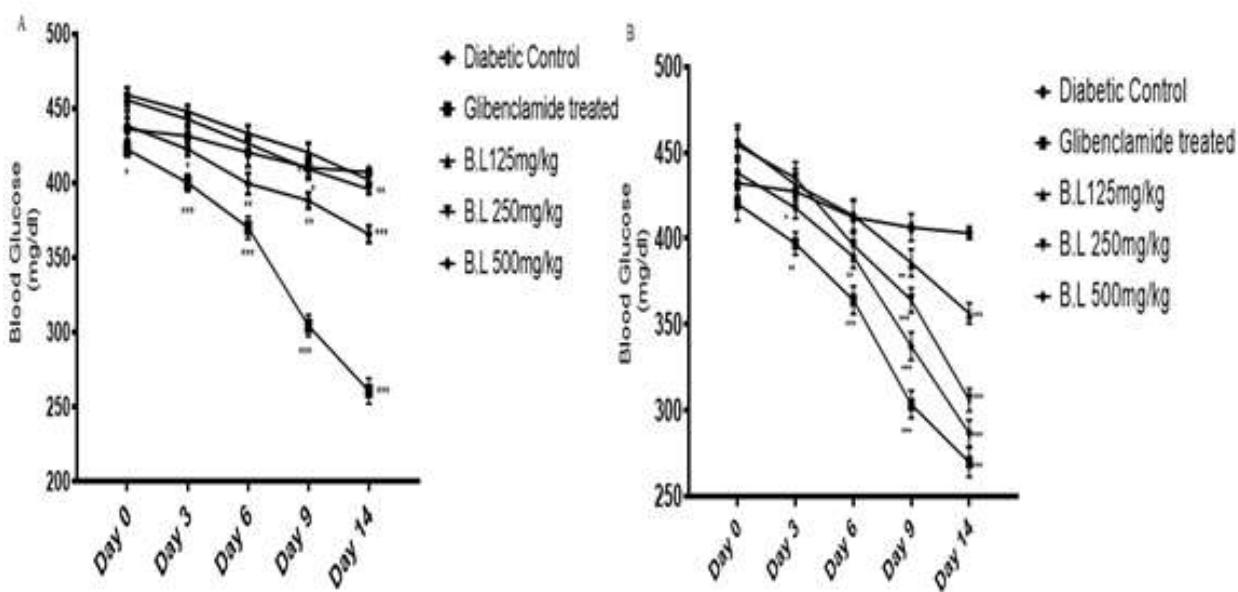


Fig. 3: Effect of Glibenclamide and different concentrations of (A) methanolic and (B) aqueous extracts of *Berberis lycium* Royle. in diabetic rats

Each value is expressed by taking mean \pm S.D and analyzed by one-way ANOVA.

*** indicates $p < 0.001$; ** indicates $p < 0.01$; * indicates $p < 0.05$ compared to diabetic control

of mobile phase. Temperature of the column was maintained at 30°C (Belwal *et al.*, 2016, Khan *et al.*, 2020).

STATISTICAL ANALYSIS

Results were represented as mean \pm SD. Linear regression curve was used to determine phenolic contents. Inhibitory concentration (IC_{50}) was calculated using GraphPad prism 5 software. Log concentration was plotted against percentage inhibition (%). All the grouped data was statistically evaluated by one-way analysis of variance (ANOVA). p values of less than 0.05 (*), 0.01 (**), and 0.001 (***) indicated the statistical significance.

RESULTS

Percentage yield of the extracts

Percentage yield of methanolic extract was greater (4.2%) as compared to aqueous extract (2.1%)

Phytochemical analysis

Qualitative phytochemical analysis of crude extract revealed the presence of phenols, flavonoids, alkaloids, glycosides, tannins and saponins.

Total phenolic contents

The methanolic extract of the *Berberis lycium* Royle. showed the maximum concentration of phenolic

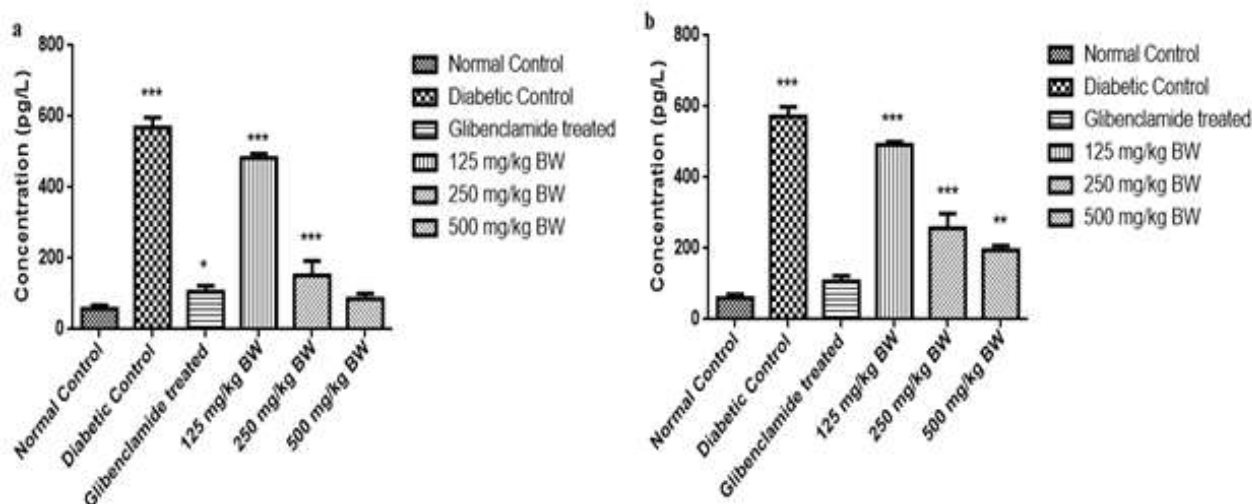
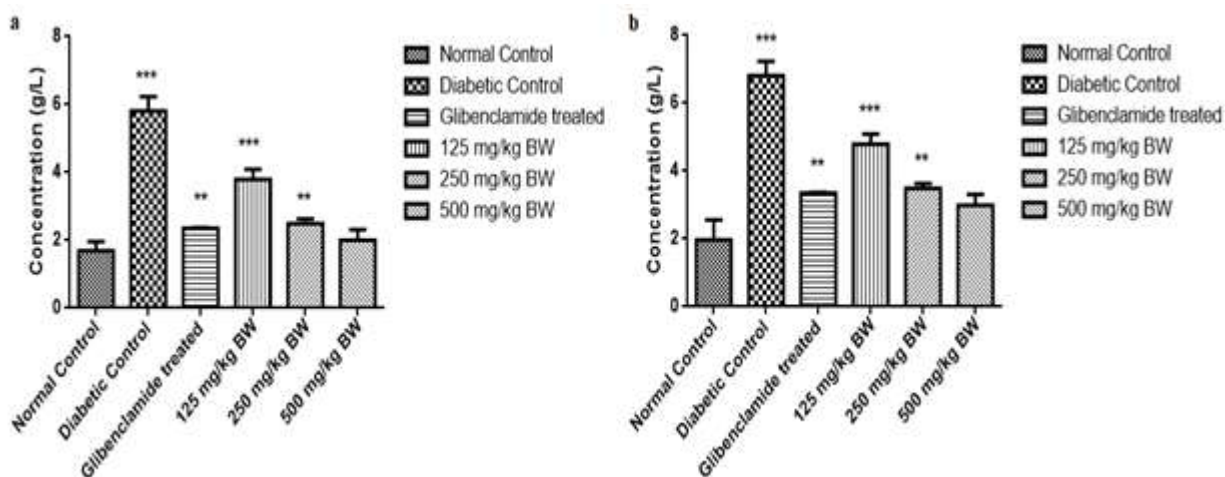


Fig. 4: Effect of *Berberis lycium* Royle. (a) methanolic and (b) aqueous extracts on levels of IL-6 in plasma of rats.



Each value is expressed by taking mean \pm S.D and analyzed by one-way ANOVA.

*** indicates $p < 0.001$

** indicates $p < 0.01$

* indicates $p < 0.05$ compared to Normal control

Fig. 5: Effect of *Berberis lycium* Royle. (a) methanolic and (b) aqueous extracts on levels of TNF- α in plasma of rats.

contents (183.5 ± 1.0 mg/g GAE) followed by crude aqueous extract (95.5 ± 1.0 mg/g GAE extract).

Antioxidant assay

Inhibitory concentration of methanol extract ($242 \mu\text{g/mL}$) was lower than that of aqueous extract ($1533 \mu\text{g/mL}$). The relationship of IC_{50} and antioxidant potency is inverse. Results are presented in table 1.

In vitro antidiabetic assay

In vitro alpha amylase inhibition potential of *Berberis lycium* Royle. methanolic extract showed the maximum inhibitory effect at a concentration of 9 mg/mL. The IC_{50} of methanolic extract was (37.26 mg/mL). Percentage inhibition of alpha amylase is depicted in fig. 1.

Oral Glucose Tolerance Test in normal rats

The effects of methanolic and aqueous extracts (125, 250 and 500 mg/kg) and standard drug glibenclamide were observed at 0, 30, 60, 90 and 120 min. Anti-hyperglycemic effect of glibenclamide group was significant ($p < 0.001$) in comparison to control group. Group receiving (500 mg/kg) of methanolic extract showed significant ($p < 0.001$) effect in lowering blood glucose at 60, 90 and 120 min. Similarly, aqueous extract also exhibited promising glucose lowering effect in normal rats as shown in fig. 2.

Anti-hyperglycemic effect in diabetic rats

Orally administered (500 mg/kg) methanolic and aqueous extracts showed significant hypoglycemic effect ($p < 0.001$) in diabetic rats in comparison to diabetic control at

day 14, whereas the anti-hyperglycemic effect of 250 mg/kg of methanolic extract treated group was significant ($p < 0.01$) in comparison to diabetic control group. A 125 mg/kg of methanolic extract treated rats were insignificant in comparison to diabetic control group as presented in fig. 3.

Inflammatory mediators

A rise in levels of TNF- α and IL-6 was seen in diabetic control group as compared to normal control group. Treatment with 500mg/kg methanolic extract significantly reduced the level of TNF- α and IL-6 showing improvement in inflammatory cytokines. Dose dependent improvement was observed among all the treatment groups. The effect of *Berberis lycium* Royle. methanolic and aqueous extracts are represented in fig. 4 and 5.

Biochemical markers

Both glibenclamide and methanolic extract (500 mg/kg) were significantly different ($p < 0.001$) in reducing the total cholesterol and triglyceride levels as compared to control. Level of HDL was improved after treatment with the plant extract. Methanolic extract (125mg/kg) treated rats showed insignificant increase in HDL level. Results are depicted in table 2. Administration of glibenclamide and crude methanolic extract of *Berberis lycium* Royle. (250 and 500 mg/kg) caused significant reduction in AST, ALP and ALT in diabetic rats whereas total protein level was raised.

Quantitation of polyphenols

The chromatographic profile of methanolic extract indicated the presence of quercetin (1.255 ppm), gallic acid (13.57 ppm), caffeic acid (4.94 ppm), ferulic acid (3.05 ppm) and p-coumaric acid (4.46 ppm). Results are shown in table 3.

DISCUSSION

The methanolic extract of the *Berberis lycium* Royle. showed maximum concentration of phenolic contents (183.5mg \pm 1.0mg/g) followed by crude aqueous extract (95.5 \pm 1.0mg/g). Extraction of plant by methanol gives higher phenolic contents, as methanol is suggested efficient solvent for the extraction of phenolic contents. Ability of methanol to inactivate polyphenol oxidase results in oxidation of phenolics and evaporate easily as compared to water, that makes it a suitable solvent for extracting large number of phenolic compounds (Banerjee and Bonde, 2011). The presence of higher phenolic contents in methanol extract contributes to its higher antioxidant and anti-hyperglycemic potential (Justino *et al.*, 2018).

The free radical scavenging potential of n-hexane, n-butanol, methanolic and aqueous extracts was studied against standard ascorbic acid by their capacity to reduce DPPH (Pal *et al.*, 2018). Both methanolic and aqueous

extracts demonstrated reducing power that increased in linear manner with concentration. Smaller value of IC₅₀ can be linked with better antioxidant potential (Saani *et al.*, 2018). Methanolic extract is found to be more active than aqueous. This might be attributed to the higher proton donating ability of methanolic extract resulting in formation of stable DPPH molecules and higher phenolic contents (Banerjee and Bonde, 2011).

In vitro alpha amylase inhibitory activity indicated that methanolic extract of *Berberis lycium* Royle. is active in inhibiting the activity of alpha amylase. Inhibition of alpha-glucosidases and alpha-amylases might be attributed to berberine. It decreases the glucose transport through the intestinal epithelium and also results in the inhibition of glucose absorption. Berberine causes an increase in the insulin sensitivity and insulin activated glucose uptake by the activation of protein kinases (Li *et al.*, 2020).

Methanolic extract of *Berberis lycium* Royle. showed significant anti-hyperglycemic effect in comparison to diabetic control group which might be associated with the presence of higher phenolic contents and antioxidant potential of the methanolic extract. Insulin-like peripheral glucose consumption was suggested in lowering blood glucose levels by the methanolic extract of *Berberis lycium*. Royle. Previously phytochemical screening of the extracts (methanolic and aqueous) showed the presence of tannins. Tannins might be involved in lowering the blood glucose level owing to its property to stimulate glucose utilization (Ajebli *et al.*, 2019). It can be proposed that insulin effect is potentiated upon treatment with methanolic extract of *Berberis lycium*. Royle. Insulin might have been released from the bound granules or remains of destructed β cells (Teraoku and Lenzen, 2017).

Present study suggested that insulin levels are affected by *Berberis lycium* Royle. methanolic extract and more than one mechanism is involved in lowering the blood glucose levels. *Berberis lycium* Royle. possesses alkaloid (berberine) mainly responsible for having hypoglycemic property. Berberine, the major active principle is a benzyl isoquinoline alkaloid found in the genus *Berberis* (Khan *et al.*, 2019).

Increase in glucose levels are associated with the initiation of the mitogen activated protein kinase (MAPK) and protein kinase C (PKC) pathways. These pathways are linked with the increase stimulation of cytokines leading to the inflammation (Robson *et al.*, 2018). It was also observed that hyperglycemia is associated with accumulation of advanced glycation products (AGP). These AGPs also promote inflammation (Yamagishi and Matsui, 2018). It can be concluded that metabolic disorders linked with the DM can cause: (a) polyol pathway initiation (b) increased cytokine levels (c) AGPs

formation (d) raised PKC levels and (e) increased oxidative stress. Oxidative stress mediated tissue injury can provoke inflammation. Thus inflammation linked to hyperglycemia causes the release of proinflammatory mediators like IL-6 and TNF- α . (Bamagous *et al.*, 2018, Malik *et al.*, 2020). Methanolic extract of *Berberis lycium* Royle. reduced the production of cytokines that was supported by the decreased oxidant and inflammatory response of the extract and might be attributed to the presence of flavonoids and phenolic components in the extracts (Robson *et al.*, 2018).

Lipids contribute majorly in the disorder of diabetes mellitus. Hyper-triglyceridemia and hypercholesterolemia are the most common lipid disorders in diabetes. Alloxan induced rats showed marked increase in serum lipid profiles including TG and TC while decrease in the levels of HDL in comparison with the normal rats. Present study is in similarity with another study exhibiting anti-hyperlipidemic potential of *Berberis lycium* Royle. extract (Madiseh *et al.*, 2014). The mechanism of the hypolipidemic actions of plant extract of *Berberis lycium* Royle. might be mediated by tissue metabolism and improvement in insulin secretion and action because insulin cause decrease in lipid levels in diabetic rats (Yang and Kang, 2018). The mechanism involved in reducing the hyperlipidemic effect is also attributed to inhibition of fatty acid synthesis. The enzyme lipoprotein lipase and hydrolyses triglycerides are activated by the insulin in normal metabolism but the deficiency of insulin causes inactivation of these enzymes resulting in hypertriglyceridemia (Samuel and Shulman, 2016). Improvement in insulin levels and action causes activation of lipoprotein lipase and hydrolyses triglycerides resulting in increased metabolism. The significant reduction of serum lipid levels in diabetic rats after treatment with *Berberis lycium* methanolic extract (500mg/kg) is directly linked with improvements in insulin levels.

Administration of alloxan caused an increase in the levels of AST, ALP, ALT and TB and decreased the TP as compared to diabetic control group that may be attributed to the formation of free radicals and membrane damage. Administration of glibenclamide and plant extract caused significant ($p < 0.001$) reversal in the enzyme serum levels that might be due to inhibition of cellular leakage and membrane stabilizing potential of the treatment resulting in the restoration of the alloxan induced elevation in enzyme levels, regeneration of damaged liver cells and maintaining the stability of cell membrane (Ashraf *et al.*, 2013).

CONCLUSION

It is concluded that *Berberis lycium* Royle. stem methanolic extract possess anti-inflammatory potential

owing to the presence of quercetin, gallic acid, ferulic acid p-coumaric acid. The anti-hyperglycemic activity may be attributed to the anti-inflammatory, antioxidant and alpha amylase inhibition activity. However, activity-based isolation of phytochemicals is necessary to identify active principles in *Berberis lycium* Royle.

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