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Comparative *in vivo* antidiabetic evaluation of leaves and bark of *Berberis lycium* Royle in alloxan induced diabetic rabbits

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Abstract

The aim of this study was to measure minerals in *Berberis lycium* leaves and bark to evaluate the effects of its methanolic extract on diabetic rabbits. For this study 15 female rabbits were used and were divided into five equal groups. For induction of diabetes in animals, Alloxan monohydrate was used. The animals were under treatment for 15 days. For healthy and diabetic control groups distilled water for treatment control group glucophage for the fourth and fifth diabetic groups *Berberis lycium* extract in respectively one gram dose were used daily. Blood samples were collected from veins of ears and glucose level was measured with autoanalyzer glucometer. The results of the study indicated that secondary metabolites level in *Berberis lycium* leaves was considerably high as compared to bark. In diabetic rabbits administered with *Berberis lycium*. The results showed that alkaloids, saponins, sterols, steroids, terpenoids, flavonoids, tannins, were present while anthraquinones and coumarins absent in the extract of *Berberis lycium*. In leaves alkaloids, saponins, sterols, steroids, terpenoids, flavonoids, tannins, were present while anthraquinones and coumarins absent in the extract of *Berberis lycium*. Blood glucose levels measured at day 15 of rabbits treated with 1g extract of leaves (T' group) were (170 ± 0.5773) and rabbits treated with 1g extract of bark (T₁ group) were 299 ± 0.57 rabbits treated with glucophage were (363 ± 2.081) are significantly lower than diabetic group (533.33 ± 0.33) . The present investigation showed that the *Berberis lycium* leaves extract alleviates lipid profile level and might be used efficiently in especially (hyperglycemia) diabetic patients.

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Introduction

Diabetes mellitus is a blend of diverse disorders presenting with episodes of hyperglycemia, as a result of defective insulin action, deficiency in insulin production or both. This type of complications take place due to disturbance in the regulatory systems for storage and metabolic fuel mobilization, including the anabolism and catabolism of proteins, carbohydrates and lipids emanating from insulin action, defective insulin secretion or both. The world health organizations (WHO) has further accepted the significance of conventional medication plus have create the strategy, guideline and standard used for botanical medicine (Votey and Peters, 2004).

Pakistan has diverse ecological zones, a big bank of natural resources and flora of nearly 6000-7000 plant species. In nearly all environmental zones of Pakistan various natural vegetation is present, aside natural vegetation many cultivated species are also present. Use of herbal medicines is rising day by day all over the world. Local people and herbalists know the traditional use of these plants and this knowledge is transferring from generation to generation. People prefer to use traditional herbal medicines due to their low cost and no side effects (Sicree *et al.*, 2006).

Berberis lycium is one of the plant species which belongs to the family Berberidaceae have great medicinal importance. *Berberis lycium* have high nutritional qualities and have great medicinal importance because it contain lots of useful compounds which are helpful for the treatment of different diseases like diabetes (Bhattacharjee *et al.*, 1990). All parts of plant have great medicinal importance specially its leaves, bark, stem, fruits and roots contain high concentration of secondary metabolites which are helpful for the treatment of different diseases like skin diseases, abdominal issues, liver disorders, diabetes mellitus etc. (Ahmed *et al.*, 2009).

Material and methods

Sampling of plant part

The leaves and barks of *Berberis lycium* was obtained in the month of July from the local areas of Kashmir (Nakyal) district Kotli.

The all parts were completely shade dried and grinded. There were extracts prepared by using the methanol as solvent. The extracts which were obtained concentrated, dried and kept in desicators for more use.

Preparations of extracts

The bark and leaves was cut into pieces, completely shade dried. The dried stem and leaves were subjected to size reduction to a coarse powder by using dry grinder. Briefly, 100 grams powder of stem and leaves individually was placed in the upper tube of soxhlet apparatus and 500ml methanol was placed in flask of apparatus. Extract was prepared for 7 days and then it is filtered after that it was placed on the hot plate on 100°C. Then it is transferred to the evaporator where the extract was finally prepared and it is saved at 4°C.

Phytochemical screening

Phytochemical analysis of methanolic extract of *Berberis lycium* was ready to check the chemicals present in it and for this qualitative analysis was done. Phytochemical analysis of tannins, saponins, flavonoids, anthraquinones and alkaloids were done according to the methods of Sofowora (2006).

Alkaloids

To make sure the existence of alkaloids 0.6 to 0.7g of the methanolic plant extract was mixed in 9ml of 1% HCl, heated and filtered. 1.5ml of the remains were treated independently with both reagents (Dragendorff's and Mayer's), later than it was observed either the alkaloids were present or absent in the turbidity or impulsive creation.

Saponins

To check the saponins existence 0.6g of the methanolic plant extract was dissolved in hot water in the test tube. On cooling test tube aqueous extracts were mixed dynamically to froth and height of the froth was calculated to find out the saponin contents in the section. 3g of the crushed plant material was boiled in distilled water in the test tube in boiling water bath and filtered. 15ml of the filtrate was assorted with 7ml of distilled water and was mixed dynamically to the formation of stable constant froth.

The frothing was mixed with 4 drops of olive lubricate and stunned dynamically for the construction of suspension thus its characteristic of saponins.

Anthraquinones

To confirm the presence of anthraquinones there is the test, is carried out in which 1.5g of methanolic *Berberis lycium* extract was heated in 5ml of 1% hydrochloric acid and filtered. Then shake the filtrate with 6ml of benzene and there benzene layer was detached. There 15% of ammonium hydroxide was further added and color at the final stage was observed there are pink or red color appears which indicates the presence of anthraquinones.

Coumarins

To verify the presence of coumarins there are 0.6g of the methanolic extract was taken in the test tube. The surface of test tube was closed with filter paper and there 1% of sodium hydroxide solution is added in the test tube and place the test tube for some minutes in hot water bowl and start boiling it after some time the filter paper removed from the test tube and observe the test tube there are yellow florescence indicates the presence of coumarins.

Sterols and Terpens

For the existence of sterol and terpenoids a joint test is performed in which 0.7g of methanolic plant extract of *Berberis lycium* was shaken with petroleum ether in organize to take away the coloring material. Remains were extracted with 15ml chloroform and chloroform layer was dehydrated over anhydrous sodium sulphate. 6ml of chloroform layer was mixed with 0.26ml of acetic anhydride and then two to three drops of concentrated sulphuric acid was added. Different colors were examined to point out the occurrence of sterol or terpenes. Green color indicates the existence of sterols, whereas pink to purple terpenes and triterpenes.

The presence of steroids is indicates when 0.6g of the methanolic extract of plant was mixed with 2.5ml of acetic anhydride followed by 2ml of sulphuric acid. The change in color from violet to blue or green which indicates the presence of steroids.

Salkowski test was performed to verify the occurrence of terpenoids. 5ml of plants extract were mixed in 2ml of chloroform follow by the suspiciously adding of 4ml intense H_2SO_4 . There are coating of the brown color was produced at the edge so it indicates the presence of terpenoids.

Flavonoids

To check the presence of flavonoids there are 0.6g of plants methanolic extract was mixed with petroleum ether for removing the fatty materials from the surface of extract. While fats are removed then exact mixed with 25ml of 75% of ethanol and filter the material then filtrate use for the further tests; there are 4ml of the filtrate was mixed with 5ml of 1% potassium hydroxide in a test tube and the color of mixture was examined. Yellow colour predicted the existence of flavonoids and it sure the presence of flavonoids. For the second test there are some drops of 1% aluminium mixture were added to the portion of each filtrate, a yellow bloom was examined thus these blooms are indicative of the presence of flavonoids.

Tannins

To check the confirmation of tannins there are mixture is prepared in 30g of methanolic plant extract was mixed with 25ml of water and then filtered the extract. There were few drops of 1% of aqueous iron chloride solution was more added to the mixture. Then observe there were blue or green colors are shows the presence of tannins in the extract. In order to make sure tannins about 0.6g extract of plant was added in 15ml of water in a test tube and then filtered. Some drops of 0.1% ferric chlorides was further added and observe the green or black color which confirm the presence of tannins.

Antidiabetic activity

Experimental animal

Female household rabbits (*Oryctolagus cuniculus*) weighing about 1 to 1.5Kg were used in this study and they were brought in the lab one week earlier to the start of research in order to reduce the stress effect. During the study they were fed with green vegetables, grains and grass (*Cynodon dactylon*).

Induction of diabetes in rabbits

Optimization of dose: Initially different doses of Alloxan were given to make the rabbits diabetic. For this purpose animals were divided into five groups. First group was given a dose of 60mg second was given a dose of 75mg, third was given a dose of 85mg and the fourth was given a dose of 95mg, and fifth was given to 100mg. None of the rabbit from first group become diabetic. Only one rabbit from the third group treated with 85mg dose become diabetic. 70% rabbits died belonging to fifth group, which is treated with 100mg dose of Alloxan. This dose was also proved effective by (Alam *et al.*, 2005). So dose was given in the ratio of 85mg by weight and this dose was proved very effective.

Alloxan induction

Alloxan monohydrate was injected intravenously to the rabbits after 12 hour fasting and the procedure followed for it was as given by Akhtar *et al.*, 1982. Rabbits were held properly but before injecting Alloxan lignocaine were applied on their ear and xylene was also applied on their ear which made their veins more prominent. Required dose of Alloxan was mixed in the measured volume of saline solution and without wasting a single minute it was injected in the marginal ear vein of rabbit with the help of 3cc. syringe. Before induction of Alloxan, 2 grams of glucose was dissolved in 10 cc. of distilled water was given orally to every rabbit in order to avoid the hypoglycemic attack. Dose of Alloxan was selected very carefully depending not only upon the weight of rabbits but also general health condition of rabbits. Required dose was dissolved in saline solution in order to make 5% Alloxan solution. After eighth day of administration of dose their blood glucose level was checked with the help of glucometer.

Treatment of diabetes

Experimental design

Experimental animals Rabbits (*Oryctolagus cuniculus*) were divided into five groups, each group consists of three rabbits.

Group I: This group was considered as normal control group (Nc). There is neither any induction of Alloxan nor plant extract.

Group II: This group was considered as diabetic control (Dc). Alloxan was given to rabbits of this group while they were not treated with plant extract.

Group III: This group is considered as treatment control group. Alloxan was injected to these rabbits and they were treated with Allopathic medicine available in market named Glucophage.

Group IV: This group is considered as treatment group (T). Alloxan was injected to these rabbits and they were treated with oral dose of 1gm of methanolic extract of bark of *Berberis lycium* per day.

Group V: This group is considered as second treatment group (T'). Alloxan was injected to these rabbits and they were treated with oral dose of 1gm of methanolic extract of leaves of *Berberis lycium* per day.

Procedure followed

After making the rabbits diabetic they were given the decided doses of *Berberis lycium* extract and these doses were 1g. Total duration of experimental work was 15 days and during these days different analysis was carried out. Their diabetes was checked after every 3 days. On 7th day and 15th day their blood samples were collected and sent for their serum insulin test from Armed Forces Institute of Pathology (AFIP) Rawalpindi. Their weight was also monitored on every third day also.

Statistical analysis

Statistical analysis was done with two-way ANOVA and "Graph Pad prism 6" software was used. The level of significance was considered to be 0.05.

Results

Phytochemical analysis

The phytochemicals present in plant bark extract were identified with different reagents. The results showed that alkaloids, saponins, sterols, steroids, terpenoids, flavonoids, tannins, were present while anthraquinones and coumarins absent in the extract of *Berberis lycium*. In leaves alkaloids, saponins, sterols, steroids, terpenoids, flavonoids, tannins, were present while anthraquinones and coumarins absent in the extract of *Berberis lycium* as shown in Table 1.

Table 1. Qualitative analysis of methanolic extract of bark and leaves of *Berberis lycium*.

Constituents	Observation		Presence/Absence	
	Bark	Leaves	Bark	Leaves
Alkaloids	Precipitation, turbidity	Precipitation, turbidity	+	+
Saponins	Formation of emulsion	Formation of emulsion	+	+
Anthraquinones	No reaction	No reaction	-	-
Coumarins	No reaction	No reaction	-	-
Sterols	Pink to purple color appear	Pink to purple color appear	+	+
Steroids	Green color appear	Green color appear	+	+
Terpenoids	Radish brown color	Radish brown color	+	+
Flavonoids	Yellow coloration appear	Yellow coloration appear	+	+
Tannins	Intense green and then black color appear	Intense green and then black color appear	+	+

Present =+ Absence = -

Measurement of blood glucose level

The blood glucose level of normal control and treatment groups were monitored at every third day by using Gluco sure Star glucometer for 15 days to estimate the effects of extract of Leaves and bark of *Berberis lycium*. The results showed that blood

glucose levels measured at day 15 of rabbits treated with 1g extract of leaves (T' group) were (170±0.5773) and rabbits treated with 1g extract of bark (T₁ group) were 299±0.57 rabbits treated with Glucophage were (363±2.081) are significantly lower than diabetic group (533.33±0.33) as shown in Table 2.

Table 2. Blood glucose level of rabbits measured after every third day during treatment.

	Nc	Dc	Tc	T	T'
D3	121±0.57	521.3±0.66	510.3±0.33	491±0.57	449±0.57
D6	120.3±0.33	525±0.57	498.6±0.33	445.33±0.33	390.3±0.33
D9	120.3±0.66	529.3±0.66	462.3±1.20	404±0.57	319.3±0.33
D12	120.3±0.33	534.3±0.33	411±2.08	331.6±0.88	239.6±0.88
D15	121±0.57	533.3±0.33	363±2.08	299±0.57	170±0.57

Nc=Normal control, Dc=Diabetic control, Tc=Treatment control, T=Treatment with bark extract, T'=Treatment with leave extract

Measurement of weight of rabbits

The weights of normal control and treatment groups were monitored at every third day by using digital weighing machine for 15 days to calculate the effects of methanolic extract of *Berberis lycium*. The results showed that weights measure at day 15 of rabbits

Treated with 1g leaves extract (T' group) were (1.09± 0.0917) and rabbit treated with 1g bark extract (T₁ group) were (1.116±0.005) and rabbits are treated with Glucophage were (1.113±0.008) significantly lower than diabetic group (1.48±0.005) as shown in Table 3.

Table 3. Weight of rabbits measured after every third day during treatment.

	Nc	Dc	Tc	T	T`
D3	1.33±0.0033	1.42±0.0033	1.47±0.0088	1.42±0.0152	1.43±0.0145
D6	1.34±0.0057	1.44±0.01	1.406±0.0066	1.306±0.0173	1.33±0.0066
D9	1.34±0.0033	1.45±0.012	1.29±0.0176	1.213±0.023	1.26±0.0088
D12	1.346±0.0033	1.45±0.02	1.226±0.0176	1.12±0.0088	1.126±0.0057
D15	1.38±0.0033	1.48±0.0057	1.113±0.0088	1.116±0.0057	1.09±0.091

Measurement of Serum insulin level

Serum insulin level of normal control and treatment groups were monitored at 7th and 15th day of experiment to calculate the effect of methanolic extract of *Berberis lycium*. The results showed that weights measured.

At day 15 of rabbits treated with 1g extract of leaves (T` group) were (5.9±0.06) and rabbits treated with 1g extract of bark (T₁ group) were (3.99±0.05) and rabbits are treated with glucophage were are significantly lower than diabetic group (0.00±0.033) as shown in Ttable 4.

Table 4. Serium insulin Level after treatment.

	NC	DC	TC	T1	T2
D7	10.47±0.133	0.03±0.033	1.43±0.033	3.47±0.088	5.47±0.066
D15	10.8±0.057	0±0	1.63±0.033	3.99±0.059	5.97±0.066

D7=day seven, D15=Day fifteen.

Discussion

Diabetes mellitus is a complex disease involving malfunctioning of pancreas along with disturbed carbohydrates and fat metabolism. It is a global trouble including developed as well as developing countries. Its victims are increasing day by day. Literature showed that it is considered as a killer disease and it affects people of any age group. Diabetic patient is on leap risk of many other diseases like blindness, coronary heart diseases, renal failure, gangrene and many other diseases (Patel *et al.*, 2006).

Hypoglycemic effect of *Berberis lyceum* is very obvious. Its leaves and bark show a remarkable hypoglycemic property (Hussain *et al.*, 2009). Compounds having hypoglycemic effect were checked by qualitative analysis of methanolic extract of *Berberis lycium*. Results showed that Alkaloids are strongly present in it while Saponins, Terpenoid and Flavonoids are moderately present. Steroids, Tannins, Phlobatannins and Cardiac glycosides are weakly present in the extract. Many studies showed that extract of *Berberis lycium* have many bioactive compounds that have hypoglycemic activity (Yibchok-Anun *et al.*, 2006). Glycoalkaloid also known as vaccine is strongly present in the extract and different

studies showed that if it is injected interperitonally to the normal organisms it cause strong hypoglycemic attack (Raman and Lau, 1996).

In the rabbits of control group, minor change in the blood glucose level was observed which is may be due to variation in food. While in group of rabbits treated with 1gm glucophage 50% decrease was observed and in the second treatment group treated with 1gm extract of bark 60% decrease in the blood glucose was observed and third group treated with 1gm extract of leaves 70% decrease in glucose level during the interval of 15 days. Chand *et al.*, (2007) also noticed more than 10% decrease in the blood glucose level after continuous administration of *Berberi lycium* extract for fifteen days. Biyani *et al.* (2003) also reported that 48% decrease in the blood glucose level by oral administration of extract.

Body weight is also affected by *Berberis lycium*. In group 1 which was considered as normal control 4.4% increase in the weight was noticed. While in diabetic control group 1.35% decrease in the weight was observed which is due to disturbed blood glucose level. 9% decrease in the body weight was observed in the group treated with 1gm glucophage and 19%

decrease in weight was noticed in the group treated with 1gm extract of bark and 37% decrease in weight was noticed in group treated with 1gm extract of leaves. All compounds present in the *Berberis lycium* normalize the blood glucose level by reducing.

The obesity. So people can normalize their blood glucose level by reducing their weights (Ramachandran *et al.*, 2012). Many scientists reported that all compounds present in the *Berberis lycium* have hypoglycemic as well as hypolipidemic property it reduces the cholesterol level of serum and liver (Mohammad *et al.*, 2014).

Blood serum insulin was also tested; decrease in the blood glucose and blood insulin is due to destruction of β -cells. Serum insulin was tested on 7th and 15th day of experiment.

There was not observed any prominent change in the serum insulin level of normal control group while insulin level of diabetic control group reached to 0.00 or 0.01 and this level persisted after 15th day.

In 3rd group treated with glucophage 13% improvement was observed between 7th and 15th day and in 4th group treated with 1gm extract of bark 17% improvement in serum Insulin level was observed between 7th and 15th day of experiment and group treated with 1gm extract of leaves 24% improvement in serum insulin level was observed. Many studies showed that blood glucose level is associated with cells and insulin level also depends upon these cells.

In Alloxan diabetic rabbits this level decreased due to destruction of cells. With the use of compound recipe of plants improvement of blood glucose and serum insulin level was observed by the slowly improvement of cells (Wadood *et al.*, 2007).

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