The extracts of *Berberis lycium* and diabetes mellitus in alloxan monohydrate induced diabetic rats.

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**ABSTRACT**

Incidence of type II diabetes is rapidly increasing worldwide. In order to identify complementary or alternative approaches to existing medications, we studied anti-diabetic properties of *Berberis lycium*-a natural health product recommended for diabetes treatment in kashmir. The hypoglycemic activity of the aqueous and methanolic extracts of this plant at two dose levels of 250 and 500mg/kg b.wt in normal as well as in rats with Alloxan monohydrate induced diabetes was studied. The oral administration of aqueous and methanolic extract causes maximum fall of blood glucose level to 58.41% and 50.80% in diabetic rats respectively. The acute oral toxicity studies of the extracts revealed no toxic effects of the extracts. The extracts also lowered the levels of serum cholesterol, serum triglycerides, serum LDL, serum VLDL, serum SGOT, serum SGPT, and Serum ALP in diabetic rats. The histological studies depict that the extracts have a protective effect on the β- cells of pancreas in diabetic rats in a dose dependent manner.

**Key words:** *Berberis lycium*; Hypoglycemic; diabetes; Alloxan monohydrate;

**INTRODUCTION**

Diabetes mellitus often simply referred to as diabetes is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Schoenfelder et al., 2006; Dairy et al., 2009). In addition to hyperglycemia diabetes is associated with the complications of hypercholesterolemia and hypertiglyceridemia. Diabetes is a predominant public health concern that has increased steadily worldwide (Honeycutt et al., 2003, Zimm et al., 2003). Diabetics is quite alarming in most of the developing countries including India. Diabetes currently affects 246 million people worldwide and is expected to affect 380 million by 2025. (International Diabetes Federation, 2007). Many of the currently available anti-diabetic agents have number of adverse effects on the body (Jung et al., 2006). Therefore, managing diabetes without any side effects is still a challenging task for health care providers (Saxena et al., 2004). Hence, the search for more effective and safer hypoglycemic agents with lesser side effects has continued to be an important area of investigation.

Today, medicinal plants are increasingly being used in most parts of the world as: hypolipidemic (Ogbonna et al., 2008; Yadav et al., 2008); contraceptive, abortifacients, or cytotoxic (Ritchie, 2001); antihypertensive (Ojewole, 2005; Ogbonnia et al., 2007; Ojemole et al., 2005; Nworgu et al., 2008). Treatment for skin diseases (Ajose, 2007), wound healing (Biswas & Mukherjee, 2003) and hypoglycemic (Eddouks et al., 2003; Maghrani et al 2003; Musabayane et al., 2006; Ogbonna et al., 2008; Patel et al., 2008; Yadav et al., 2008; Ajao et al., 2009; Farswan et al., 2009; Lee et al., 2009). Hypoglycemic agents have been used in the management of diabetes mellitus.

In the present study, an attempt has been made to investigate clinically the antidiabetic activity *Berberis lycium* roots. *Berberis lycium* belonging to family Berberidaceae is a semi-deciduous shrub, 2 to 4 meter high, leaves lanceolate or narrowly obovate-oblong, entire or with few large spinous teeth, arranged alternately on stem. Inflorescence a racemes, flowers yellow born in axillary clusters longer then the leaves. Fruit, berries black. The roots are aperent, carminative, febrifuge and ophthalmic (Chopra et al., 1986). They are used in the treatment of eye complaints, menorrhagia, chronic diarrhoea and piles. The leaves have been used in the treatment of jaundice (Duke et al., 1985). Berberine, universally present in rhizomes of Berberis species, has marked antibacterial activities. Since it is not appreciably absorbed by the body, it is used orally in the treatment of various enteric infections, especially bacterial dysentery (Duke et al., 1985). *Berberidaceae* is a famous family of medicinal and edible values and is included in British and Indian pharmacopeias (Srivastava et al., 2005). One of the species called *Berberis aristata* is reported to have hepatoprotective and cutative effects in rats (Janbaz et al., 2000). The fruit of *Berberis vulgaris* has anticholinergic and antihistaminic activities (Shamas et al., 1999). *Berberis lycium* is a well known medicinal plant with equal edibility and medicinal rating 3 (Nadkarni, 1992; Monnin, 1987; Grover et al., 2000; Srivastava et al., 2005).

**Material and methods**

I.Plant material:

The roots of *Berberis lycium* were collected in the month of June from the local areas of Kashmir and were identified by the Centre of Taxonomy, University of Kashmir. Sample specimen (voucher specimen No: 709-KASH) was deposited in the herbarium of Centre of Taxonomy, University of Kashmir. The material was completely shade dried and coarsely ground. The extracts were prepared by continuous hot extraction using methanol and water respectively as solvents. Extracts obtained were concentrated, dried and kept in desiccators for further use.

II.Preparation of extracts:

The root material was cut into pieces, completely dried. The dried roots were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into s生效tlet apparatus and extracted successively with methanol and distilled water (yield 2, 8, and 8%, respectively). All the extracts were kept in a desiccator till whole moisture was removed and then were stored in airtight containers in refrigerator below 10°C. The yield was found to be 12.5% and 2.3% respectively for methanolic and aqueous solvents.

III.Preliminary phytochemical analysis of the extracts:

The extracts so obtained were subjected to preliminary phytochemical screening to identify the chemical constituents. Phytochemical screening was performed using standard procedures (Harborne, 1988; Kokate, 1994).

IV.Ash value determination:

2 g of the grinded, air dried material, weighed accurately was taken in a clean and tared crucible. The crucible was placed in a muffle furnace. The material was ashed by gradually increasing the heat up to 800°C until it is white ash. The crucible was cooled in a desiccator and weighed. The cooled crucible containing the ash obtained earlier was moistened with 2ml of water or HCl (10%) (2ml), then stirred and filtered the solution, the residue was dried on the filter paper and then ignited it again in furnace in order to determine the water insoluble or acid insoluble ash value.
VI. Sample collection: Blood samples were collected by retro-orbital plexus puncture method.

VII. Acute toxicity study: Acute toxicity study was performed for aqueous and methanolic extract according to the acute toxic classic method as per guidelines of Organisation for Economic Co-operation and Development (OECD). The rats were kept fasting for overnight providing only water, after which the extract was administered orally at different dose levels i.e. 100, 200, 500, 1000, 1500, 2000 mg/kg of body weight. The rats were observed continuously for 24 hr for behavioral, neurological and then at 24 hrs and 72 hrs for any lethality.

VIII. Effect of Berberis lycium on Oral Glucose Tolerance Test (OGTT): The oral glucose tolerance test (Bonner-Weir, 1988) was performed in overnight fasted (18 hr) normal rats. Rats divided into three groups (n=6) were administered drinking water, Berberis lycium (250 and 500 mg/kg of b. wt) respectively. Glucose (2g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 60, 90, 120 and 150 min of glucose administration and glucose levels were estimated using the standard glucose reagent kit.

IX. Experimental design: All the animals were randomly divided into the six groups with six animals in each group. Group A, B, and E were served as saline, diabetic, and standard drug (glibenclamide, 30 mg/kg per day) control, respectively. While as group D and F served as Extract treated groups (250 mg/kg of body weight and 500 mg/kg of body weight).

X. Assessment of effect of extracts on alloxan-induced diabetic animals: Diabetes was induced in rats by a freshly prepared single intraperitoneal injection of alloxan monohydrate (Sigma Aldrich: 160 mg/kg), as per the methods Aruna et al. (1999). Alloxan was first weighed individually for each animal according to the weight and then solubilized with normal saline (0.9 w/v NaCl) just prior to injection. Two days after alloxan injection, rats with fasting blood glucose levels of >200 mg/dl were included in the study. Treatment with plant extracts was started 72 hr after alloxan injection. Blood samples were drawn at weekly intervals till the end of study (i.e. 2 weeks). Effect of the extracts on physiological parameters like food intake, fluid intake and urine excreted was observed and recorded as per the standard operating procedures (SOP) of the Drug Standardisation Unit, Srinagar. Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. On day 14, blood was collected by terminal bleeding under mild ether anesthesia from overnight fasted rats and fasting blood sugar (Trinder et al., 1989) was estimated. Serum was separated and analyzed for serum cholesterol, serum triglycerides, serum albumin, serum globulin (VLDL), serum Glucose, serum oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP). The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, followed by alcohol treatments in 70%, 90%,100% isopropyl alcohol for an hr each, then by xylene treatment (one hr) and immediately processed by the paraffin technique as per the SOP (Standard Operating Procedure) named as “SOP for Necropsy, collection and weighing of tissues” and “SOP for Tissue fixation, processing and embedding”. Sections of 5μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination as per the SOP for “Section cutting and staining”.

XI. Statistical analysis: All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard deviation (S.D) and analyzed for ANOVA and post Dunnet’s t-test. Differences between groups were considered significant at P < 0.05 levels.

RESULTS: Phytochemical investigation of Berberis lycium root extract revealed the presence of alkaloids, tannins, glycocides, flavonoids, saponins, and pheno-

There was a considerable reduction in the body weight of the diabetic rats as considered to normal ones. While as extract treated (aqueous and methanolic; 500mg/kg of b.wt) showed an increase in body weight (Table I). The extracts (Aqueous and methanolic; 500mg/kg of b.wt) significantly reduced the fluid intake of rats. An elevated level of fluid intake, food intake and volume of urine excreted by diabetic rats was lowered by the treatment with extracts (Aqueous and methanolic; 500mg/kg of b.wt) and also by the glibenclamide (30 mg/kg of b.wt) Table II.

Table 1: Effect of extracts of Berberis lycium on body weight in Alloxan monohydrate treated diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>6th day</th>
<th>7th day</th>
<th>14th day</th>
<th>% Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>180±4</td>
<td>184±5</td>
<td>189±5</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>diabetic control</td>
<td>185±3</td>
<td>183±5</td>
<td>181±5</td>
<td>-2.16</td>
<td></td>
</tr>
<tr>
<td>Met. Ext (B. lycium) 500mg/kg</td>
<td>180±6</td>
<td>186±3</td>
<td>174±4</td>
<td>3.88</td>
<td></td>
</tr>
<tr>
<td>Aq.ext. (B. lycium) 500mg/kg</td>
<td>175±4</td>
<td>178±3</td>
<td>182±5</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide(30mg/kg)</td>
<td>165±8</td>
<td>168±6</td>
<td>171±7</td>
<td>3.63</td>
<td></td>
</tr>
</tbody>
</table>

Values are represented as mean±S.D of 6 animals in each group. *P<0.05 (Dunnet t-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

Table 2: Effect of the extracts on food intake, fluid intake and urine excreted by the rats.

<table>
<thead>
<tr>
<th>Table 2: Effect of the extracts on food intake, fluid intake and urine excreted by the rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Fluid intake (ml/day)</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
</tr>
<tr>
<td>Urine (wetting of saw-bedding)</td>
</tr>
</tbody>
</table>

Values are represented as mean±S.D of 6 animals in each group.
Table 5: Effect of extract of Berberis lycium roots on serum lipid profile of alloxan monohydrate induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>V.L.D.L (mg/dl)</th>
<th>L.D.L (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>129.06 ± 7.2</td>
<td>89.83 ± 4.8</td>
<td>38.00 ± 2.8</td>
<td>96.23 ± 6.8</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>252.73 ± 3.5*</td>
<td>204.34 ± 9.3*</td>
<td>75.74 ± 5.4*</td>
<td>196.64 ± 4.5*</td>
</tr>
<tr>
<td>Aqueous extract (250 mg/kg b.wt)</td>
<td>204.45 ± 5.99</td>
<td>144.73 ± 3.92</td>
<td>49.25 ± 4.75</td>
<td>144.33 ± 7.1</td>
</tr>
<tr>
<td>Aqueous extract (500 mg/kg b.wt)</td>
<td>161.49 ± 5.3*</td>
<td>92.58 ± 4.6*</td>
<td>40.36 ± 2.3*</td>
<td>101.364 ± 2.8*</td>
</tr>
<tr>
<td>Methanolic extract (250 mg/kg b.wt)</td>
<td>211.46 ± 7.47</td>
<td>157.75 ± 4.94</td>
<td>49.26 ± 5.67</td>
<td>144.357 ± 7.24</td>
</tr>
<tr>
<td>Methanolic extract (500 mg/kg b.wt)</td>
<td>172.45 ± 4.7*</td>
<td>109.93 ± 3.7*</td>
<td>46.57 ± 3.4*</td>
<td>93.776 ± 7.7*</td>
</tr>
<tr>
<td>Gilbenclamide (30mg/kg)</td>
<td>168.61±5.4*</td>
<td>105.634.6*</td>
<td>40.23±1.9*</td>
<td>95.48±3.3*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. *P < 0.05 (Dunnet t-test), diabetic control was compared with the normal and extract treated groups were compared with the diabetic control.

Table 6: Effect of extract of Berberis lycium roots on SGOT, SGPT and ALP of alloxan monohydrate induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPTT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22.37 ± 4.47</td>
<td>25.74 ± 5.33</td>
<td>96.67 ± 10.42</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>46.74 ± 6.57*</td>
<td>49.48 ± 7.64*</td>
<td>175.49 ± 16.57*</td>
</tr>
<tr>
<td>Aqueous extract (250 mg/kg b.wt)</td>
<td>28.53 ± 4.55</td>
<td>34.48 ± 5.47</td>
<td>139.63 ± 7.36</td>
</tr>
<tr>
<td>Aqueous extract (500 mg/kg b.wt)</td>
<td>23.39 ± 4.42*</td>
<td>24.81 ± 6.26*</td>
<td>95.46 ± 13.75*</td>
</tr>
<tr>
<td>Methanolic extract (250 mg/kg b.wt)</td>
<td>29.93 ± 7.95</td>
<td>36.73 ± 24.8</td>
<td>145.57 ± 14.38</td>
</tr>
<tr>
<td>Methanolic extract (500 mg/kg b.wt)</td>
<td>24.63 ± 3.72*</td>
<td>27.76 ± 3.73*</td>
<td>98.58 ± 13.13*</td>
</tr>
<tr>
<td>Gilbenclamide (30mg/kg)</td>
<td>23.34 ± 4.48*</td>
<td>26.47 ± 9.53*</td>
<td>97.59 ± 6.83*</td>
</tr>
</tbody>
</table>

Data represented as mean ± S.D values of 6 animals each. *P < 0.05 (Dunnet t-test), diabetic control was compared with the normal and extract treated groups were compared with the diabetic control.

Fig. (1) showed normal acini, and normal cellular population in the islets of langerhans in pancreas of vehicle-treated rats (A). Extensive damage to the islets of langerhans and reduced dimensions of islets (B), restoration of normal cellular population size of islets with hyperplasia by Glibenclamide (C) was also shown. The partial restoration of normal cellular population and enlarged size of ß-cells with hyperplasia was shown by methanol and aqueous extracts. (Fig. 1:D–F).

DISCUSSION

Phytochemical screening of the plants revealed the presence of flavonoids, alkaloids, glycosides, Phenolics and tannins in the plant and are likely to be responsible for the antidiabetic effect effects observed. (Cherian et al., 1992; Hakim et al., 1995; Manickam et al., 1997; Akowuah et al., 2002). Alloxan monohydrate is a drug that selectively destroys ß-cells of pancreas and thus induces experimental diabetes ( Badole et al., 2006; Sun et al., 2008; Xu et al., 2008). In our study we found that the continuous treatment with the extracts of Berberis lycium produced significant decrease in fasting blood glucose levels. Although the exact mechanism of extracts of Berberis lycium in diabetes process is not well understood, one possible underlying mechanism may be the extracts are potentiating the insulin effect by of plasma by increasing either pancreatic secretion of insulin from ß-cells of pancreas or direct protection of ß-cells of pancreas. It has been reported that berberine- a quaternary protoberberine alkaloid and constituent present in number of clinically important medicinal plant families like Berberidaceae family of which Berberis lycium is a member, inhibits apoptosis and necrosis in pancreatic cell lines invitro (Issac et al., 2006; Kwon et al., 2005; wikipedia.org/wiki/Berberine).

CONCLUSION:
The extracts of Berberis lycium showed improvement in parameters like body weight, food intake, fluid intake and urine excreted. Berberis lycium extracts lowered fasting blood glucose levels significantly, the extracts also lowered, serum SGPT, SGOT, and ALP levels which show the protective effect and normal functioning of liver in reversing the organ damage due to diabetes which is clearly observed by high levels of SGOT and SGPT in diabetic control. The extracts also lowered the levels of serum lipids like triglycerides, LDL, VLDL, and cholesterol. The extracts of Berberis lycium did not show any toxic effects upto the dose level of 2000mg/Kg of b. wt.
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