Pharmacological Evaluation for Antidiabetic Activity of *Blepharis repens* (Valh) Roth Extract

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INTRODUCTION: Nature has generously endowed the world with a rich variety of plants, each harbouring valuable medicinal properties. Therefore, there is a necessary to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, now a day’s diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Blepharis repens* for the area for diabetes. For the evaluation of antidiabetic activity alloxan induced diabetes rat model was used. Glimiperide was used as standard. Selected plant extracts showed good antidiabetic activity. Blood glucose levels in diabetic rats and different parameters of diabetic complications like serum SGOT, SGPT, Triglyceride, Cholesterol, LDL and HDL as a marker for liver abnormalities, kidney abnormalities (Nephropathy) and hyperlipidemia showed significant change (P<0.05 to P<0.001) with methanol extract of *Blepharis repens* as compared to diabetic control groups and comparable activity with that of standard.

KEYWORDS: Diabetes mellitus, *Blepharis repens*, Metabolic disorder, Antidiabetic

ABSTRACT: Nature has generously endowed the world with a rich variety of plants, each harbouring valuable medicinal properties. Therefore, there is a necessary to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, now a day’s diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Blepharis repens* for the area for diabetes. For the evaluation of antidiabetic activity alloxan induced diabetes rat model was used. Glimiperide was used as standard. Selected plant extracts showed good antidiabetic activity. Blood glucose levels in diabetic rats and different parameters of diabetic complications like serum SGOT, SGPT, Triglyceride, Cholesterol, LDL and HDL as a marker for liver abnormalities, kidney abnormalities (Nephropathy) and hyperlipidemia showed significant change (P<0.05 to P<0.001) with methanol extract of *Blepharis repens* as compared to diabetic control groups and comparable activity with that of standard.

INTRODUCTION: Diabetes mellitus (DM) constitutes a cluster of metabolic disorders characterized by elevated blood sugar levels persisting for extended periods. This heightened blood sugar leads to symptoms like frequent urination, heightened thirst, and increased appetite.[1] When left untreated, diabetes can give rise to various complications, including diabetic ketoacidosis and nonketotic hyperosmolar coma. Additionally, severe long-term consequences encompass heart disease, stroke, kidney failure, foot ulcers, and eye damage.[2] Diabetes is a metabolic disorder affecting the body’s utilization of digested food for growth and energy. When we consume food, it breaks down into glucose, a form of sugar found in the bloodstream, serving as the primary source of fuel for our body’s cells. To enter the cells, glucose requires insulin, a hormone produced by the pancreas, located behind the stomach. In a healthy individual, the pancreas automatically releases the right amount of insulin to transport glucose from the blood into the cells. However, individuals with diabetes experience a deficiency in insulin production or the cells’ improper response to the insulin produced. As a result, glucose accumulates in the blood, eventually spilling into the urine and being excreted from the body. This condition leads to the loss of the body’s primary source of fuel despite the presence of high glucose levels in the blood. Diabetes can arise from either insufficient insulin production by the pancreas or inadequate cellular response to the available insulin.[3] The two main types of diabetes are:
Type 1 DM or Insulin dependent diabetes mellitus (IDDM) or juvenile diabetes.
Type 2 DM or non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes.

*Blepharis repens* (Vahl) Roth is a significant plant belonging to the Acanthaceae family, widely distributed across India, Sri Lanka, and Africa. This large-sized plant is extensively cultivated in both India and Sri Lanka, where it serves as a food source for humans. *Blepharis repens* (vahl) Roth is gaining much importance in diabetic control as it has been used as a traditional medicine for diabetes; since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, phytosteroids, tannins and saponins, alkaloids, fixed oil. Numerous researchers reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic properties. flavonoids have been shown to aid in the regeneration of impaired beta cells in alloxan-induced diabetic rats and function as insulin secretagouges. Saponin has been found to decrease the absorption of specific nutrients like glucose and cholesterol in the gastrointestinal tract through a physicochemical reaction within the gut. As a result, it has been documented to possess a hypocholesterolemic effect, potentially assisting in reducing the metabolic strain on the organ. [4]
MATERIALS AND METHOD:

Extraction of plant material

Selection of Solvent: As per the nature of phytochemical present in drug and literature survey revealed solvents were selected for the extraction of the whole plant Blepharis repens like Petroleum ether, Ethyl acetate and Methanol.

Selection of Extraction method: According to nature of phytochemical present in drug and literature review, solvent and extraction method was selected. The extraction method selected for extraction for the leaves of Blepharis repens was continuous hot extraction method using Soxhlet apparatus.

Material: Soxhlet apparatus and heating mental, powdered drug, petroleum ether, ethyl acetate and methanol.

Preparation of Methanolic extract:

Methanolic extract of powdered plant was prepared in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered leaves and 1000 ml Methanolic was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

Experimental animals: Wistar albino rats procured for studying the ability of aqueous extract of Blepharis repens for anti-diabetic activity.

Anti-diabetic activity
Species: Rat
Strain: Wistar
Sex: Either sex
Body weight: 350-400 g

**Figure: Wister Albino Rats**

**Experimental Methodology**

**Principle:**
Alloxan: is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death.
When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both its inhibitory and cytotoxic effects.

**Procedure:**
Albino rats of either sex [200-300g] are injected with a single dose of alloxan monohydrate [120 mg/kg body weight] dissolved in normal saline by i.p. route.
The diabetic state was confirmed 48 hours after alloxan injection by weight loss and hyperglycemia.
The blood glucose was measured by one touch glucometer (One touch Select Simple).
The alloxan induced rats were allowed to drink 5% glucose solution during 48 hrs of alloxan treatment.
Blood glucose levels show triphasic response with hyperglycemia for one hour followed by hypoglycemia that lasts for six hours & stable hyperglycemia after 48 hours.
Animals showing fasting blood glucose level above 200 mg/dl after 48 hour of alloxan administration are considered diabetic & selected for the further study.
For a period of seven days, drug samples to be screened are administered orally.
After seven days of treatment, blood samples are collected from 8 hour fasting animals through a retro orbital vein.
Serum is separated by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes.
The serum glucose level is estimated by glucose oxidase-peroxidase method [GOD-POD kit] using autoanalyser.

**Experimental grouping for allowance induced diabetes**
Group 1: Control group
Group 2: Alloxan induced diabetes group
Group 3: Standard group (Glimiperide)
Group 4: Methanolic extract of BR 200 mg/kg
Group 5: Methanolic extract of BR 100 mg/kg

**Collection of Blood Sample and Blood Glucose Determination:**
Blood samples was drawn from retro–orbital plexus at weekly intervals till the end of study (i.e., 2 weeks).
Fasting blood glucose estimation and body weight measurement was done on day 1, 7, and 14 of the study.
Blood glucose estimation was done by using GOD-POD method on day 14, blood collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats.
Centrifuge the blood sample at 3000 RPM for 10 min to separate serum and fasting blood sugar was estimated.

**Following parameters estimated during study**
1) body weight
2) blood glucose level
3) LDL level
4) HDL level
5) Total cholesterol level
6) Triglyceride level

**Statistical analysis:** The data were expressed as mean ± Standard Deviation of Mean (SDM). Statistical analysis was performed by one way analysis of variance (ANOVA).
RESULTS:
Each values represents as mean S.D.; n=6; *: Significant difference (P<0.05 or less) & **: highly significant difference (P<0.001) when compared with control; a: No significant difference when compared with standard. Methanolic extracts when compared with control by ANOVA shows significant difference (P<0.05); E.A. Blepharis repens ethyl acetate extract; GLU Glucose; SGPT Serum glutamate pyruvate transaminase, = SGOT Serum glutamate oxaloacetate transaminase; TC- Total cholesterol; TG- Triglyceride; LDL Low density lipoprotein, HDL= High density lipoprotein.

DISCUSSION:

<table>
<thead>
<tr>
<th>Group</th>
<th>Control group</th>
<th>Alloxan induced diabetes</th>
<th>Standard group</th>
<th>M.E of BR 100 mg/kg</th>
<th>M.E of BR 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU</td>
<td>97.78±4.42</td>
<td>297.68±40.9</td>
<td>111.9±9.02</td>
<td>196.66±7.36</td>
<td>172.33±6.65</td>
</tr>
<tr>
<td>SGPT</td>
<td>57.8±5.73</td>
<td>128.33±2014</td>
<td>77.26±11.81</td>
<td>101.16±4.0</td>
<td>88.13±5.02</td>
</tr>
<tr>
<td>SGOT</td>
<td>50.05±3.07</td>
<td>150.37±11.06</td>
<td>67.87±1.89</td>
<td>103.99±8.14</td>
<td>98.69±4.60</td>
</tr>
<tr>
<td>TC</td>
<td>91±9.26</td>
<td>253.9±0.09</td>
<td>176.27±0.45</td>
<td>211.6±0.19</td>
<td>205.5±0.22</td>
</tr>
<tr>
<td>TG</td>
<td>70.9±6.71</td>
<td>144.2±32.79</td>
<td>75.85±5.98</td>
<td>99.52±11.22</td>
<td>89.37±20.12</td>
</tr>
<tr>
<td>LDL</td>
<td>27.38±3.51</td>
<td>81.03±3.22</td>
<td>48.59±2.79</td>
<td>61.34±5.98</td>
<td>57.34±2.1</td>
</tr>
<tr>
<td>HDL</td>
<td>52.96±8.69</td>
<td>19.11±5.62</td>
<td>47.89±3.39</td>
<td>36.92±2.40</td>
<td>42.18±16.9</td>
</tr>
</tbody>
</table>

In traditional medicine system, many plants and herbs are claimed to have antidiabetic and antioxidant efficacy of Blepharis repens is used traditionally as folk medicine to treat a number of illnesses including in disorders where its antidiabetic potential is claimed to be useful. The whole plant as well as specific parts (leaves, fruits, stems) of plant extract has been used for the treatment of large number of human diseases. Blepharis repens tested for antidiabetic activity by researchers. Antidiabetic activity and further diabetic complications of methanolic extracts of Blepharis repens are not available. Therefore it was thought worthwhile to explore this indigenous plant for its antidiabetic activity and further diabetic complications. Three extracts were prepared with petroleum ether, ethyl acetate and methanol. Preliminary phytochemical evaluation of all three extracts was carried out for the identification of presence of phytoconstituents. All extracts shows presence of saponins, tannins, carbohydrates, glycosides, flavonoids, and steroids were confirmed by qualitative analysis.

Present study investigates the antidiabetic potential of extracts of Blepharis repens. The results showed that the highest dose (200mg/kg) of methanolic & ethyl acetate extracts of Blepharis repens possesses significant antidiabetic activity when given orally in a daily single dose for seven days. The findings suggest effect of two different doses of different extracts i.e. ethyl acetate and methanolic extract (100 mg/kg and 200 mg/kg) of Blepharis repens is probably mediated through its ability to cause a significant decrease in blood glucose level of diabetic rats.

The antidiabetic activity of Blepharis repens was evaluated in alloxan-induced diabetic rats by testing its effect on fasting blood glucose level using autoanalyser glucose kit. Alloxan monohydrate, at a dose of 120 mg/kg body weight, caused sufficient damage to pancreatic  Β cells so that secreted insulin was not enough to regulate blood glucose and resulted to a significant increase in blood glucose levels. Alterations of lipid profile are common in diabetic conditions. In diabetes, since blood glucose is not utilized by tissue, the fatty acid from adipose tissue are metabolized for energy purpose and excess fatty acids are accumulated in the liver and converted to triglycerides. Under diabetic condition, cholesterol level was increased due to decreased cholesterol absorption and increased cholesterol biosynthesis. Chronic insulin deficiency is associated with a diminished level of LDL receptor which causes the increase in LDL particles and results to an increase in LDL-cholesterol levels in diabetes mellitus.

The result of study indicates that the methanolic leaf extract (200 mg/kg) of Blepharis repens at the test dose used and the reference drug Glimiperide (2 mg/kg) exhibited a time dependent significant (p < 0.0001) reduction of the blood glucose levels of the alloxan-induced diabetic rats. The medical literature extensively covers the mechanism by which antidiabetic medications address diabetes mellitus. They work by reducing the levels of glucose in the bloodstream. The result obtained from this preliminary study clearly shows that Blepharis repens caused marked antihyperglycemic activity in alloxan-induced diabetic rat model which indicates antidiabetic potentials of the extract. The dose of the extract (200 mg/kg) produced the highest antidiabetic effect and this may suggest that this dose may be the effective antidiabetic dose of the crude extract. However Glimiperide was superior in activity when compared to the test doses of the extract which may be attributed to the crude nature of the plant extract. At the same time different parameters of diabetic complications like serum SGOT, SGPT, Triglyceride, Cholesterol, LDL, HDL, as a marker for liver abnormalities, kidney abnormalities (Nephropathy) hyperlipidemia were performed and result reveals that methanolic extract of Blepharis and repens showed significant curative activity (P<0.001) against diabetic complications.
CONCLUSION:
Blepharis repens is used traditionally as folk medicine to treat a number of illnesses including in disorders where its antidiabetic potential is claimed to be useful. The whole plant as well as specific parts (leaves, fruits, stems) of plant extract and its active constituents have been used for the treatment of large number of human diseases. Ethanolic extract of Blepharis repens already tested for antidiabetic activity by researchers. Therefore it was thought worthwhile to explore the antidiabetic activity of ethyl acetate & methanolic extracts of Blepharis repens.
Antidiabetic activity was carried out on rats by using alloxan induced diabetes model. Equal numbers of rats in group were treated with 100 mg/kg and 200 mg/kg, of plant extracts and against Glimiperide as standard. Observations were made for the decrease in blood glucose level in diabetic rats. The result of antidiabetic activity and diabetic complications protective activity against nephropathy and hepatic complications along with antihyperlipidemic activity reveals methanolic extracts has promising and significant activity (from P<0.05 to P<0.001).

REFERENCE:
7. Chauhan A. et al, Plants having potential antidiabetic activity pharmaceutical chemistry research lab, Department of pharmaceutical technology, 2010, pp.369-387.