EVALUATION OF QUERCUS INFFECTORIA (GALLS) EXTRACTS FOR THE MANAGEMENT OF DIABETES

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ABSTRACT

The objective of this study was to investigate the Antidiabetic activity of galls of Quercus infectoria in rats. The antidiabetic activity of methanolic & aqueous extract was evaluated in Glucose tolerance test & Sucrose tolerance Test model in wistar rats. The galls methanolic & aqueous extract was prepared by maceration extraction process using methanol & water as solvent. Phytochemical analysis & acute oral toxicity were carried out using standard methods. The preliminary phytochemical screening of Quercus infectoria revealed the presence of sterols, protein, alkaloid, tannins, phenolic compounds & flavonoids. The methanolic & aqueous extract at a concentration of 500 mg/kg exhibit a blood glucose lowering effect on diabetes models & was compared with standard drug Acarbose. The percentage reduction of blood glucose level within 4 hr in Glucose tolerance test for methanolic extract & aqueous extract was 18.5% & 2.58% respectively and for Sucrose tolerance test percentage reduction of blood glucose level within 4 hr was 16.92% & 9.7% respectively. Among the two extracts studied methanolic extract 500 mg/kg body weight was found to be optimum for significant blood glucose lowering.

KEYWORDS: Antidiabetic activity, acarbose, Glucose, Quercus infectoria.

INTRODUCTION

According to the IDF (International Diabetes Federation) 285 million people affected the diabetes corresponding to 6.4 percentage of the world adult population will live with diabetes in 2010. The number is expected to grow 438 million in 2030 corresponding to 7.8 percent of the adult population.[1] Diabetes mellitus is characterized by abnormally high levels of glucose in the blood. In people with diabetes, blood sugar levels remain high. This may be
because insulin is not being produced at all, is not made at sufficient levels, or is not as effective as it should be. The most common forms of diabetes are type-1 diabetes (5%), which is an autoimmune disorder, and type-2 diabetes (95%), which is associated with obesity.\[2\]

Galls of *Quercus infectoria* was used in diabetes traditionally and the methanolic fraction of dried methanol extracts of galls dissolved in acetone and filtered, the filtrate was partially purified material, pale yellow amorphous powder showed hypoglycemic activity\[^{3,4,5,6,7,8}\] in non diabetic rabbits. Galls of *Quercus infectoria* had been reported to inhibit alpha-glycosidases such as sucrase, maltase and isomaltase *in vitro* with inhibitory effect being comparable to acarbose. Hence an attempt has been made to investigate the antidiabetic activity of aqueous & methanolic extract of *Quercus infectoria* galls in experimental *In-vivo* animal models for diabetes.

**MATERIALS AND METHODS**

**CHEMICALS**

Glucose, Sucrose, Acarbose was obtained from Oxford lab Mumbai and other chemicals and reagents used were of analytical grades.

**INSTRUMENTS**

Blood glucose levels of the rats were estimated using Elegance CT-X12 with Glucose strips.

**PLANT COLLECTION AND IDENTIFICATION**

The Galls of *Quercus infectoria*, Fagaceae\[^{9,10}\] were collected from Ayurvedic crude drug shop (kanthaliya) Khargone (M.P). Galls were shade dried to prevent any type of moisture presence and degradation of active constituents. Authentication was done by Dr. Zia-ul Hasan, Professor & head of department botany, Saifia college of science, Bhopal (MP), India. (Voucher specimen No. 307|Bot|Saifia|2012). The galls were grinded by an electric mill to produce coarse powder. Powdered drug were kept in air tight containers until use.
PREPARATION OF EXTRACT
The collected galls of *Quercus infectoria* were shade dried & powdered coarsely & then passed through 40 mesh sieve. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of *Quercus infectoria* was macerated with the solvent distilled water & methanol for 5-7 days. The extract was concentrated, frozen and lyophilized by lyophilizer. The extract obtained was stored in labeled, airtight, amber colored bottle in the refrigerator until use for phytochemical analysis & pharmacological studies. The extract obtained was weighed to a constant weight & the percentage yield w/w basis was calculated.

**Determination of percentage yield**

\[
\% \text{ Yield} = \frac{\text{weight of extract}}{\text{Weight of powdered drug}} \times 100
\]

PHYTOCHEMICAL SCREENING
The screening was carried out on the Methanolic & aqueous extracts of galls of *Quercus infectoria* to determine the active principles or secondary plant constituents. Tests were carried out for carbohydrates, reducing sugar, tannins, polyphenols, flavanoids, alkaloids, gum, saponins, amino acid, resins & steroids.

ANIMALS
Healthy female albino wistar rats between 2 and 3 months of age weighing 150-250 g were used for the evaluation of acute oral toxicity test and antidiabetic activity. The animals were housed in polypropylene cages, maintained under standard conditions (12 hr light; 12 hr dark cycle; 25 ± 30°C, 35-60% humidity). They were fed with standard rat pellet diet (Hindustan lever Mumbai) and water. Ethical clearance for the handling of animals and procedure used
in study was obtained from institutional animal ethical committee prior to the beginning of
the study.

**ACUTE TOXICITY STUDY**

The rats were fasted overnight, divided into each dose groups (n=6) and were fed with
increasing doses (1, 4 and 8 g/kg body wt) of two extracts by gastric intubations and observed
for 14 days. If mortality was observed in two out of three animals, then the dose administered
was assigned as toxic dose. If mortality was observed in one animal, then the same dose was
repeated again to confirm the toxic dose. If mortality was not observed, the procedure was
repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

**ANTIDIABETIC ACTIVITY**

Two models (OGTT & OSTT) with effective elevation of blood glucose experimentally in
rats were employed to evaluate the antidiabetic activity of methanolic & aqueous extract of
*Quercus infectoria*.

**ORAL GLUCOSE TOLERANCE TEST (OGTT)**

The rats were placed on fasting, each group (6 each) of the animals in a separate fresh cage
with no food, but make sure they have water bottles. Use cages with woodchip bedding and
not corncob bedding. The animals eat the corncob (containing starch) when they are hungry
and are not fasted. After 16 hr acarbose or the water extract of *Quercus infectoria* was orally
administered to groups of six rats each. 60 min later, glucose (1.5 g/kg) was orally
administred to each rat. Blood samples were taken from tail veins at 0 hr (just before the
water extract administration), 1 hr (just before glucose the administration), 2h, 4h for the
assay of glucose.

The rats were segregated into five groups of six rats in each.

**Group I**- Positive control: rats received only distilled water.

**Group II**-Negative control: rats received only glucose solution. This group was used for
studying the baseline value of the parameters studied.

**Group III**- Rats received acarbose (50 mg/kg p.o) suspended in distilled water and Glucose
solution.

**Group IV**- Rats received methanolic extract (500 mg/kg p.o) suspended in distilled water
and Glucose solution.
Group V- Rats received aqueous extract (500 mg/kg p.o) suspended in distilled water and glucose solution.

**ORAL SUCROSE TOLERANCE TEST**
The fasting condition and protocol same as oral glucose tolerance test. Sucrose was taken at the place of glucose. Sucrose (1.5 g/kg) in distilled water was orally administrated to each rat.

The rats were segregated into five groups of six rats in each.

**Group I**- Positive control: rats received only distilled water.

**Group II**-Negative control: rats received only sucrose solution. This group was used for studying the baseline value of the parameters studied.

**Group III**- Rats received acarbose (50 mg/kg p.o) suspended in distilled water and sucrose solution.

**Group IV**- Rats received methanolic extract (500 mg/kg p.o) suspended in distilled water and sucrose solution.

**Group V**- Rats received aqueous extract (500 mg/kg p.o) suspended in distilled water and sucrose solution.

**STATISTICAL ANALYSIS**
Statistical significance between the different groups was determined using one way analysis of variance (ANOVA) followed by Dunnett test. The results were considered statistically significant if p-values were less than 0.05.

**RESULTS**
The percentage yield of methanolic & aqueous extract were 25% & 30 % respectively. Phytochemical screening showed that the extracts contain carbohydrates, alkaloids, sterols, tannins & flavonoids. Acute toxicity results showed that the LD$_{50}$ was greater than 2000mg/kg.

*Oral glucose tolerance test*
In Table 1, Blood glucose lowering was evident in treatment groups of the methanolic & aqueous extract of *Quercus infectoria* compared to the negative control. However statistically significant blood glucose lowering (18.5% *p*< 0.01) could be seen at dose 500 mg/kg for the methanolic extract.
**Oral sucrose tolerance test**

In Table 2, the methanolic & aqueous extract of *Quercus infectoria* at 500mg/kg dose provided reduction in blood glucose level. The methanolic extract provides statistically significant protection (19.92% *p<0.01) when compared with negative control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Positive control</td>
<td>85.33±3.24</td>
<td>84±3.05</td>
<td>83.33±3.49</td>
<td>82.83±3.30</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>83±2.97</td>
<td>86±3.22</td>
<td>87.33±3.34</td>
<td>86.16±3.44*</td>
</tr>
<tr>
<td>III</td>
<td>Acarbose</td>
<td>88.83±5.02</td>
<td>82.16±4.61</td>
<td>77.83±5.06*</td>
<td>72.33±4.14**</td>
</tr>
<tr>
<td>IV</td>
<td>Methanolic Ext</td>
<td>80.33±3.27</td>
<td>78.16±3.32</td>
<td>76.66±3.07*</td>
<td>75.66±3.25*</td>
</tr>
<tr>
<td>V</td>
<td>Aqueous Ext</td>
<td>84±5.50</td>
<td>82.66±5.39</td>
<td>82.16±5.44</td>
<td>81.83±5.41</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM (n=6), Statistical significance: (*) p < 0.05, when compared group II with group I, (**) p < 0.01 when group III, IV, V, compared with group II.

**DISCUSSION**

Diabetic Mellitus (DM) is a common disorder associated with increased morbidity and mortality is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced.

The Antidiabetic activity of the methanolic & aqueous extract of *Quercus infectoria* against oral glucose tolerance test & oral sucrose tolerance test were established. Our finding showed that the methanolic extracts at doses 500 mg/kg body weight had better antidiabetic effect than aqueous extract on acute experimental diabetes models. The present finding concluded that both methanolic & aqueous extract had potential blood glucose lowering effect, which was not superior to the respective effect observed with standard drug (Acarbose).
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REFERENCES