ANTIDIABETIC ACTIVITY OF BAUHINIA VARIEGATA EXTRACTS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Bauhinia variegata commonly known as kachnar tree and is cultivated throughout India. Literature survey revealed that the leaf of Bauhinia variegata is traditionally used as an astringent in diabetes. Flowers are laxative. The present study was undertaken to evaluate the antidiabetic activity of ethanol extract of leaf of Bauhinia variegata. The extract was evaluated using albino wister rat i.e. alloxan induced diabetes in albino wister rat by glucometer method, with 50 mg/kg, 100 mg/kg and 200 mg/kg and higher doses showed significant value represented at table no and figure 1,2,3,4 respectively with different successive extract and show the significant p value.

Keyword: Bauhinia Variegata, Ethanolic Extract, Antidiabetic, Alloxan, Albino Wister Rat

INTRODUCTION

Diabetes mellitus is a group of endocrine syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease [1]. Insulin dependent diabetes most commonly afflicts juveniles, but it can also occur in adults. The disease is characterized by an absolute deficiency of insulin caused by massive β-cell lesions or necrosis. Loss of β-cell function may be due to invasion by viruses, the action of chemical toxin or usually through the actions of autoimmune antibodies directed against the β-cells [2]. The metabolic alterations observed are milder than those described for IDDM (for example, NIDDM patients typically are not ketosis) but the long term clinical consequences can be just as devastating (for example, vascular complications and subsequent infection can lead to amputation of the lower limbs) [3].

Many remedial plants have been explored and their therapeutic efficacy has been widely exploited in complex disorders such as diabetes [4]. The mountain ebony, Bauhinia variegata belongs to the family Fabaceae. It is commonly known as Kachnar in Hindi. Transverse section of the leaf petiole shows single layered epidermis covered with thin cuticle. Covering trichomes showed uni to multicellular broad at the base and pointed at the apex & thin wall multicellular ballon shaped glandular trichomes [5]. The leaf is astringent, tonic and antidiuretic. It is useful in scrofula and skin diseases. It is also used for ulcers and leprosy. The juice of bark is used in the treatment of amoebic dysentry, diarrhoea & other stomach disorders [6]. The crude extract from Bauhinia variegata have been shown to posses biological activities viz., Antihyperlipidemic activity [7], Antistress/Adaptogenic Activity [8].
antibacterial, antioxidant, immunomodulatory activities, hepatoprotective activity, hypoglycemic activity. The plant Bauhinia variegata has been reported to contain Quercetin, lupeol, β-sitosterol, tannins, kaempferol-3-glucoside, amides, carbohydrates, reducing sugar. Thus, the major objective of this study was to active constituent from the plant and to assess the anti-diabetic potential of Bauhinia variegata in vivo.

MATERIAL AND METHOD

Chemicals
Alloxan of CDH, New Delhi was used for the induced of diabetes and was obtained from Department of Pharmacy and the standard drug i.e. glibenclamide was received by Sun Pharmaceutical Industries, Jammu & Kashmir.

Plant material
Fresh and young green leaves of Bauhinia Variegata were collected from the hill Gandoli Konch Distt Jalaun (U.P.) and got identified by Dr. R.V. Singh Scientist & Head, Herbarium and Museum Central Council Research Ayurveda & Shidha Gwalior (M.P.) with (Reference No. CCRAS/10/036).

Preparation of plant extract
Extraction of plant
The freshly collected Leaf Bauhinia Variegata were shaded dried until cracking sound was observed during breakage, and then these are made in to coarsely powdered by using dry grinder. The powdered Leaf of the plant (600 gm.) was packed in soxhlet apparatus and continuously extracted with ethanol till complete extraction, after completion of extraction the solvent was removed by distillation and then concentrated extract obtained was dried under reduced pressure using rotatory evaporator at temperature not exceeding 40°C and then give moderate heating on water bath. A green extract approximate 450 gm.

Anti-diabetic studies
Experimental Animals
The adult male albino rats of weight 180-240 gm were selected for the study. All animals were procured from disease free animal house, Institute of Pharmacy, Bundelkhand University, Jhansi with Institutional ethical number (IAEC No.BU/Pharm/16/01A).

The animals were fasted overnight with water ad libitum. The starting dose of 300 mg/kg of methanolic extract was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 200 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, expect where they need to be removed from the study and humanely killed for animal welfare reasons or were found dead.

Preparation of Dose
The Dose of 200 mg/kg and 400 mg/kg of methanol extract was selected for the test. All the doses were given orally after making emulsion in vehicle i.e. 1% acacia gum and the standard drug i.e. glibenclamide was given orally (10 mg/70kg) in the vehicle.

The Antihyperglycemic Effect of Ethanolic Extract of Leaves of Bauhinia Variegata on Alloxan Induced Diabetic Rats
A) Induction of experimental diabetes:
Diabetes mellitus was induced by administering intraperitoneal injection of alloxan monohydrate 120 mg/kg. to the overnight fasted rats. Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was induced by a single i.p injection of 120 mg/kg of alloxan monohydrate in sterile saline after 2 days of alloxan injection, the hyperglycemia rats (glucose level >250 mg/dl) were separated and divided into different groups comprising of 4 rat each for the anti-diabetic study. The treatment (P.O) was started from the same except normal control and diabetic control group for a period of 10 days. During this period, animal in all group had free access to standard diet and water. Body weight and blood glucose level were estimated on 4th, 7th and 10th day of the treatment.

B) Sample collection:
Blood sample were collected from tail nipping and glucose level was determined by an automatic electronic glucometer (Accuchek comfort).

C) Procedure:
After checking the fasting blood glucose in overnight fasted diabetic rats; they were divided into five groups of five rats reach and one group of non-diabetic rats.

All the doses were given in the following manner:
- Group A served as normal control and did not receive any treatment.
- Group B served as diabetic control and received alloxan monohydrate and vehicle (0.2 ml of 2% aqueous gum acacia)
- Group C glibenclamide (10 mg / kg p.o) and served as standard
- Group D Ethanolic extract (100 mg / kg p.o)
- Group E Ethanolic extract (250 mg / kg p.o)
- Group F Ethanolic extract (500 mg / kg p.o)

The treatment was continued for 3 hour. During this period, food and water was supplied ad libitum. All the doses were administered orally by the oral feeding needle. The effect of extract on blood glucose levels was estimated on overnight fasted rats on hour 0, 1, 2, and 3 by the method described before. The basal values are
those of the day on which extract was started to give. The general behaviors of the animals were recorded daily. The blood glucose level in (Mean ± S.E.M.) is shown in the Table 1 and Table 2.

Table 1: The Antihyperglycemic Effect of Ethanolic Extract of Leaves of Bauhinia Variegata on Alloxan Induced Diabetic Rats:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Basal value</th>
<th>4 th day</th>
<th>7 day</th>
<th>10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>90.46 ± 3.80</td>
<td>92.32 ± 2.92</td>
<td>92.32 ± 1.73</td>
<td>88.29 ± 3.44</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control (vehicle)</td>
<td>293.8 ± 5.27</td>
<td>286.9 ± 5.05</td>
<td>291.8 ± 5.41</td>
<td>289.4 ± 9.75</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide (10 mg)</td>
<td>285.86 ± 6.92</td>
<td>205.25 ± 7.06</td>
<td>183.18 ± 6.35</td>
<td>178.13 ± 6.20</td>
</tr>
<tr>
<td>D</td>
<td>Ethanolic extract (100 mg/kg)</td>
<td>291.76 ± 4.79</td>
<td>277.76 ± 5.65</td>
<td>266.23 ± 8.19</td>
<td>255.42 ± 7.71</td>
</tr>
<tr>
<td>E</td>
<td>Ethanolic extract (250 mg/kg)</td>
<td>284.48 ± 5.32</td>
<td>258.23 ± 6.66</td>
<td>255.85 ± 9.97</td>
<td>252.06 ± 9.19</td>
</tr>
<tr>
<td>F</td>
<td>Ethanolic extract (500 mg/kg)</td>
<td>287.48 ± 5.32</td>
<td>212.61 ± 5.07</td>
<td>198.36 ± 3.52</td>
<td>189.83 ± 3.31</td>
</tr>
</tbody>
</table>

Value are mean ± SEM, n=6, *P < 0.05, **P < 0.01 and ***P < 0.01 vs diabetic control

Table 2: The Antihyperglycemic Effect of Ethanolic Extract of Leaves of Bauhinia Variegata on Glucose Loaded Rats:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>1</td>
<td>Control (2mg/kg)</td>
<td>78.2 ± 3.20</td>
</tr>
<tr>
<td>2</td>
<td>Glibenclamide (120mg/kg)</td>
<td>76.5 ± 2.45</td>
</tr>
<tr>
<td>3</td>
<td>Bauhinia variegata Ethanolic extract (100 mg/)</td>
<td>72.2 ± 2.37</td>
</tr>
<tr>
<td>4</td>
<td>Bauhinia variegata Ethanolic extract (250 mg/)</td>
<td>73.3 ± 3.23</td>
</tr>
<tr>
<td>5</td>
<td>Bauhinia variegata Ethanolic extract (500 mg/)</td>
<td>79.3 ± 3.30</td>
</tr>
</tbody>
</table>

NS= not significant, **P< 0.01, show significant when compare with control
Statistical Analysis
The data were statistically evaluated using one way Anova. expressed as Mean ± S.E.M. followed by Tukey test using the Graph pad instant Demo (Data set 1.IS) version P. values of 0.05 or less were considered to be significant.

RESULT AND DISCUSSION
Ethanol extract of Bauhinia variegata leaves was subjected to antidiabetic activity in rats where alloxxan monohydrats (120 mg/kg, b.w, i.p) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compared to normal control groups. Ethanol extract of Bauhinia variegata (at 250 and 500 mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on 4th, 7th, and 10th day part treatment. The extract dose of 100 mg/kg also caused reduction in blood glucose level but the result was found statistically insignificant. The antihyperglycemic effect of ethanol extract at was found less effective than the reference standard. Glibenclamide produced a significant reduction in blood glucose compare to diabetic control. When the activity of extract was done by glucose tolerance test in glucose loaded rats, ethanol extract should significant effect on the blood glucose level but extract of 100mg/kg did not show the significant result. Ethanol extract 250mg/kg and 500mg/kg showed the significant decrease in blood glucose level.

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