EVALUATION OF ANTI-DIABETIC ACTIVITY OF Justicia adhatoda (Linn.) LEAVES IN DIABETIC WISTAR RATS

Roshan Kumar Mehta, Rupkala Thapa, Mukesh Kumar Chaudhary

ABSTRACT

Diabetes mellitus is defined as the increase in blood glucose level resulting from defects in insulin secretion, insulin action, or both. The present study was conducted to evaluate anti-diabetic activity of Justicia adhatoda (Linn.) leaves extract in diabetic Wistar rats. Justicia adhatoda, commonly known as vasaka is rich in phytochemicals like alkaloids, saponins, steroids, flavonoids and glycosides. It is used in asthma, jaundice, wound healing, typhus fever, cough, chronic bronchitis and inflammatory swellings treatment.

INTRODUCTION

The extract was obtained by maceration process with the use of methanol as solvent. 30 Wistar rats of 150-250 gm were used taken as study animal. Diabetes was induced by single intraperitoneal injection of Alloxan 150 mg/kg body weight. The methanolic extract of low dose (50 mg/kg) and high dose (100 mg/kg) were given orally for anti-diabetic activity. The standard drug glimepiride 0.5 mg/kg body weight was used.

MATERIAL AND METHODS

The extract treated 50mg/kg and 100mg/kg showed significant \( p<0.01 \) reduction in blood glucose level while the standard 0.5 mg/kg showed less significant \( p<0.05 \). The standard and extract showed less significant \( p<0.05 \) body weight recovery in comparison to negative control group. The extract 50 mg/kg, 100 mg/kg and standard showed significant \( p<0.01 \) improvement in food intake when compared to negative control group. The extract 100 mg/kg treatment showed significant \( p<0.01 \), highly significant \( p<0.001 \) improvement in water intake on 1st, 2nd and 3rd week respectively when compared to negative control group.

RESULTS

The present study concludes that the extract possesses potent and significant anti-diabetic activity of Justicia adhatoda leaves.

CONCLUSION

KEYWORDS

Justicia adhatoda, Anti-diabetic, Alloxan, Glimepiride, Methanolic extract

1. Department of Pharmacology, Universal College of Medical Sciences, Bhairahawa, Nepal.
2. Department of Pharmacy, Universal College of Medical Sciences, Bhairahawa, Nepal.
3. Department of Pharmacy, Crimson College of Technology, Butwal-11, Nepal.

https://doi.org/10.3126/jucms.v11i01.54641

For Correspondence
Roshan Kumar Mehta
Department of Pharmacology
Universal College of Medical Sciences
Bhairahawa, Nepal.
Email: roshan3mehta@gmail.com
INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Hyperglycemia and its attendant metabolic complications lead to various biochemical changes leading to complications such as nephropathy, retinopathy and peripheral neuropathy. Since 2000, the International Diabetes Federation (IDF) has reported the national, regional and global occurrence of diabetes. In 2019, 463 million of the population was suffering from diabetes globally which is 62% increment from 2009 (285 million). About 79% of adults with diabetes were found in low- and middle-income countries and 50.1% (232 million) of the cases remain undiagnosed leading to cause 4.2 million deaths. More than 1.1 million children and adolescents are suffering from type-1 diabetes while the proportion of people with type-2 diabetes is increasing at an alarming rate in most of the countries.

Alloxan (2, 4, 5, 6-tetraoxypyrimidine) is an oxygenated pyrimidine derivative available in aqueous solution as Alloxan hydrate. It is widely used diabetogenic agent used to induce type 1 diabetes in experimental animals due to its selective toxicity towards pancreatic ß-cells. The dose of Alloxan varies according to experimental animal species, route of administration (intravenous, intra-peritoneal or subcutaneous) and nutritional status.

Justicia adhatoda (Acanthaceae), is an important medicinal plant widespread throughout the tropical regions of Southeast Asia. It is native to Afghanistan, Assam, Bangladesh, East Himalaya, India, Myanmar, Nepal, Pakistan, Sri Lanka and Vietnam. In Nepal, it is distributed throughout the country up to the altitude of 1300 meters. The chemical constituents present in vaska are alkaloids, flavonoids, sterols, glycosides, tannins, proteins, phenols, etc. It has multiple uses in traditional, unani and ayurvedic system of medicines. It is used in the treatment of asthma, chronic bronchitis, cold cough and whooping cough. It is also used as antibacterial, anti-inflammatory, diuretic, antiperiodic and purgative. Synthetic derivative of vasicine (Bromhexine HCl) is used as expectorant.

Traditional medicines system is a holistic approach mainly focusing on prevention of disease, rejuvenation of the body systems and lengthening the life span of an individual. Locally available plants can be used to develop newer therapeutic agents after proper research and investigation. Justicia adhatoda is local plant available in Nepal with therapeutic activity.

The main aim of the study is to evaluate the anti-diabetic activity of methanolic extract of leaves of Justicia adhatoda. Therefore the present study is focused with the objective to provide background for minimizing adverse effects of conventional hypoglycemic drugs by using herbal medicine.

MATERIAL AND METHODS

Collection and authentication of plant material
The plant was collected from Ranigao, Bhairahawa. The herbarium was prepared with fresh plant material and was submitted for its identification sand certification. The certificate of plant identification was issued by Assistant professor, Mr. Rukmagat Pathak, Department of Horticulture and Plant Protection (IAAS, Paklihawa Campus).

Preparation of extract
The fresh leaves of Justicia adhatoda were collected, washed thoroughly with distilled water and shade dried completely. Then the leaves were grinded to fine powder using electrical grinder and allowed to pass through sieve size 80. The fine powder plant material was extracted with methanol in the ratio of 1:6 in terms of gm/ml in a suitable container. The container was plugged with cotton wool, wrapped in aluminium foil, shaken vigorously on periodic basis and allowed to stand for 14 days. The extract was filtered through Whatman’s No.1 filter paper and then condensed to dryness using rotary evaporator at 30°C. The thick extracted mass was then dried at 25°C using hot air oven and the extract was stored in refrigerator in reagent bottle at 4°C.

Animals
Wistar rats of either sex weighing between 150-250 g, 30 in number were used in present study. The animals were housed in cages under standard conditions (25 ± 2 °C, 55 ± 5 % relative humidity, and 12 h light and dark cycles) in the Department of pharmacology at Universal College of Medical Sciences. The animals were allowed free access to water and standard food. The care and handling of rats was in accordance with the internationally accepted standard guidelines for use of animals and the protocol was approved by IRC (Institutional Review Committee).

Preparation and Dose selection
Alloxan: Freshly dissolved in the normal saline and administered orally
Glimepiride: In the present study the dose of Glimepiride 0.5 mg/kg/day was taken as per the earlier studies and was administered orally
Justicia adhatoda methanolic extract: In the present study animals received methanolic extract of Justicia adhatoda 50 mg/kg (low dose) and 100 mg/kg (high dose) were taken as per the earlier studies and was administered orally

Induction of diabetes mellitus
Diabetes was induced in rats after an overnight fasting by single i.p. of 150 mg/kg of alloxan and 5% glucose solution was kept for 14 hours to prevent fatal hypoglycemia. The development of diabetes was checked by measuring glucose level after 2 days of Alloxan injection by using glucometer. Those showing blood glucose level of 200 mg/dl were used for the study.

Experimental design
The study was conducted for 28 days. The experimental animals were branched into 5 groups (N=6 Rats in each group) as follows:

- Group I: Normal control - Animals received balanced diet only
- Group II: Negative control - Diabetic animals received normal saline
- Group III: Standard - Animals received Glibenpiride 0.5 mg/kg/day.
- Group IV: Low dose - Animals received methanolic extract of Justicia adhatoda 50 mg/kg/day dissolved in normal saline.

Measurement of blood glucose level
After overnight fasting of animals, fasting blood glucose level was measured by using glucometer on 1st, 7th, 14th, 21st and 28th day.15

Measurement of physiological parameters
Body weight: Body weight of each animal was measured by using precision balance after overnight fasting on 1st, 7th, 14th, 21th and 28th day to measure either gain or loss of body weight.16

Food intake and water intake: Water and food intake were measured daily during the experimental period by using weighing balance and measuring cylinder respectively.17

Table 1. Phytochemicals present in Justicia adhatoda

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Phytosterols</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 2. Effect of methanolic extract Justicia adhatoda (MEJA) on blood glucose level (BGL) in Alloxan induced diabetic rats on different weeks of study period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>±0.76</td>
<td>±0.92</td>
<td>±0.97</td>
<td>±1.02</td>
<td>±1.16</td>
</tr>
<tr>
<td>Negative control</td>
<td>±1.03</td>
<td>±1.20</td>
<td>±1.16</td>
<td>±1.12</td>
<td>±1.21</td>
</tr>
</tbody>
</table>

Table 3. Effect of MEJA on body weight (gram) in alloxan induced diabetic rats on different weeks of study period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>±0.58</td>
<td>±0.63</td>
<td>±0.66</td>
<td>±0.70</td>
<td>±0.75</td>
</tr>
<tr>
<td>Negative control</td>
<td>±0.70</td>
<td>±0.77</td>
<td>±0.79</td>
<td>±0.83</td>
<td>±0.86</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, Justicia adhatoda was used to evaluate anti-diabetic activity. Preliminary phytochemical screening of the extract of Justicia adhatoda revealed the presence of alkaloids, flavonoids, saponins, tannins and glycosides. The present study showed that the methanolic extract of Justicia...
**ADHATODA LEAVES POSSESSES SIGNIFICANT ANTI-DIABETIC ACTIVITY.**

In the study, the BGL of negative control group is increased significantly more than the BGL of normal control group. The food intake of negative control group when compared to normal control group is reduced significantly more than the food intake of normal control group. The water intake of negative control group when compared to normal control group is reduced significantly more than the water intake of normal control group.

The body weight variation of animals during study period showed the persistent significant loss in body weight of negative control group when compared to normal control group throughout the study period due to effect of Alloxan. There is significant loss in body weight of standard, low and high dose group on 1st day of experiment but with continuous treatment there is significant weight gain (recovery) in standard (157.40±0.81 to 161.75±0.62), low dose (157.16±0.92 to 160.37±0.37) and high dose group (157.80±1.20 to 160.83±0.25) respectively. The significant recovery in body weight in standard, low and high dose group was observed which may be due to their protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis.

The above result is similar to previous study.14

The food intake of negative control group is increased significantly more on 1st and 2nd week and more significantly increased on 3rd and 4th week when compared to normal control group. The food intake of standard group is more in 1st week but decreased significantly more on 2nd, 3rd and 4th week when compared to negative control group. The food intake of low dose group is more in 1st and 2nd week but decreased significantly more on 3rd and 4th week. The food intake of high dose group is more on 1st week but decreased less significantly more on 2nd week and significantly increased on 3rd and 4th week. The water intake of negative control group is less significantly increased on 1st week and significantly more in 2nd week respectively in comparison to normal group. On 3rd and 4th week, the water intake of negative control group is more significantly increased when compared to normal control group. The low and high dose group also have high water intake in 1st week when compared with negative control group. The water intake of low dose group is reduced less significantly increased on 3rd week and more significantly increased on 4th week. The water intake of high dose group is reduced significantly more on 2nd and 3rd week and more significantly increased on 4th week respectively. The extract diminished water and food consumption when compared to diabetic control rats which is similar to previous study.19

The main limitation of the present study was that the main active chemical constituents which is responsible for anti-diabetic activity was not isolated and identified due to lack of availability of instruments and presence of multiple chemical constituents. This study opens the avenue to identify the actual phytoconstituent responsible for the anti-diabetic activity.

**CONCLUSION**

The results of the study showed that the methanolic extract of leaves of *Justicia adhatoda* possesses potent and more significant anti-diabetic activity. The presence of phyto-constituents like flavonoid, alkaloids, saponins, phenols may be responsible for hypoglycemic effect and considered as bioactive phytochemicals for antidiabetic activity. The result also suggests that the extract of leaves of *Justicia adhatoda* may be an alternative therapeutic drug for conventional pharmacotherapy of diabetes treatment.

**CONFLICT OF INTEREST**

None

**REFERENCES**


