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

Roshan Kumar Mehta,[1] Rupkala Thapa,[2] Mukesh Kumar Chaudhary[3]

ABSTRACT

INTRODUCTION

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.

MATERIAL AND METHODS

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.

RESULTS

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.

CONCLUSION

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

KEYWORDS

Justicia adhaotda, 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.

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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.[1] Hyperglycemia and its attendant metabolic complications lead to various biochemical changes leading to complications such as nephropathy, retinopathy and peripheral neuropathy.[2] 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.[3]

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.[4]

Justicia adhatoda (Acanthaceae), is an important medicinal plant widespread throughout the tropical regions of Southeast Asia.[5] It is native to Afghanistan, Assam, Bangladesh, East Himalaya, India, Myanmar, Nepal, Pakistan, Srilanka and Vietnam. In Nepal, it is distributed throughout the country up to the altitude of 1300 meters.[6] The chemical constituents present in vaska are alkaloids, flavonoids, sterols, glycosides, tannins, proteins, phenols, etc.[7] It has multiple uses in traditional, unani and ayurvedic system of medicines.[5] 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.[8] Synthetic derivative of vasicine (Bromhexine HCl) is used as expectorant.[9]

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 Ranigaon, 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 grinded 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.[10] The container was plugged with cotton wool, wrapped in aluminum 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 30ºC. The thick extracted mass was then dried at 25ºC using hot air oven and the extract was stored in refrigerator in reagent bottle at 4ºC.[11]

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 with a single dose of 150 mg/kg body weight, intraperitonealy (i.p.)

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.[12]

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 Glimepiride 0.5 mg/ kg/day [13]
- Group IV: Low dose - Animals received methanolic extract of Justicia adhatoda 50 mg/kg/day dissolved in normal saline [14]
-Group V: High dose - Animals received methanolic extract of Justicia adhatoda 100 mg/kg/day dissolved in normal saline [14]

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]

Statistical analysis : Results of experiment was expressed as mean ±SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnet's Multiple range test using Graph Pad Prism version 5. The values of $p<0.05$ was considered as significant.

Table 1. Phytochemicals present in Justicia adhatoda

S.N.	Test	Result
1	Alkaloids	Positive
2	Saponins	Positive
3	Glycosides	Positive
4	Phyto-sterols	Positive
5	Phenols	Positive
6	Tannins	Positive

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

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	95.5 ±2.27	97.67 ±2.81	94.50 ±2.01	96.17 ±2.43	96.50 ±1.50
Negative control	261.67 ±4.81###	273.33 ±3.04###	276.17 ±2.05###	280.83 ±2.56###	281.67 ±3.04###
Standard (GLM)	262.50 ±2.87	271.50 ±2.46	267.33 ±1.85*	193.83 ±3.87**	167.33 ±2.61***
Low dose MEJA	265.17 ±2.88	272.83 ±2.62	269.33 ±2.77	200.17 ±2.53**	188.16 ±2.71**
High dose MEJA	268.50 ±2.91	271.33 ±2.18	270.67 ±1.79	205.17 ±4.49**	186.33 ±1.92***

All values are expressed as Mean±SEM (N=6). Significant at ###$p<0.001$ vs normal control group and significant at ***$p<0.001$, **$p<0.01$ vs negative control group by One Way Anova followed by Dunnet's multiple comparison test.

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

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	161.56 ±2.34	161.86 ±2.54	162.10 ±2.30	162.72 ±2.15	162.75 ±1.94
Negative control	156.78 ±1.73##	155.22 ±2.04##	154.51 ±2.23###	154.10 ±2.19###	154.10 ±2.19###
Standard (GLM)	157.40 ±0.81	156.33 ±0.96	158.15 ±0.91	161.22 ±0.63*	161.75 ±0.62*
Low dose MEJA	157.16 ±0.92	157.44 ± 0.76	158.26 ±0.53	158.73 ±0.60	160.37 ±0.37*
High dose MEJA	157.80 ±1.20	158.18 ±0.83	159.48 ±0.43	160.58 ±0.28*	160.83 ±0.25*

All values are expressed as Mean±SEM (n=6). Significant at ###$p<0.001$, ##$p<0.01$ vs normal control group and significant at *$p<0.05$ vs negative control group by One Way Anova followed by Dunnet's multiple comparison test.

Table 4. Effect of MEJA on food intake in Alloxan induced diabetic rats on weekly basis during study period

Groups	Week 1	Week 2	Week 3	Week 4
Normal control	82.4 5±2.37	85.84 ±1.95	84.34 ±1.98	82.23 ±2.75
Negative control	107.21 ±2.15##	103.92 ±1.46##	108.71 ±1.70###	108.75 ±2.60###
Standard (GLM)	102.29 ±1.84	97.05 ±57**	91.24 ±2.10**	89.89 ±2.43**
Low dose MEJA	105.64 ±2.58	101.23 ±1.83	97.52 ±1.12**	91.12 ±2.24**
High dose MEJA	103.68 ±2.80	98.15 ±0.68	97.65 ±1.22**	92.09 ±1.80**

All values are expressed as Mean±SEM (n=6). Significant at ###$p<0.001$, ##$p<0.01$ vs normal control group and significant at **$p<0.01$, *$p<0.05$ vs negative control group by One Way Anova followed by Dunnet's multiple comparison test.

Table 5. Effect of MEJA on water intake in Alloxan induced diabetic rats on weekly basis during study period

Groups	Week 1	Week 2	Week 3	Week 4
Normal control	16.08 ±0.34	14.33 ±1.17	15.83 ±0.19	13.17 ±0.93
Negative control	19.17 ±0.60#	19.67 ±0.90##	20.42 ±0.30###	20.12 ±0.29###
Standard (GLM)	17.17 ±0.28*	16.33 ±0.45*	16.50 ±0.39***	14.17 ±0.37***
Low dose MEJA	18.67 ±0.77	18.33 ±0.56	18.92 ±0.38*	17.58 ±0.18***
High dose MEJA	18.75 ±0.78	17.83 ±0.80**	17.08 ±0.34**	16.25 ±0.28***

All values are expressed as Mean±SEM (N=6). Significant at ###$p<0.001$, ##$p<0.01$, #$p<0.05$ vs normal control group and significant at ***$p<0.001$,**$p<0.01$,*$p<0.05$ vs negative control group by One Way Anova followed by Dunnet's multiple comparison test.

DISCUSSION

In the present study, *Justicia adhatoda* was used to evaluate *antidiabetic* 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 more significantly ###p<0.001 when compared to normal control on 1st, 7th, 14th, 21st and 28th days due to Alloxan administration. There is no significant reduction of BGL of standard, low and high dose group on 1st and 7th day when compared to negative control group. There is less significant reduction *p<0.05 in BGL of standard group in comparison to negative control on 14th day and on continuous treatment the significant *p<0.01effect is seen on 21st and 28th day in comparison to negative control. The BGL of low and high dose groups also showed significant *p<0.01 reduction on 21st and 28th day when compared to negative control group. Therefore the significant anti-diabetic effect is observed. The possible mechanism by which plants extracts brings anti-hyperglycemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue and was obvious by increased level of insulin in diabetic rats when treated with plants extracts. The above result is similar to previous study.[14]

The body weight variation of animals during study period showed the persistent significant ##p<0.01, ###p<0.001 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 their protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. The above result is similar to previous study.[18]

The food intake of negative control group is increased significantly #p<0.01 on 1st and 2nd week and more significantly ##p<0.001on 3rd and 4th week when compared to normal control group. The food intake of standard group is more in 1st week but decreased significantly **p<0.01 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 **p<001 on 3rd and 4th week. The food intake of high dose group is more on 1st week but decreased less significantly *p<0.05 on 2nd week and significantly **p<0.01 on 3rd and 4th week. The water intake of negative control is less significantly #p<0.05 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 ###p<0.01 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 *p<0.05 on 3rd week and more significantly *p<0.001 on 4th week. The water intake of high dose group is reduced significantly *p<0.01 on 2nd and 3rd week and more significantly ***p<0.001 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 chemicals 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

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