EVALUATION OF ANTI-DIABETIC ACTIVITY OF DATURA STRAMONIUM LEAVES EXTRACT IN DIABETIC SWISS ALBINO MICE

Roshan Kumar Mehta,[1] Ramesh Kohar,[2] Vivek Kumar Mehta,[3] Narayan Gautam,[4] Pradyumna Chaudhari,[2] Mukesh Kumar Chaudhary[5]

ABSTRACT

INTRODUCTION

Diabetes mellitus is defined as the increase in blood glucose levels associated with a relative insulin deficiency, insulin resistance or both. *Datura stramonium* is commonly known as Jimson weed or Datura belongs to family Solanaceae containing different types of alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides.

MATERIAL AND METHODS

Extraction was done by maceration process using ethanol as solvent. Swiss albino mice of 20-35 gram were taken as study animal. Diabetes was induced by using Alloxan 20 mg/kg body weight and Glibenclamide 10 mg/kg body weight was used as standard. Ethanolic extract of low dose (50 mg/kg) and high dose (100 mg/kg) were subjected for Anti-diabetic activity.

RESULTS

The extract treated 50 mg/kg, 100 mg/kg and standard 10 mg/kg showed highly significant reduction in blood glucose level (BGL) on 21st and 28th day respectively when compared to negative control group. Extract treated 100 mg/kg showed significant improvement on high density lipoprotein when compared to negative control group. Extract treated 50 mg/kg, 100 mg/kg and standard 10 mg/kg showed highly significant improvement in lipid profile when compared to negative control group. Extract treated 50 mg/kg, 100 mg/kg and standard 10 mg/kg showed significant body weight gain when compared to negative control group. Extract treated 50 mg/kg and 100 mg/kg showed significant improvement in food intake. Extract treated 50 mg/kg and 100 mg/kg showed highly significant but standard treated 10 mg/kg showed significant improvement in water intake.

CONCLUSION

The present study concludes that the extract possesses potent and significant anti-diabetic activity of *Datura stramonium* leaves.

KEYWORDS

Datura stramonium, Anti-diabetic, Alloxan, Glibenclamide

1. Department of Pharmacology, Universal College of Medical Sciences, Bhairahawa, Nepal
2. Department of Pharmacy, Universal College of Medical Sciences, Bhairahawa, Nepal
3. Biratnagar Oro Dental Care, Biratnagar, Nepal
4. Department of Biochemistry, Universal College of Medical Sciences, Bhairahawa, Nepal
5. Department of Pharmacy, Crimson College of Technology, Butwal-11, Nepal

https://doi.org/10.3126/jucms.v12i01.65584

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

INTRODUCTION

Diabetes mellitus (DM) is defined as elevation of blood glucose levels. High blood glucose levels are also associated with the decreasing levels of High-Density Lipoprotein (HDL) cholesterol and increasing of Low-Density Lipoprotein (LDL) cholesterol, thus increasing risk of coronary heart diseases.[1] The estimated health expenditure of global diabetes was 966 billion USD in 2021 and the projected expenditure will reach to 1,054 billion USD by 2045.[2] Diabetes is becoming the third 'killer' of mankind beside cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality.[3] Hyperglycaemia and its attendant metabolic complications lead to various biochemical changes leading to complications like nephropathy, retinopathy and peripheral neuropathy.[4] The treatment for diabetes consists of the administration of either insulin or other hypoglycaemic agents with recommendations for dietary control and physical exercise. But these hypoglycaemic agents can cause adverse effects such as hypoglycaemia, gastrointestinal disorders, renal toxicity and hepatotoxicity. To overcome these limitations medicinal plants have become highly important in primary health care. In fact, the WHO estimated that 80% of the population living in the developing countries like Nepal relies exclusively on herbal medicine for their primary health care. Many of these plant species have largely been used in traditional medicinal systems over hundreds of years to treat DM.[1]

D. stramonium is commonly known as Jimson weed or *Datura* belonging to family solanaceae, probably originated in Caspian Sea territories and spreaded to Europe in the first century and is cultivated in Germany, France, Hungary, South America and throughout the world.[5,6] D. stramonium contains different type of alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides.[7] Dry leaves contain less than 0.25 %, stem contain 0.3-0.4 % and seeds contain more than 25 % of alkaloids of stramoniu.[8,9] Leaves extract contain different types of secondary metabolites such as glycosides, phenol, lignins, saponins, sterols and tannins. The constituents of D. stramonium are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. The phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory effect.[10] So our study believe that it may have anti-diabetic activity potential which will be fruitful source of inspiration for the development of new drug to treat diabetes diseases.

MATERIAL AND METHODS

The present study was carried out from December 2019 to March 2020 in the department of pharmacology, Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Nepal, after taking approval from Institutional Review Committee with IRC No: UCMS/IRC/228/19.

Animals

Swiss albino mice of either sex weighing between 24-35 gm of eight weeks age were used in this study. The animals were housed in cages under standard conditions (25 ± 2 °C, 55 ± 5 % relative humidity, and 12 h light and dark cycles). The animals were allowed free access to water and standard food. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals and the protocol was approved by Institutional Review Committee, UCMS, Bhairahawa, Nepal.

Collection and authentication of plant material

Plant materials were collected from Siyari Gaon Palika -5, Banghusari, Rupandehi, Nepal. Herbarium was prepared with fresh plant material and was submitted for identification and certification of plant. Certificate was issued by Assistant professor Subodh khanal, Department of Soil and Environment Science, Institute of Agriculture and Animal Science, Paklihawa Campus, Bhairahawa.

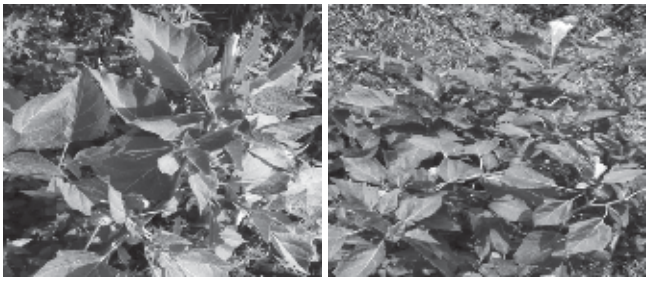


Figure 1. ***Datura stramonium*** **leaves**

Extraction

The coarse powder and 95% ethanol was taken in the ratio of 1:10 in terms of gm/ml. 250 gm powder was soaked in ethanol in a flask. The flask was plugged with cotton wool, wrapped in aluminium foil, shaken vigorously on periodic basis and allowed to stand for 7 days. The extract obtained was evaporated to viscous semisolid mass using a rotary evaporator. The obtained extract was stored in refrigerator in reagent bottles at 4^{o}C and percentage yield was calculated.[11]

Induction of diabetes mellitus

All mice were fastened an overnight. Then the body weight and fasting blood glucose of each mice was measured. Diabetes was induced by single intraperitoneal injection of freshly prepared 20 mg/kg, body weight alloxan dissolved in normal saline and 5% glucose solution was kept for 24 hours to prevent fatal hypoglycemia. The food and water were provided to the animals. 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 more than 200 mg/dL were included in the study.[12,13]

Experimental design[13, 14]

The drug glibenclamide and extracts were given orally. The study was conducted for 28 days. The experimental animals were branched into 5 groups (n=6) as follows:
Group I: Normal control- Received balanced diet only
Group II: Negative control- Received only vehicle i.e 1% tween 80, 10 mL/kg distilled water
Group III: Standard received- Received glibenclamide, 10 mg/kg/day
Group IV: Low dose- Received extract 50 mg/kg/day, dissolved in tween 80
Group V: High dose- Received extract 100 mg/kg/day, dissolved in tween 80
Tween 80 increases the extract solubility like a surfactant.

Measurement of blood glucose level

After overnight fasting of animals, fasting blood glucose level was measured by using glucometer and strips of glucometer by tail vein pricking method on 1st, 7th, 14th, 21st and 28th days regularly.[15]

Measurement of lipid profile
On 28th day blood was collected by heart puncturing method. The collected blood was allowed to coagulate at room temperature for 30 mins. The serum was separated by centrifugation at 4000 rpm for 20 min at 4˚ C in biochemistry laboratory of UCMS. The separated serum was analysed for TC, TG, HDL, LDL and VLDL levels on last day in the laboratory.[16, 17]

Measurement of physiological parameters
a) Body weight
Body weight of each animal was measured by using electronic balance after overnight fasting on day 1, 7, 14, 21 and 28 to measure either body weight gain or loss regularly.[15]
b) Food intake and water intake
Food and water intake were measured daily during the experimental period by using electronic weighing balance and measuring cylinder respectively.[18]

Statistical analysis
Results of experiment were plotted with graph pad prism 5 and using Microsoft excel, 2007 values were expressed as mean ± SEM. Statistical analysis were performed using one-way ANOVA followed by Dunnet's multiple comparision test using graph pad prism 5. The value of $p < 0.05$ was considered as significant.

RESULTS

Blood glucose level
The BGL of negative control group was increased more significantly when compared to normal control. There was significant reduction of BGL of standard and high dose group respectively when compared to negative control group on 14th day of treatment. With continuous treatment on 21st and 28th day BGL was reduced in standard, low dose and high dose group more significantly when compared to negative control group.

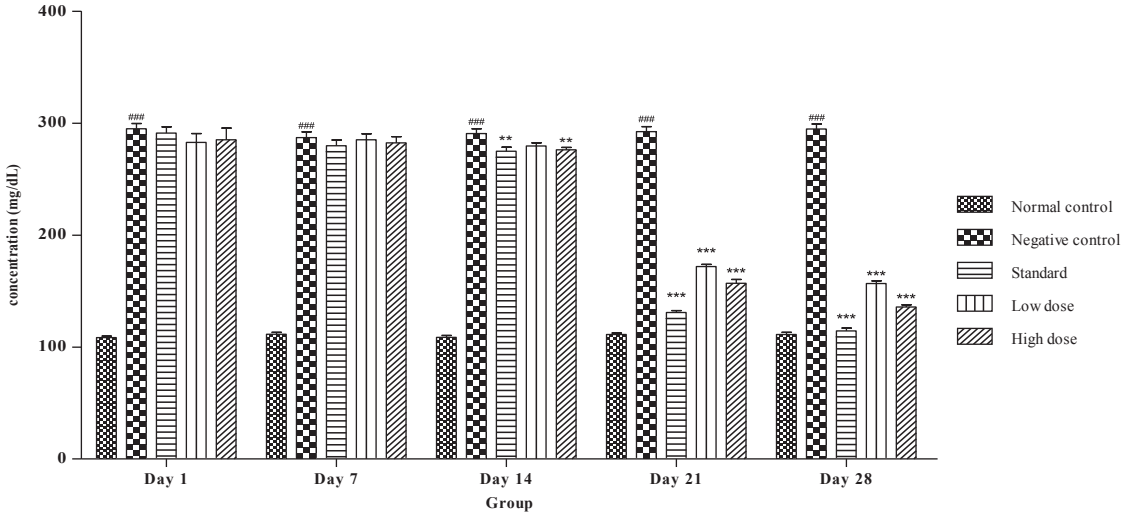
Lipid profile
In our study, the TC, TG, LDL and VLDL are more significantly increased but HDL is significantly decreased in negative control group when compared to normal control group. The TC, TG, LDL and VLDL are decreased more significantly in standard group, low dose group, and high dose group when compared to negative control group. HDL is significantly increased in high dose group and nonsignificantly increased in standard group and low dose group when compared to negative control.

Physiological parameters
The negative group showed significant loss in body weight but standard, low dose and high dose group showed significant gain (recovery) in body weight on complete treatment. The food intake by low dose and high dose group was found significantly less during study period. The water intake by standard, low dose and high dose group showed significantly less than negative group in every week of study.

DISCUSSION

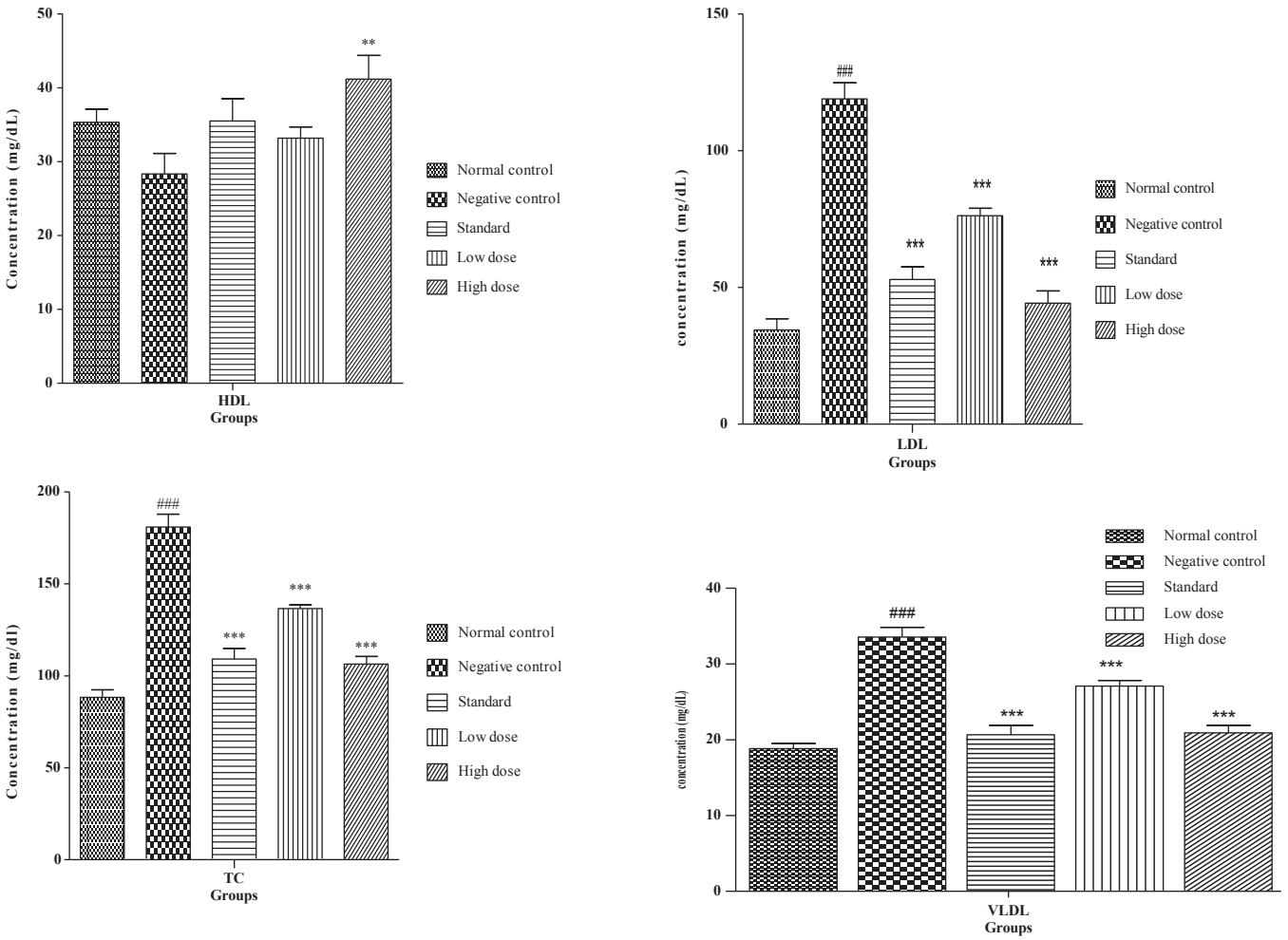
Diabetes mellitus is one of the most challenging metabolic disorders which affect essential biochemical pathways in the body such as carbohydrate, protein and lipid metabolism. Alloxan 20 mg/kg body weight via i.p. route is considered most effective dose for the induction of diabetes.[13,19] Many study claimed that the phytochemicals such as flavonoid, phenols, terpenoids, alkaloids, saponines, tannins and amines have significant antidiabetic activity.[20]

In our study the BGL of negative control group was increased more significantly (p<0.001) when compared to normal control on all days. There was significant (p<0.01) reduction of BGL of standard and high dose group respectively when compared to negative control group on 14th day of treatment. With continuous treatment on 21st and 28th day BGL was reduced in standard, low and high dose group more significantly (p<0.001) when compared to negative control



(Level of significance: ###/*** p<0.001, ##/** p<0.01 & #/* p<0.05)

Figure 2. Bar diagram representing effect of EEDS on blood glucose level in Alloxan induced diabetic mice on different weeks of study period



(Level of significance: ###/*** $p<0.001$, ##/** $p<0.01$ & #/* $p<0.05$)

Figure 3. Bar diagram representing Effect of EEDS on HDL, TC, TG, LDL and VLDL in Alloxan induced diabetic mice.

group. This indicates that the ethanolic extract of *Dadura stramonium* leaves possesses significant antidiabetic effect. The mechanism of antidiabetic effects may be due to presence of well-known antioxidant phytoconstituents like flavonoids, polyphenols, and tannins that acts as free radical scavengers. The mechanism of action of these antioxidants is because of an insulin mimetic effect on the peripheral tissues by either stimulation of regeneration process or release of pancreatic secretion of insulin from undamaged or existing β-cells of pancreas.[13]

The change on serum lipid profile in diabetic rats was studied in order to elucidate the possible effect of *Azadirachta indica* leaf extract. It was observed that *A.indica* leaf extract significantly reduced the TC, TG, LDL and VLDL levels in serum of streptozotocin induced diabetic rats but HDL levels remained unchanged.[21] In our present study, the TC, TG, LDL and VLDL levels are more significantly ($p<0.001$) increased but HDL is significantly decreased in negative control group when compared to normal control group. The TC, TG, LDL and VLDL levels are decreased more significantly ($p<0.001$) in standard group, low dose

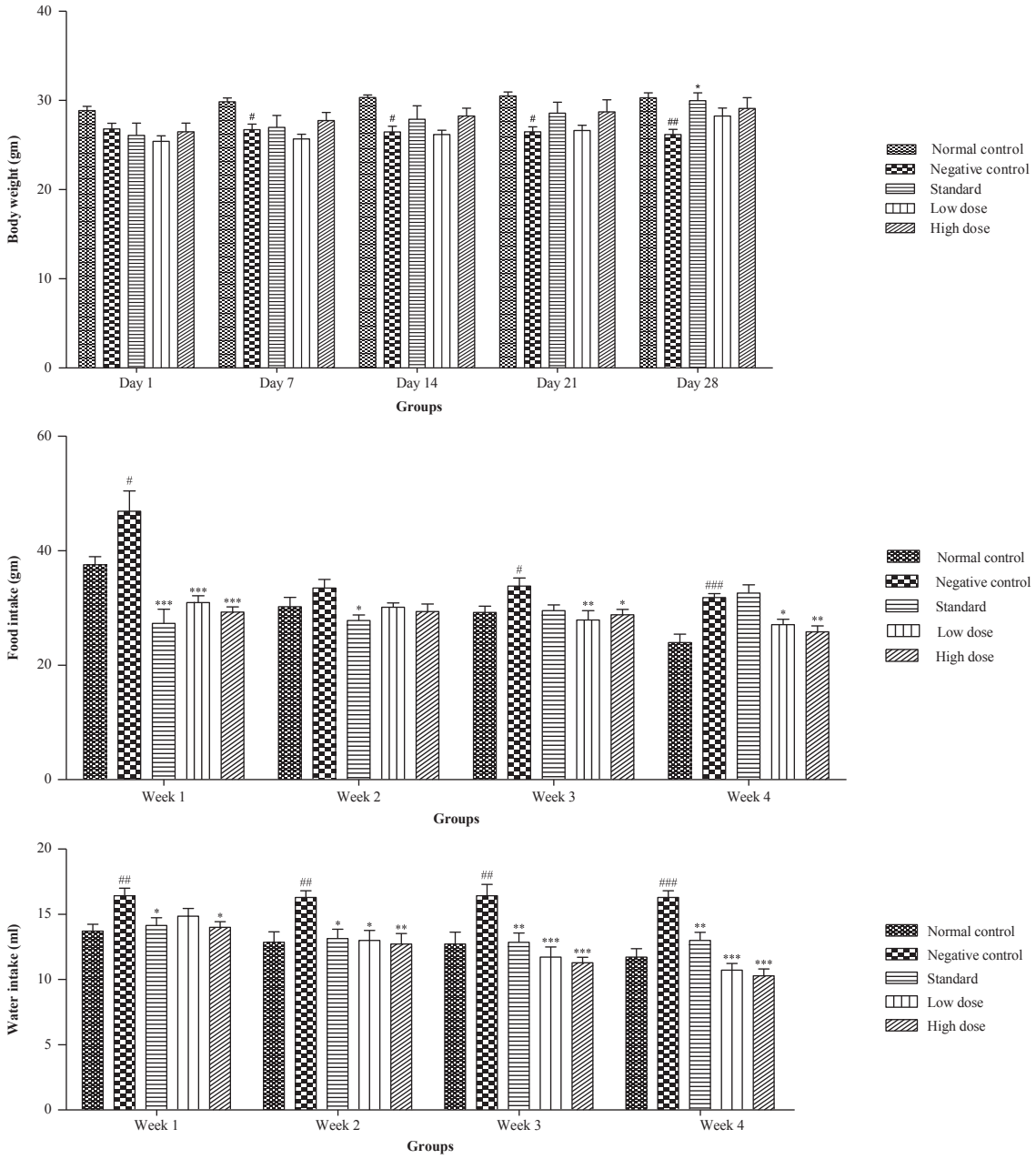


Figure 4. Bar diagram representing effect of EEDS on body weight, food intake and water intake in Alloxan induced diabetic mice

group, and high dose group when compared to negative control group. HDL is significantly (P<0.01) increased in high dose group and non-significantly increased in standard group and low dose group when compared to negative control. As our above result, the previous study also showed significant increase in HDL level and decrease in TC, TG, LDL and VLDL level in leaves extract treated group.[15] This indicates that the extract of *Datura stramonium* possed significant effect on lipid profile.

The study of body weight loss or gain of animals during study period showed that there is significantly (p<0.05, p<0.01) persistent loss in body weight of negative control group throughout study period and 4th week respectively as compared to normal control group. Standard dose, low dose and high dose group showed significant loss in body weight in 1st day of experiment but with continuous treatment showed significant weight gain (recovery) in standard (26.08±1.37 to 29.98±0.88), low dose (25.42±1.62 to 28.25±0.90) and high dose group (26.48±0.97 to 29.10±1.26) respectively on complete treatment. The significant recovery in body weight in high dose, standard and low dose group may be due to improved level of insulin in these groups. The above result is similar to previous study.[22, 23]

The changes on food intake and water intake in alloxan induced diabetic rats were studied in order to elucidate the possible effect of Justicia adhatoda leaves extract. It was

observed that Justicia adhatoda leaves etract showed significant improvement in food intake and water intake.[24] In our study food intake by negative control group is less significantly (p<0.05), more significantly (p<0.001) high in 3rd and 4th week respectively. Food intake by standard group is nonsignificant, by low dose group is significantly (p<0.01) and by high dose group is less significantly (p<0.05) lower when compared with negative control in 3rd week. Standard group have nonsignificantly more, low dose group have less significantly (p<0.05) low and high dose group have significantly (p<0.01) low food intake in 4th week.

Water intake by negative control group is significantly (p<0.01) high in 1st, 2nd and 3rd week respectively and more significantly (p<0.001) high in 4th week when compared to normal control group. Both standard group and high dose group have less significantly (p<0.05) and low dose group have non-signifiacntly low water intake in 1st week when compared to negative control group. In 2nd week standard and low dose group have less significantly (p<0.05) low but high dose group have significantly (p<0.01) low water intake when compared to negative control group. As this in 3rd and 4th week standard group have significantly (p<0.01) low but both low dose and high dose group have more significantly (p<0.001) low water intake when compared to negative control group. As per the previous study our study also showed significant improvement in food intake and water intake result. The study confirmed that plant extract could be used effectively to manage diabetes.

CONCLUSION

The results of the study showed that the ethanolic leaves extract of *D. stramonium* possesses potent and more significant anti-diabetic activity. The phytoconstituents such as flavonoid, phenols, terpenoids, alkaloids, saponines and tannins may be concidered as bioactive phytochemicals responsible for anti-diabetic activity. The antidiabetic activity may also be due to the antioxidant property of the ethanolic leaves extract of the plant. The results also suggest that the leaves extract of *Datura stramonium* may serve as an alternative therapeutic drug for conventional pharmacotherapy of diabetes.

ACKNOWLEDGEMENT

We would like to thank Assistant Professor Subodh Khanal, Course in-charge medicinal and aromatic plants, Institute of Agriculture and Animal Science, Paklihawa Campus, Bhairahawa, Rupandehi, Nepal for plant identification. We also thank the administration and Pharmacy department of Universal College of Medical Sciences, Bhairahawa for all the support during this research.

CONFLICT OF INTEREST

None

REFERENCES

1. Sudasinghe HP, Peiris DC. Hypoglycemic and hypolipidemic activity of aqueous leaf extract of Passiflora suberosa L. Peer J. 2018;6:e4389.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice. 2022;183:109119.
3. Li W, Zheng H, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. Journal of Ethnopharmacology. 2004;92(1):1-21.
4. KD T. Essentials of Medical Pharmacology. 7th edition. Ansari Road, Daryaganj, New Delhi 110002, India: Jaypee Brothers Medical Publishers (P) Ltd; 2015.
5. Stace C. New flora of the British Isles: Cambridge University Press; 2010.
6. Soni P, Siddiqui AA, Dwivedi J, Soni V. Pharmacological properties of Datura stramonium L. as a potential medicinal tree: an overview. Asian Pacific Journal of Tropical Biomedicine. 2012;2(12):1002-1008.
7. Shagal MH MUaLA. Pharmacological justification for the ethnomedical use of datura stramonium stem-bark extract in treatment of diseases caused by some pathogenic bacteria. Int Res Pharm Pharmacol. 2012;2(1):016-019.
8. Kulkarni K. Handbook of Experimental Pharmacology. 3rd Editon. Hilton and Company, New Delhi; 2005:182-183.
9. Gaire BP. Monograph on Datura stramonium. 1st Edition. Kaski, Nepal: Pokhara University Press; 2008.
10. Nain J, Bhatt S, Dhyani S, Joshi N. Phytochemical screening of secondary metabolites of Datura stramonium L. International Journal of Current Pharmaceutical Research. 2013;5(2):151-153.
11. Banso A, Adeyemo S. Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, Bacopa monnifera and Datura stramonium. Biokemistri. 2006;18(1):39-44.
12. Merina AJ, Sivanesan D, Begum VH, Sulochana N. Antioxidant and hypolipidemic effect of Plumeria rubra L. in alloxan induced hyperglycemic rats. Journal of Chemistry. 2010;7(1):1-5.
13. Tafesse TB, Hymete A, Mekonnen Y, Tadesse M. Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice. BMC Complementary and Alternative Medicine. 2017;17(1):243.
14. Abbas DA. Analgesiac, anti-inflammatory and antidiarrhoeal effects of Datura stramonium hydroalcoholic leaves extract in mice. IJRRAS. 2013;14(1):193-9.

15. Ben Younes A, Ben Salem M, El Abed H, Jarraya R. Phytochemical screening and antidiabetic, antihyperlipidemic, and antioxidant properties of anthyllis henoniana (Coss.) Flowers extracts in an Alloxan-induced rats model of diabetes. Evidence-Based Complementary and Alternative Medicine. 2018;2018:1-14.

16. Nagappa AN, Thakurdesai, PA, Rao NV and Singh, J. Antidiabetic activity of Terminalia catappa Linn fruits. Journal of Ethnopharmacology. 2003;88(1):45-50.

17. Yadav H, Jain S, Sinha P. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition. 2007;23(1):62-8.

18. Sudasinghe HPaP, D.C. Hypoglycemic and hypolipidemic activity of aqueous leaf extract of Passiflora suberosa L. Peer J. 2018;6:e4389.

19. Jothi M, BSV AK, Parameswari C, Vincent S, Sivasubramanian S. Antidiabetic activity of Cinnamomummacrocarpum Hook. f. leaves on alloxan-induced diabetic swiss albino mice. International Journal of Plant, Animal and Environmental Sciences. 2015;5(3):16-9.

20. Bharti SK, Krishnan S, Kumar A, Kumar A. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. Therapeutic advances in Endocrinology and metabolism. 2018;9(3):81-100.

21. Chattopadhyay R, Bandyopadhyay M. Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African Journal of Biomedical Research. 2005;8(2):101-4.

22. Hammeso WW, Emiru YK, Ayalew Getahun K, Kahaliw W. Antidiabetic and antihyperlipidemic activities of the leaf latex extract of Aloe megalacantha baker (Aloaceae) in streptozotocin-induced diabetic model. Evidence-Based Complementary and Alternative Medicine. 2019;2019:1-9.

23. Belayneh YM, Birru EM, Ambikar D. Evaluation of hypoglycemic, antihyperglycemic and antihyperlipidemic activities of 80% methanolic seed extract of Calpurnia aurea (Ait.) Benth.(Fabaceae) in mice. Journal of Experimental Pharmacology. 2019;11:73-83.

24. Mehta RK, Thapa R, Chaudhary MK. Evaluation of anti-diabetic activity of Justicia adhatoda (linn.) leaves in diabetic wistar rats. Journal of Universal College of Medical Sciences. 2023;11(1):50-54.