IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2015 Vol.:4, Issue:2

Antidiabetic Effects of *Clerodendrum inerme* (L) Gaertn

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ISSN 2349-7203
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Submission: 1 September 2015
Accepted: 7 September 2015
Published: 25 September 2015

Keywords: antihyperglycemic, streptozotocin, normoglycemic, *Clerodendrum inerme*

ABSTRACT

The objective of the present study was to evaluate the antihyperglycemic activity of aerial parts of *Clerodendrum inerme* (L) Gaertn. The plant extracts were found with low toxicity. The methanolic extract of the plant at the dose of 400 mg/kg body weight produced a significant decrease in fasting blood glucose level by 54.32% with respect to initial FBG level after 10 hours of treatment. In the oral glucose tolerance test this extract at the dose level of 400 mg/kg decreased hyperglycemia was found to be potent in restoration of the elevated glucose levels to normal, thereby indicating good antihyperglycemic activity of the extract of *Clerodendrum inerme.*

INTRODUCTION

Medicinal plants continue to provide valuable therapeutic agents, both in modern & traditional medicine. As powders, extracts, decoctions or infusions, plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities for the control, management and/or treatment of a variety of ailments. The current worldwide trends towards utilisation of plant derived natural remedies have therefore, created a dire need for accurate & up to date information on the properties, uses, efficacy, safety and quality of medicinal plants products[1].

Diabetes mellitus is a chronic metabolic disorder, mainly characterized by disruption in carbohydrates, protein & fat metabolism caused by the complete or relative deficiency of insulin action[2]. Besides hyperglycemia, several other factors including dyslipidemia or hyperlipidemia are involved in the development of micro & macro vascular complications of diabetes which are the major cause of morbidity & death[3]. Several oral hypoglycemic agents are the primary forms of treatment of diabetes. However prominent side effects of such drugs are the main reason for an increased number of people seeking alternate therapies that may have less severe or no side effects[4].

Clerodendrum inerme (L) Gaertn (verbenaceae) well known under vernaculars as Glory Bower, Garden quinine in English & phuljholi in odia[5]. Different parts of the plant are used in the ayurvedic medicine for the treatment of rheumatism, skin disease, beri beri and tumors[6]. Traditionally, in Indian medicine, the tincture or decoction of the leaves are used for the treatment of remittent & intermittent fevers[7].

It is reported to have antibacterial, hepatoprotective, anticancer, antioxidant & antihypertensive properties[8,9,10].

Various species of the plant like *C. phlomidis, C. calamitosum, C. trichotomum* have been reported to have antidiabetic & antihypertensive properties[11].

The objective of the present investigation is to evaluate the antihyperglycemic activity of the aerial parts *of Clerodendrum inerme* in streptozotocin -induced diabetic rats.

MATERIALS & METHODS

Plant materials

The plant materials of *Clerodendrum inerme (L) Gaertn* were collected from Utkal University campus, Bhubaneswar during the month of October. The plant was identified by DR.K.B.Satpathy, Head, P. G. Dept. of Botany, Utkal University, Bhubaneswar. A voucher specimen (SVN-572) was deposited in the departmental herbarium.

Preparation of plant extracts

The aerial parts of the plant *Clerodendrum inerme (L)* Gaertn were collected in bulk quantity, dried under shade and ground to a coarse powder. The coarse powder was extracted successively with petroleum ether (60-80^{0}C) & methanol in a Soxhlet apparatus. The percentage yields of the methanol and petroleum ether extracts were found to be 25.78 & 1.92 respectively.

Drugs & chemicals

Streptozotocin (Merck) & glibenclamide (S.N chemicals) were used for these investigations. All other chemicals & solvents used were of analytical grade & obtained from S. D. Fine Chem. Ltd., Mumbai.

Preliminary phytochemical screening[12,13]

Preliminary phytochemical screening of petroleum ether extract (CIP) & methanolic extract (CIM) of *Clerodendrum inerme* was carried out by the standard methods. Small amount of both the extracts were appropriately treated to prepare sample solutions & then subjected to phytochemical tests. Qualitative chemical tests were carried out in order to identify the phytoconstituents present in various extracts as per the standard procedures & using suitable reagents. These were identified by the characteristic colour changes.

Animals

Wistar albino rats (200-220 g) and albino mice (20-25 g) of both sexes were selected & procured from the animal supplier. They were kept in clean propylene cages. They were allowed free access to food (standard pellets) and water *ad libitum*. They were maintained under standard

condition at a temperature of 25±2^{0}C with a 12/12 hour-light and dark cycle. Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethical committee of S.I.P.S Jharpokharia, Mayurbhanj vide approval no. A1/15/IAEC/SIPS.

Acute toxicity test'[14,15,16]

The lethal dose (LD_{50}) of the extracts was assessed by using albino mice of either sex weighing about 20-25 g. The animals were fasted overnight prior to the experimental procedures with free access to water. Different doses of extracts were separately administered to different groups of animals by intraperitonial route. The LD_{50} was calculated by Miller & Tainter. 1/10th of the lethal dose was taken as the screening dose.

Study on healthy normal animals (Normoglycemic)

The rats were kept fasting overnight with free access to water. The fasting blood glucose (FBG) level of each animal was determined at the beginning of the experiment. The animals of control group received only the vehicle & the test groups were treated with the suspension of methanolic extract and petroleum ether extract of *Clerodendrum inerme* at different dose levels. Glibenclamide (10 mg/kg body wt.) was administered as the standard drug. Blood sugar levels were determined at 0, 1, 2, 4 & 8 hours after the oral administration of test samples to assess the effect of the test samples on normoglycemic rats.

Study on glucose loaded animals (OGTT) oral glucose tolerance test:

The overnight fasted rats were divided into different groups (n=6). The first group received only vehicle. Glibenclamide was administered as the standard drug to the second group. The methanolic (CIM) & petroleum ether (CIP) extracts of *Clerodendrum inerme* (CI) were administered to the animals of the test groups at the dose of 400 mg/kg body wt.

The rats of different groups were loaded with glucose (2 g/kg p.o.) 30 mins. after the administration of test substances. Blood glucose levels were measured at 1, 2 and 4 hrs after glucose loaded to assess the effect of different doses of extract on blood glucose level of the glucose loaded animals.

Induction of experimental diabetes:

The rats were kept fasting for 24 hrs and thereafter diabetes was induced by intraperitonial injection of streptozotocin, freshly dissolved in citrate buffer (Ph 4.5) immediately before use. Streptozotocin was given at dose of 65 mg/kg of body wt. In order to avoid the streptozotocin induced hypoglycemic mortality, 5% glucose solution was given for 24 hrs. to streptozotocin treated rats. After 72 hrs of streptozotocin administration, the blood glucose levels were measured and the rats showing blood glucose level >220 mg/dl were considered to be diabetic & were used for the study.

Effects of extracts on streptozotocin induced Diabetic rats:

The rats were divided into different groups (n=6). The treatment was given orally. Control group received the vehicle (2 ml/kg body wt.) & glibenclamide (10 mg/kg body wt.) was given as the standard drug. The animals of the test groups received the methanolic and petroleum ether extracts of *C. inerme*. The blood glucose level was examined at 0, 1, 2, 4, 8, 10 hour following the treatment.

Collection of blood & determination of blood glucose:

Blood samples were collected from the tip of the tail vein of each rat & blood glucose levels were measured using Glucometer (DR. Morepen, DG03).

Statistical analysis:

All the results are expressed as mean ±SEM. Comparison between the test groups with control was made. The data were statistically analysed by one way analysis of variance (ANOVA), followed by Dunnet's t-test. P values less than 0.05 and 0.01 were considered significant.

RESULTS & DISCUSSIONS

The preliminary phytochemical screening of the extracts were carried out. The study indicates the presence of alkaloids, glycosides, tannins, steroids, triterpenoids, flavonoids, & carbohydrates in methanolic extract and steroids and triterpenoids in the petroleum ether extract of the plant as shown in the Table-1.

Acute oral toxicity study revealed that the extracts of *Clerodendrum inerme* did not show any sign of toxicity and mortality up to 14 days of the study period in the dose level of 4000 mg/kg and hence the dose of the extracts for the animal study were fixed at 400 mg/kg body weight of the animal.

The effect of extracts on blood glucose level of normoglycemic rats is presented in Table-2. Both the extracts did not produce any significant hypoglycemia in the normoglycemic rats till the end of the study .The extracts have no significant effect on normoglycemic animals as compared to solvent control group.

The effect of methanolic and petroleum ether extracts of *Clerodendrum inerme* (CI) on blood glucose level in glucose loaded rat is given in Table-3. Methanolic extract and pet. ether extract of the plant were effective in the oral glucose tolerance test. There was a significant reduction in the blood glucose level with a dose of 400 mg/kg at the end of 4 hour following the administration of test substances. Methanol extract exhibited maximum reduction of blood glucose and better glucose tolerability.

The anti-hyperglycemic effect of methanolic extract and petroleum ether extract of *CI* in streptozotocin induced diabetic rats is depicted in Table-4. The methanolic extract of *CI* at the dose of 400 mg/kg showed significant reduction (54.32%) in fasting blood glucose level as compared to diabetic control group at the end of 10 hour after administration of test substance whereas the petroleum ether extract showed a mild to moderate decrease of blood glucose level (33.15%) at the same dose. It indicates that the methanolic extract exhibited similar effect as that of the standard drug Glibenclamide.

CONCLUSION

The experimental results of the present investigation conclude that the methanolic extract of *Clerodendrum inerme* has more potent anti-hyperglycemic activity than the pet. ether extract in streptozotocin induced diabetic animals. The effect of this plant was also found to be much comparable to that of the standard drug glibenclamide treated rats.

Table 1: Preliminary Phytochemical screening of *Clerodendrum inerme*

Test for Extract	Pet ether extract	Methanol extract
Alkaloids	-ve	+ve
Glycosides	-ve	+ve
Tannins	-ve	+ve
Saponins	-ve	-ve
Steroids	+ve	+ve
Triterpenoids	+ve	+ve
Flavonoids	-ve	+ve
Amino acids, proteins	-ve	-ve
Carbohydrate	-ve	+ve

'+' denotes present, '-' denotes absent

Table 2: Effect of *C. inerme* extract on normoglycemic rats

Groups and treatments	Blood glucose levels (mg/dl)				
	0 hr	1 hr	2 hr	4 hr	8 hr
Solvent control (Tween+water)	98.16±0.47	98±0.57	98.16 ±0.70	97.83±0.30	95.16±0.70
Glibenclamide (10 mg/kg)	92.16±0.70	89.5±0.42	87.66±0.66	78.83±0.60	88.66±0.33
Methanol extract (400 mg/kg)	93.66±0.66	90.16±0.47	89±0.51	82.66±0.88	90.50±0.42
Pet ether (400 mg/kg)	95.33±0.49	91.5±0.76	90±0.56	87.16±0.54	92.66±0.33

Values are expressed in mean±SEM of six animals. One –way ANOVA followed by Dunnet's t-test, t-value denotes statistical significance at *p<0.05, **p<0.01 and ***p<0.001, respectively, in comparison to Group-I, SEM: Standard error of the mean, *C. inerme:Clerodendrum inerme*.

Table-3: Effect of *C. inerme* extracts on glucose-loaded hyperglycemic rats

Groups and treatments	Blood glucose levels(mg/dl)				
	Pre-treatment	Post-treatment			
		1hr	2hrs	4hrs	% age decrease at the end of 4hrs
Solvent control (Tween+water)	85.83±1.30	140.50±0.76	133.00±0.36	124.33±0.49	
Glibenclamide (10mg/kg)	79.66±0.55	126.17±1.44	99.83±0.54	76.66±0.76	38.34
Methanol extract (400mg/kg)	80.83±0.60	131.67±0.21	120.00±0.73	85.33±0.71	31.36
Pet ether (400mg/kg)	89.16±0.30	135.33±0.33	129.00±0.57	91.33±0.98	26.54

Values are expressed in mean+SEM of six animals. One –way ANOVA followed by Dunnet's t-test, t-value denotes statistical significance at *p<0.05, **p<0.01 and ***p<0.001, respectively, in comparison to Group-I, SEM: Standard error of the mean, *C. inerme: Clerodendrum inerme*.

Table 4: Effects of *C. inerme* extracts on STZ-induced diabetic rats

Groups and treatments	Blood glucose levels(mg/dl)						
	0hr	1hr	2hrs	4hrs	8hrs	10hrs	% age decrease at the end of 10 hrs
Solvent control (Tween+water)	273.17±1.30	276.01±0.73	279.50±0.76	281.50±0.56	283.17±0.70	283.50±0.76	
Glibenclamide (10mg/kg)	266.50±0.763	229.83 ±0.60	195.50±1.11	143.83±0.79	121.33±0.71	116.17±0.47	59.02
Methanol extract (400mg/kg)	260.50±1.17	241.17±0.60	219.67±1.05	188.0±0.57	139.83±0.60	129.50±0.76	54.32
Pet ether (400mg/kg)	261.83±0.60	249.17±0.70	237.50±1.05	220.50±0.76	194.33±1.56	189.50±0.42	33.15

Values are expressed in mean+SEM of six animals. One –way ANOVA followed by Dunnet's t-test, t-value denotes statistical significance at *p<0.05, **p<0.01 and **p<0.001, respectively, in comparison to Group-I, SEM: Standard error of the mean, *C. inerme:Clerodendrum inerme*, STZ-Streptozotocin.

ACKNOWLEDGEMENT

The authors wish to thank the H.O.D, UDPS, Utkal University, Bhubaneswar and the Principal, SIPS, Jharpokharia, Orissa, India, for providing the necessary facilities to carry out the research work. The funding support through a "UGC research award" project to DR.S.K.Mishra is gratefully acknowledged.

REFERENCES

1. Ojewole J.A.O, Analgesic, anti-inflammatory & hypoglycemic effects of Rhus chirindensis (Baker F) stem.bark aqueous extract in mice & rats. J. of Ethnopharmacology, vol 113, no.2, 2007,338-345.
2. Amiral Diabetes association "Diagnosis & Classification of diabetes mellitus, "Diabetes Care, vol-30, suppl-1,2007, s42-s46.
3. Thiruvenkata subramaniam, R and Jayakar, B.Antihyperglycemic and antihyperlipidemic activities of Premna corymbosa(BurnF) Rott L on streptozocin induced diabrtic rats, Der pharmacia lettre, 2, 2010, 505-509.
4. Mentreddy S.R. : Medicinal plant species with potential antidiabetic properties, J. Sci Food agric., 87(5), 2007,743.
5. Saxena H, Brahmam M, The flora of Orissa, vol-3, Allied publishers limited, New Delhi, 1994, 1406.
6. Manoharan S, Kavita K. Senthil N. Raju G L. Singapore medicinal journal, 2006, 47, 1038.
7. Farooqui M.A. Chauhan P.S. Krishnamoothy P. Shaikh J. Digest J. Nanomater Bio struct. 5, 2010, 43.
8. Bhusan B. Sardana S. Gulshan B. Phytochemical & pharmacognostical studies of leaves of Clerodendrum inerme. Der Pharmacia letter, 7(4), 2015, 157-161.
9. Anonymous, wealth of Indiam vol-2, NISCOM & and CSIR, New Delhi, 2001, 67-68
10. Bhakuni, O.S. Dhar; ML, Dhar. MM, & Mehrotra BN screening of Indian plants for biological activities part II, Ind. J. of Exp biology, 7, 1969, 250-262.
11. Singh, V.P. Sharma, S.K. Khan, V.S. Medicinal plants from Ujain district of Madhya Pradesh part II, Indian drugs & pharmaceutical industry, 5, 1980, 7-12.
12. Kokate CK. Practical Pharmacognosy, 4th edn. ,New Delhi,Vallabh Prakashan, India, 2001(reprint), 107-111.
13. Harborne JB. Phytochemical methods-A guide to modern techniques of plant analysis,3rd edn.(Indian reprint),New Delhi, Springer,2005,40-249.
14. Turner, R.A. Screening methods in Pharmacology,1st India reprint, Noida (U.P.), Academic press (Elsevier),2009, 60-68.
15. Kulkarni S K.Handbook of Experimental Pharmacology , 3rd edn., Delhi,Vallabh Prakashan, 1999,168-170.
16. Ghosh MN.Fundamentals of Experimental Pharmacology,3rd edn.,Kolkata,Hilton & company,2005,190-195.