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Original Article

Antidiabetic effects of Clerodendrum viscosum, vent

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ABSTRACT

The objective of the present study was to evaluate the antihyperglycemic activity of leaves of *Clerodendrum viscosum,vent*(Verbenaceae). The plant extracts were found with low or no toxicity as seen in the acute toxicity study. The methanolic extract of the plant at the dose of 400mg/kg body weight produced a significant decrease in fasting blood glucose level in the streptozotocin induced diabetic rats by 46% approximately with respect to initial FBG level after 10 hrs of treatment. In the oral glucose tolerance test this extract at the dose level of 400mg/kg decrease hyperglycemia. But in the normoglycemic animals the extracts did not produce any significant hypoglycemia as shown in the corresponding table and was found to be potent in restoration of the elevated glucose levels to normal, thereby indicating good antihyperglycemic activity of the extract of *Clerodendrum viscosum*.

Keywords: antihyperglycemic, streptozotocin, normoglycemic, Clerodendrum viscosum

INTRODUCTION

Medicinal plants contain inherent active ingredients to cure diseases. They are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or, folk medicine practice base on the use of plants & plant extracts. [1, 2]Diabetes mellitus is the most common heterogenous metabolic disorder, which currently affects an estimated 143 million people worldwide & its incidence is increasing steadily with changes in life style. [3] 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 & mortality. [4] 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.[5] *Clerodendrum viscosum*, vent.(verbenaceae), known as Bhant in hindi, Ghentu in bengali, Bhania in oriya, is a terrestrial shrub occurring throughout the plains of india. The shrub is 2-4 feet in height. [6,7] The plant is bitter tonic, antipyretic & anthelmintic leaves & roots are used in asthma, tumours & certain skin diseases. Infusion of the leaves is used as bitter tonic. Expressed juice of the leaves is laxative & chola gogue. Leaves are also used in chest complaint with cough & difficult expectoration. Alcoholic extract of the leaves possesses strong antibacterial & poor antifungal properties. [8,9] The antioxidant, antimicrobial, antimalaria, anthelmintic & analgesic activities of the plant have further created an upsurge in investigations on the plant. [10,11] Various species of the plant like C.phlomidis, C. calamitosum, C.trichotomum have been reported to have antidiabetic & antihypertensive properties. [12] Based on the above perspective, an effort was made to ascertain the possible role of C.viscosum,vent, in streptozocin-induced diabetes mellitus.

MATERIALS AND METHODS

Plant material: The leaves of *Clerodendrum viscosum*, vent were collected from Naharkanta village, near Bhubaneswar during October. The plant was identified by Dr. K.B. Satpathy, Head, Dept. of Botany, Vani Vihar, Utkal University Bhubaneswar. A voucher specimen (no.SVN-571) was submitted to the Herbarium PG department of Botany, Utkal University, Bhubaneswar.

Preparation of plant extracts: Fresh leaves & twigs were separated, washed with tap water, rinsed with distilled water, air dried for 1 hour &

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dried under shade. They were ground into powder (coarsely). The coarse powder was extracted successively with petroleum ether (60-80 ^{0}c) & methanol in a soxhlet apparatus. The percentage yields of the petroleum ether & methanol extracts were found to be 1.8 & 16.6% respectively.

Drugs & Chemicals: Streptozotocin & glibenclamide (Merck & S.N.Chemicals respectively) were used for these investigations. All other chemicals, solvents & drugs used were of analytical grade.

Preliminary Phytochemical Screening of C.V[13,14]: Preliminary phytochemical screening of petroleum ether extract (CVP) & methanolic extract (CVM) of *Clerodendrum viscosum*, vent 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.

Experimental animals: The Wistar albino rats (200-220g) & albino mice (20-25g) of both sexes were procured from the animal supplier. They were housed in clean propylene cages & were fed with standard pellet diet (Hindustan lever, Kolkata) & water *ad libitum*. The animals were housed under standard condition at a temp. of 25±2^0, with 12/12-light/ dark cycle & 35-60% humidity. The conditions in the animal house & the study protocols were approved by the IAEC, of SIPS, Jharpokharia (vide approval no.A1/15/IAEC/SIPS).

Acute toxicity study[15,16,17]**:** The lethal dose (LD_{50}) of the extracts was assessed by using albino mice of either sex weighing about 20-25g.The animals were fasted overnight prior to the experimental procedures, with free access to water. Different doses of extracts were separately administered in increasing dose levels up to 4000mg/kg body weight of the mouse to the animals by the intraperitoneal route. The LD_{50} was calculated by the method reported by Miller & Tainter. 1/10th of the lethal dose was taken as the screening dose.

Study on normoglycemic rats: The effect of extracts on blood glucose level was studied in normal rats.[18]The rats were divided into different groups (n=6) and fasted for 12 hours with free access of water. The treatments were made orally as : Gr.I : solvent control (Tween20+distilled water) ; Group II Glibenclamide (10mg/kg) Group III Methanolic extract (400mg/kg) Group IV Petroleum ether extract (400mg/kg). The blood glucose level was determined at 0,1,2,4,6,8 & 10 hr. following the oral administration of test samples to assess the effect of the test samples on normoglycemic rats.

Study on extracts on glucose loaded animals (oral glucose tolerance test/OGTT): 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 extract (CVM) & petroleum ether extracts (CVP) of Clerodendrum viscosum were administered to the animals of the test groups , orally , at a dose of 400mg/kg body wt.The rats of different groups were loaded with glucose (2g/kg p.o.) ,30 minutes after the administration of test substances . Blood glucose levels were measured at 0,1,2 & 4 hours after glucose load of extract on blood glucose level of the glucose loaded animals.

Induction of experimental Diabetes: The rats were kept fasting for 12 hours with access to water and there after diabetes was induced by intra peritoneal injection of Streptozotocin (STZ), freshly dissolved in citrate buffer (pH 4.5) immediately before use. Streptozotocin was given at dose of 65mg/kg of body wt. In order to avoid STZ-induced hypoglycemic mortality 5% glucose solution was given for 24hr.to STZ treated rats. After 72 hours of STZ administration the blood glucose levels were measured and the rats showing blood glucose level >220mg/dl were considered to be diabetic & were used for the study.

Study on STZ induced diabetic rats: The rats were divided into different groups (n=6). The treatment was for 10hrs. Control group received the vehicle (2ml/kg) & glibenclamide (10mg/kg body wt.) was given as the standard drug to the second group. The animals of the test group received the methanolic and petroleum ether extracts of *Clerodendrum viscosum*(CV). at a dose of 400mg/kg.

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 on 0,1,2,4,8 & 10 hours using glucometer (Dr.Morepen,DG03).

Statistical analysis: All the result were expressed as mean ± SEM. The statistical analysis was carried out using one way ANOVA followed by Dunnet's t-test. P values less than 0.05 & 0.01 were considered significant.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening (as shown in the Table.1) showed the presence of alkaloid, glycosides, tannins, steroids, and triterpinoids, carbohydrate in methanolic extract & alkaloids, glycosides, saponin, steroids in petroleum ether extract. Acute oral toxicity study revealed that the extracts of *Clerodendrum .viscosum* did not show any sign of toxicity and mortality upto 14 days of the study in the dose level of 4000mg/kg & hence the dose of the extracts for animal study was fixed at 400mg/kg. The effect of methanolic extract (CVM) & petroleum ether extract (CVP) on normal healthy rats is shown in Table 2. The test extracts do not have any significant effect on normoglycemic animals, as they did not produce any significant hypoglycemia at the end of 4 and 8 hours as compared to the initial blood glucose level. The effect of extracts on blood glucose level of glucose loaded animals is presented in table 3. The test extracts at 400mg/kg body weight were effective in the oral glucose tolerance test (OGTT). Both the extracts showed a significant fall of blood glucose level when compared with solvent control group at the end of 4 hours. The effect of methanolic extract was more pronounced as compared to the pet ether extract. Among them methanol extract exhibited the highest reduction of blood glucose level with percentage reduction of 31.50 followed by petroleum ether extract of 25.87. Antihyperglycemic effect of methanolic extract and pet.ether extract of *Clerodendrum viscosum* (CV) in STZ induced diabetic rats after single dose (acute effect) is depicted in table 4. The methanol extract exhibited highest reduction of blood glucose level with the percentage reduction of 46.99 followed by petroleum ether extract of 36.69 when compared to diabetic control group at the end of 10hrs experimental period.

Conclusion

The results of the current study showed that the extracts of the plant *Clerodendrum viscosum,* vent has antihyperglycemic activity as it is capable of lowering blood glucose level in STZ induced diabetic rats. Further studies are warranted to isolate & characterize the antidiabetic principles from the plant *Clerodendrum viscosum VENT.*

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Table-1 Preliminary Phytochemical screening of *Clerodendrum viscosum*:

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.viscosum extract on normoglycemic rats**

Groups and treatments	Blood glucose levels(mg/dl) 0hr	1hr	2hr	4hr	8hr
Solvent control (Tween+water)	98.16±0.47	98.33±0.49	99.16±1.01	97.83± 0.30	98.50±0.99
Glibenclamide (10mg/kg)	92.16±0.70	89.50±0.42*	87.50±0.76**	82.33±0.88*	92.83±2.13**
Methanol extract (400mg/kg)	94.50±0.76	93.16±0.47	91.66±0.42	92.50±2.02*	93.83±1.95*
Pet ether (400mg)	95.33±1.20	94.50±0.76	92.16±0.70*	93.66±1.40*	94.33±1.56*

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 comparision to Group-I, SEM: Standard error of the mean, C.viscosum:Clerodendrum viscosum

Table-3: Effect of C.viscosum extracts on glucose-loaded hyperglycemic rats(OGTT)

Groups and treatments	Blood glucose levels(mg/dl) Pre-treatment	Post-treatment 1hr	2hr	4hr	% age decrease at the end of 4hrs
Solvent control (Tween+water)	85.83±1.30	140.50±0.76	132.67±0.49	124.33±0.49	
Glibencamide (10mg/kg)	79.66±0.55	126.17±1.44	99.83±0.54*	76.66±0.76 **	38.34
Methanol extract (400mg/kg)	84.50±1.54	132.33±1.33	121.33±0.91*	85.16±0132**	31.50
Pet ether (400mg/kg)	86.50±1.72	135.17±1.24	128.50±0.76 *	92.16±1.29 *	25.87

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 comparision to Group-I, SEM: Standard error of the mean, C.viscosum: Clerodendrum viscosum.

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

Groups and treatments	Blood glucose levels(mg/dl) 0hr	1hr	2hr	4hr	8hr	10hrs	%age decrease at the end of 10hr
Solvent control (Tween+water)	234.33±1.38	235.67±1.94	237.33±1.33	239.50±0.99	242.33±0.66	244.33±1.33	
Glibencamide (10mg/kg)	243.33±0.88	224.67 ±1.58	196.33±0.88*	158.83±1.16 **	127.83±1.35**	116.83±0.94**	52.18
Methanol extract (400mg/kg)	238.50±1.17	229.67±0.88	203.67±1.14*	172.83±0.94**	140.17±1.13 **	129.50±0.76 **	46.99
Pet ether (400mg/kg)	236.17±1.07	231.83±1.40	212.50±0.76	191.17±0.94 **	164.67±0.66*	154.67±0.88*	36.69

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 comparision to Group-I, SEM: Standard error of the mean, C.Viscosum: Clerodendrum viscosum, STZ-Streptozotocin.

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