WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 5.210

Volume 4, Issue 09, 216-224 Research Article ISSN 2278 – 4357

ANTIHYPERGLYCEMIC AND ANALGESIC ACTIVITY STUDIES WITH *CLERODENDRUM VISCOSUM* VENT. (VERBENACEAE) LEAVES

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Article Received on
08 July 2015,

Revised on 29 July 2015,
Accepted on 23 Aug 2015

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ABSTRACT

Background. *Clerodendrum viscosum* is found in the wild and fallow lands of Bangladesh and is known for its multiple medicinal uses including use for alleviation of diabetic high sugar and pain. It was of interest to determine the antihyperglycemic and analgesic properties of the leaves of the plant. **Methods**. Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT). Analgesic activity was determined by observed decreases in abdominal constrictions (writhings) in intraperitoneally administered acetic acid-induced pain model in mice. **Results.** Administration of methanol extract of leaves led to significant dose-dependent reductions in blood glucose levels in glucose-loaded mice at doses of 200 and 400 mg per kg body weight. At these two doses, the extract reduced blood glucose levels by 25.2 and 33.3%, respectively compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 33.3%. In analgesic activity tests, the extract at doses of 100, 200 and 400 mg per kg body weight significantly reduced the number of abdominal constrictions by 29.6, 37.0, and 59.3%, respectively. A standard pain relieving (analgesic) drug, aspirin, reduced the number of writhings by 33.3 and 51.9%, respectively, when administered at doses of 200 and 400 mg per kg body weight. **Conclusion**. The leaves can be beneficial in lowering blood glucose and for alleviating pain.

KEYWORDS: Antihyperglycemic, *Clerodendrum viscosum*, analgesic, Verbenaceae.

BACKGROUND

Clerodendrum viscosum Vent. is a large shrub belonging to the Verbenaceae family and can be found growing in the wild and fallow lands of Bangladesh. The plant has reportedly multiple ethnomedicinal uses. The Jaintia tribe of North Cachar Hills district of Assam, India, uses the leaves for treatment of diabetes, high blood pressure, and asthma.[1] Leaves are used by the Bodo people of Manas Biosphere Reserve in Assam, India, for treatment of malaria, a disease characterized by high fever and body pain0.[2] The roots and leaves are used for treatment of helminthiasis, body pain, boils, burns, cuts, skin diseases, sores, swelling, ulcer, and wounds by the people in Sonebhadra district of Uttar Pradesh, India.[3] Root and leaf juice is taken for stomach disorders by the Mog and Reang communities of south district of Tripura, India. [4] Tender twigs are used for menstrual dysfunction by the Karbi ethnic group in Assam State, India. [5] People of Rourkela Steel City and its surroundings in Sundargarh, Odisha, India, take fruits of the plant internally and apply fruit paste externally for dog bite; they also use roots for mumps, mouth ulcers and angular stomatitis.[6]

In two mouzas of Kurigram district, Bangladesh, folk medicinal practitioners use leaves for helminthic infections, loss of appetite, sprain, and fracture.[7] The plant is used to treat diabetes by folk and tribal medicinal practitioners in Sylhet district, Bangladesh.[8] The Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh, also use the plant to treat diabetes.[9] Our interest lies in screening medicinal plants of Bangladesh for their blood sugar lowering and analgesic activities[10-21] for diabetes and pain are common afflictions in Bangladesh, and for which the rural people suffer from lack of accessibility or affordability of allopathic medicines. The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of *Clerodendrum viscosum*, which is widely available in rural areas.

METHODS

Plant material collection

Leaves of *C. viscosum* were collected during May 2014 from Srimangal in Sylhet Division, Bangladesh. The plant was taxonomically identified at the Bangladesh National Herbarium (Accession Number 39,587).

Preparation of methanolic extract of seeds

Leaves were thoroughly dried in the shade and 100g of dried and powdered seeds were extracted with methanol (w:v ratio of 1:5, final weight of the extract 3.967g).

Chemicals and Drugs

Glibenclamide, aspirin, and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 12-15g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Oral glucose tolerance tests for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999)[22] with minor modifications. Briefly, fasted mice were grouped into six groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received methanolic leaf extract of *C. viscosum* (MECV) at doses of 50, 100, 200 and 400 mg per kg body weight. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method.[23] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 - W_e/W_c) \times 100$, where W_e and W_c represents the blood glucose concentration in glibenclamide or MECV administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test

Analgesic activity of MECV was examined as previously described.[24] Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MECV at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MECV, all mice were intraperitoneally

injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid[25], following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

Percent inhibition = $(1 – W_e/W_c) X 100$

where W_e and W_c represents the number of writhings in aspirin or MECV administered mice (Groups 2-7), and control mice (Group 1), respectively.

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.[17]

RESULTS

Antihyperglycemic activity evaluation results

In oral glucose tolerance tests (OGTT), MECV at doses of 200 and 400 mg/kg caused, respectively, 25.2 and 33.3% reductions in blood glucose levels. The results were dose-dependent and statistically significant. There were no statistically significant reductions in blood glucose levels at doses of 50 and 100 mg per kg. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg lowered blood glucose by 33.3%. The results are shown in Table 1 and suggest that the extract possess antihyperglycemic activity, with the highest dose of the extract being comparable to glibenclamide.

Analgesic activity evaluation results

Dose-dependent reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MECV. At doses of 100, 200 and 400 mg per kg body weight, MECV was observed to reduce the number of constrictions, respectively, by 29.6, 37.0, and 59.3%. The percent reduction observed with 50 mg/kg MECV (11.1%) was not statistically significant. A standard analgesic drug, aspirin, when administered to experimental animals at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions by 33.3 and 51.9%, respectively. Thus, a dose of 200 mg/kg MECV was better than 200 mg/kg aspirin, while a dose of 400 mg/kg MECV gave

better analgesic activity than 400 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties.

Table 1: Effect of crude methanol extract of *C. viscosum* leaves (MECV) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	4.68 ± 0.26	-
Glibenclamide	10 mg	3.12 ± 0.33	33.3*
(MECV)	50 mg	5.02 ± 0.22	-
(MECV)	100 mg	4.64 ± 0.30	0.9
(MECV)	200 mg	3.50 ± 0.38	25.2*
(MECV)	400 mg	3.12 ± 0.32	33.3*

All administrations were made orally. Values represented as mean ± SEM, (n=5); $^{*}P < 0.05$; significant compared to hyperglycemic control animals.

Table 2: Analgesic effect of crude methanol extract of *C. viscosum* leaves (MECV) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.4 ± 0.24	-
Aspirin	200 mg	3.6 ± 0.40	33.3*
Aspirin	400 mg	2.6 ± 0.51	51.9*
(MECV)	50 mg	4.8 ± 0.49	11.1
(MECV)	100 mg	3.8 ± 0.37	29.6*
(MECV)	200 mg	3.4 ± 0.40	37.0*
(MECV)	400 mg	2.2 ± 0.20	59.3*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control.

DISCUSSION

Although in preliminary studies like this, the bioactive components have not been isolated or identified, it is always of interest to perusethe literature to find out what sort of phytochemicals have been reported for the plant, and whether any such phytochemical(s) may give the observed antihyperglycemic and analgesic effects. It is of interest that acetamide and 5-hydroxymethylfurfural has been reported from leaves of the plant.[26] 5-hydroxymethylfurfural has been shown to have a protective effect on high glucose induced oxidative stress in human umbilical vein endothelial cells.[27] 2-(substituted phenoxy)

acetamide derivatives have been shown to possess analgesic properties.[28] It has been reported that β-sitosterol is present in the plant, and antinociceptive activity reported in methanolic extract of leaves,[29] the latter in agreement with our present findings. Interestingly, β-sitosterol has reportedly both antidiabetic and analgesic properties.[30, 31] Thus β-sitositerol can be a possible component for the observed antihyperglycemic and analgesic effects in the present study.

CONCLUSION

The results suggest that methanolic extract of *C. viscosum* leaves can be used for lowering blood glucose and for alleviating pain.

Conflicts of interest

The author(s) declare that they have no competing interests.

REFERENCES

1. Sajem AL, Gosai K: Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. J Ethnobiol Ethnomed., 2006; 2: 33-39.
2. Paul S, Devi N, Sarma GC: Ethnobotanical utilization of some medicinal plants by Bodo people of Manas Biosphere Reserve in the treatment of malaria. Int Res J Pharm., 2013; 4(6): 102-105.
3. Singh A, Dubey NK: An ethnobotanical study of medicinal plants in Sonebhadra district of Uttar Pradesh, India with reference to their infection by foliar fungi. J Med Plants Res., 2012; 6(14): 2727-2746.
4. Debnath B, Debnath A, Shilsharma S, Paul C: Ethnomedicinal knowledge of Mog and Reang communities of south district of Tripura, India. Indian J Adv Plant Res., 2014; 1(5): 49-54.
5. Terangpi R, Basumatary TK, Teron R: Ethnomedicinal plants of the Karbi ethnic group in Assam state (India) for management of gynaecological disorders. Int J of Pharm Life Sci., 2014; 5(10): 3910-3916.
6. Mallick SN, Ram JP, Parida N: Study of ethnomedicinal values of some shrubs in Rourkela Steel City and its surroundings, Sundargarh, Odisha. Int J Appl Biol Pharmaceut Technol., 2014; 5(3): 123-130.
7. Azad AK, Mahmud MR, Parvin A, Chakrabortty A, Akter F, Moury SI, Anny IP, Tarannom SR, Joy SK, Chowdhury SY, Akter S, Rahmatullah M: Ethnomedicinal

surveys in two mouzas of Kurigram district, Bangladesh. World J Pharm Pharmaceut Sci., 2014; 3(10): 1607-1620.

8. Shaheen MEK, Syef MA, Saha SS, Islam MS, Hossain MDA, Sujan MAI, Rahmatullah M: Medicinal plants used by the folk and tribal medicinal practitioners in two villages of Khakiachora and Khasia Palli in Sylhet district, Bangladesh. Adv Nat Appl Sci., 2010; 5(1): 9-19.
9. Rahmatullah M, Azam MN, Khatun Z, Seraj S, Islam F, Rahman MA, Jahan S, Aziz MS: Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. Afr J Tradit Complement Altern Med., 2012; 9(3): 380-385.
10. Morshed A, Hossain MH, Shakil S, Nahar K, Rahman S, Ferdausi D, Hossain T, Ahmad I, Chowdhury MH, Rahmatullah M: Evaluation of antinociceptive activity of two Bangladeshi medicinal plants, *Kalanchoe pinnata* (Lam.) Pers. and *Lagerstroemia speciosa* (L.) Pers. Adv Nat Appl Sci., 2010; 4(2): 193-7.
11. Rahmatullah M, Sultan S, Toma TT, Lucky SS, Chowdhury MH, Haque WM, Annay MEA, Jahan R: Effect of *Cuscuta reflexa* stem and *Calotropis procera* leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. Afr J Trad Complement Altern Med., 2010; 7(2): 109-12.
12. Ahmed F, Rahman S, Ahmed N, Hossain M, Biswas A, Sarkar S, Banna H, Khatun MA, Chowdhury MH, Rahmatullah M: Evaluation of *Neolamarckia cadamba* (Roxb.) Bosser leaf extract on glucose tolerance in glucose-induced hyhperglycemic mice. Afr J Trad Complement Altern Med., 2011; 8(1): 79-81.
13. Shahreen S, Banik J, Hafiz A, Rahman S, Zaman AT, Shoyeb MA, Chowdhury MH, Rahmatullah M: Antihyperglycemic activities of leaves of three edible fruit plants (*Averrhoa carambola*, *Ficus hispida* and *Syzygium samarangense*) of Bangladesh. Afr J Trad Complement Altern Med., 2012; 9(2): 287-91.
14. Rahmatullah M, Hosain M, Rahman S, Rahman S, Akter M, Rahman F, Rehana F, Munmun M, Kalpana MA: Antihyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of *Amaranthus tricolour* L. (Amaranthaceae). Afr J Trad Complement Altern Med., 2013; 10(5): 408-11.
15. Rahmatullah M, Hossain M, Mahmud A, Sultana N, Rahman SM, Islam MR, Khatoon MS, Jahan S, Islam F: Antihyperglycemic and antinociceptive activity evaluation of 'khoyer' prepared from boiling the wood of *Acacia catechu* in water. Afr J Trad Complement Altern Med., 2013; 10(4): 1-5.

16. Haque ME, Rahman S, Rahmatullah M, Jahan R: Evaluation of antihyperglycemic and antinociceptive activity of *Xanthium indicum* stem extract in Swiss albino mice. BMC Complement Alternat Med., 2013; 13: 296-9.
17. Hossain AI, Faisal M, Rahman S, Jahan R, Rahmatullah M: A preliminary evaluation of antihyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. BMC Complement Alternat Med., 2014; 14: 169-73.
18. Tazin TQ, Rumi JF, Rahman S, Al-Nahain A, Jahan R, Rahmatullah M: Oral glucose tolerance and antinociceptive activity evaluation of methanolic extract of *Vigna unguiculata* ssp. *unguiculata* beans. World J Pharm Pharmaceut Sci., 2014; 3(8): 28-37.
19. Rahman S, Jahan R, Rahmatullah M: Effect of paddy husk extracts on glucose tolerance in glucose-induced hyperglycemic mice. World J Pharm Pharmaceut Sci., 2014; 3(8): 111-120.
20. Jahan S, Rahmatullah M: Methanolic extract of aerial parts of *Raphanus sativus* var. *hortensis* shows antihyperglycemic and antinociceptive potential. World J Pharm Pharmaceut Sci., 2014; 3(8): 193-202.
21. Ghosh D, Mandal I, Rumi JF, Trisha UK, Jannat H, Ahmed M, Rahmatullah M: Effect of *Allium sativum* leaf extracts on glucose tolerance in glucose-induced hyperglycemic mice. Adv Nat Appl Sci., 2014; 8(8): 66-69.
22. Joy KL, Kuttan RJ: Anti-diabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol., 1999; 67(2): 143-148.
23. Venkatesh S, Reddy GD, Reddy YSR, Sathyavathy D, Reddy B: Effect of *Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. Fitoterapia., 2004; 75(3-4): 364-367.
24. Shanmugasundaram P, Venkataraman S: Anti-nociceptive activity of *Hygrophilous auriculata* (Schum) Heine. Afr J Tradit Complement Altern Med., 2005; 2(1): 62-69.
25. Akter M, Mitu IZ, Proma JJ, Rahman SM, Islam MR, Rahman S, Rahmatullah M: Antihyperglycemic and antinociceptive activity evaluation of methanolic extract of *Trichosanthes anguina* fruits in Swiss albino mice. Adv Nat Appl Sci., 2014; 8(8): 70-74.
26. Panda P, Rath M, Pal A, Sharma T, Das D: GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Phcog Res., 2015; 7: 110-113.
27. Cao G, Cai H, Cai B, Tu S: Effect of 5-hydroxymethylfurfural derived from processed *Cornus officinalis* on the prevention of high glucose-induced oxidative stress in human umbilical vein endothelial cells and its mechanism. Food Chem., 2013; 140(1-2): 273-279.

28. Rani P, Pal D, Hegde RR, Hashim SR: Anticancer, anti-inflammatory, and analgesic activities of synthesized 2-(substituted phenoxy) acetamide derivatives. Biomed Res Int 2014, 2014: Article ID: 386473.
29. Rahman MM, Rumzhum NN, Zinna KEK: Evaluation of antiuoxidant and antinociceptive properties of methanolic extract of *Clerodendrum viscosum* Vent. S J Pharm Sci., 2011; 4(1): 74-78.
30. Karan SK, Mishra SK, Pal D, Mondal A: Isolation of β-sitosterol and evaluation of antidiabetic activity of *Aristolochia indica* in alloxan-induced diabetic mice with a reference to *in-vitro* antioxidant activity. J Med Plants Res., 2012; 6(7): 1219-1223.
31. Bhalke RD, Pal SC: Anti-inflammatory and antinociceptive activity of *Pterospermum acerifolium* leaves. Asian J Pharmaceut Clin Res., 2012; 5(2): 23-26.