European Journal of Medicinal Plants
6(3): 175-180, 2015, Article no.EJMP.2015.053
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

Pharmacological Potential of *Tricosanthes tricuspidata* and *Clematis montana* for Hypoglycemic and Antioxidant Activity

Sanjay Singh[1*], Sadath Ali[2] and Mamta Singh[3]

[1]Department of Pharmacy, Siddhartha group of Institutions, Dehardun, Uttarakhand, India.
[2]Department of Pharmacy, Azad Institute of Pharmacy & Research, Lucknow, Uttar Pradesh, India.
[3]Department of Pharmacy, Sardar Bagwan Singh (PG) Institute, Dehradun, Uttarakhand, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SS and SA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/12769
Editor(s):
(1) Ana Ribeiro, Senior Researcher Habilitation and Deputy Director of Biotrop – Environment, Agriculture and Development Center, Tropical Research institute (IICT), Portugal.
(2) Marcello Iriti, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Nigeria.
(2) Anonymous, Czech Republic.
(3) Anonymous, Malaysia.
(4) Anonymous, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=913&id=13&aid=7764

Original Research Article

Received 17th July 2014
Accepted 1st November 2014
Published 13th January 2015

ABSTRACT

The aim of present study was to evaluate the antidiabetic and antioxidant potential of *Tricosanthes tricuspidata and Clematis montana* leaf and root extracts. On the basis of toxicity studies a dose of 200 mg/kg body weight was selected for antidiabetic activity. Diabetes was induced by the administration of streptozotocin at a dose of 50 mg/kg, intra peritoneal. Among all the extracts, ethanolic extract (EtOH) of *T. tricuspidata* (197.5±1.31**) and *C. montana* (183.8±3.79**) have shown significant reduction in blood glucose level in SZT induced diabetic Wister rats as compared to control and the standard drug glipizide (5 mg/kg body weight). Aqueous extract of *T. tricuspidata* (22.35%) and ethanolic extract of *C. montana* (26.23%) have shown significant antioxidant activity when compared with standard (Ascorbic acid & BHT) at 30 µg/ml concentration. The results of the

**Corresponding author: E-mail: sanjaymph@gmail.com;*

study concluded that *Tricosanthes tricuspidata and Clematis montana* leaf and root extracts have potential antidiabetic and antioxidant properties.

Keywords: Antidiabetic; antioxidants; Clematis montana; Tricosanthes tricuspidata.

1. INTRODUCTION

Diabetes mellitus is a disease characterized by elevated blood glucose level and leads to disturbance of carbohydrates, fat and protein metabolism. Insulin and oral hypoglycemics are mostly used to reduce the elevated blood sugar but their use is associated with various side effects. Nowadays people are frequently using herbal medicines to alleviate the problems associated with diabetes than the allopathic medicines. Constantly there is a need to search for safer hypoglycemic agents from plant origin, which will tackle the issue. The literature survey revealed that a number of plants possess antidiabetic and antioxidant activity. However, many of them lack proper validation and systemic evaluation [1].

This study deals with the evaluation of *Tricosanthes tricuspidata* (*Cucurbitaceae*) *and Clematis montana* (*Ranunculaceae*) plants for their antidiabetic & antioxidant activity. *T. tricuspidata* grows at a height of 1200 to 2300 meters, a large climber attaining a height of 9-10 meters. The root of the plant contains methyl palmitate, palmitic acid, suberic acid, α-spinasterol, stigmasterol, cucurbitacin B, isocucurbitacin B, used to treat lung diseases, diabetes, epilepsy, fever and headache [2].

C. montana is commonly known as Clematis 'Tetrarose' available at height 1500-2000 meters in the region of the Himalaya, Kashmir to Bhutan and Afghanistan to China. Flower and fruits, mainly appear in the month of March- August. The literature survey revealed that its leaf extract is used to cure diabetes and urinary troubles [3]. Further *C. montana* have reported number of chemical constituents like Clemontanoside-A, B and C Saponins [4-7]. It also has a novel mannose-binding lectin with antiviral and apoptosis-inducing activities and an Oleanolic acid based Bisglycoside [8,9]. Its antidiabetic activity of alloxan method has also reported [10]. Although both of the plant posses incredible medicinal properties, but a number of the activities are yet to be discovered.

Therefore, the present study was aimed to evaluate aqueous and ethanolic extracts from *T. tricuspidata* and *C. montana* for its antidiabetic & antioxidant activity.

2. MATERIALS AND METHODS

All the animal studies were approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India (SIP/IAEC/10/2011).

2.1 Plant Material

The fresh roots of *T. tricuspidata* and leaves of *C. montana* were collected from Dehradun, Uttarakhand and authenticated at Tirupati University, Andra Pradesh, India.

2.2 Chemicals

All solvents used for extraction were analytical grade. Streptozotocin (STZ), Ascorbic acid, BHT and glipizide were purchased from Himgiri Chemicals, Dehradun, Uttarakhand, India.

2.3 Extraction

Dried powder of *T. tricuspidata* roots and *C. montana* leaves (2.0 kg) were extracted with ethanol (EtOH) at 48°C under reflux and hot maceration with water. Extracts were concentrated to dryness under reduced pressure to obtain a slurry (200 and 230 gm). Then extracts were kept in well closed air tight container for further use [11].

2.4 Antidiabetic Activity

2.4.1 Animal selection

Healthy adult Wister rats of either sex weighing 150-180 gm were selected for the study. The study was carried in accordance with the rules and regulations laid by the Institutional Animal Ethics Committee. The animals were housed with free access to food and water. The basal food intake and body weights to the nearest gram were noted. Rats were fasted 24 hrs prior to the study [12].

2.4.2 Acute toxicity study

The acute oral toxicity study was carried out in mice as per OECD guidelines. At a dose of (2000

mg/kg) 50% mortality was observed. Hence 200 mg/kg body weight of *T. tricuspidata* and *C. montana* of both EtOH and aqueous extracts were taken as effective dose for an evaluation of antidiabetic [13].

2.4.3 Preparation of doses

The plant extracts (200 mg/kg body weight orally) were suspended in 5% aqueous acacia solution. The standard drug glipizide (5 mg/kg body weight orally) was also given as a suspension in 5% normal saline. The control group received normal saline orally [14].

2.4.4 Induction of diabetes

STZ has been widely used to induce type-2 diabetes in animal models, especially rats & mice. The animals were deprived for food 24 hours prior to administration of STZ. Diabetes was induced by an intraperitoneal injection of STZ at a dose of 50 mg/kg (Prepared in 0.01 M citrate buffer pH 4.5). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of STZ. After the seven day stabilization period, the animals which have the fasting blood-glucose level ≥ 250 mg/dl were selected for the studies [15].

2.4.5 Treatment protocol

Diabetic animals were divided into six groups each containing six animals and one group of normal non diabetic animals. Animals were fasted 18 hrs prior to dosing and 3-4 hours after administration of the plant extracts. The plant extracts were given at a dose of 200 mg/kg, p.o. to the diabetic animals for a period of 21 days.

Group I animals received normal saline (1 ml/kg, body weight orally).
Group II diabetic animals received normal saline (1 ml/kg, body weight orally).
Group III diabetic animals received the EtOH extracts of *T. tricuspidata* (200 mg/kg, p.o.).
Group IV diabetic animals received the aqueous extracts of *T. tricuspidata* (200 mg/kg, p.o.).
Group V diabetic animals received the EtOH extracts of *C. montana* (200 mg/kg, p.o.).
Group VI diabetic animals received the aqueous extracts of *C. montana* (200 mg/kg, p.o.).
Group VII diabetic animals received standard drug glipizide (5 mg/kg, p.o.).

The animals were fasted for 18 hrs before the experiment and blood-glucose levels were checked. It was considered as a 0^{th} day reading. The blood-glucose levels were checked at 0, 7, 14 & 21 day period. The blood was collected from snipping of tail with a sharp razor in rats. The collected blood was centrifuged at 2000 rpm for 15 minutes and determination of blood-glucose levels were carried out using GOD-POD kit method in semi autoanalyser [16].

2.5 Antioxidant Activity

The antioxidant activity of EtOH and aqueous extracts of *T. tricuspidata* and *C. Montana* were determined by 1, 1 diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay. All the assays were carried out in triplicate and average values were considered [17-19].

2.5.1 DPPH radical scavenging assay

DPPH solution (0.004% w/v) was prepared in 95% EtOH. Et OH and aqueous extract of *T. tricuspidata* and *C. montana* were mixed with 95% EtOH to prepare the stock solution (10 mg/100 ml). From this stock solution 1ml, 2 ml & 3 ml of solution were taken in three- three test tubes and by serial dilution with same solvent, the final volume of each test tube was made up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml and 30 µg/ml. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer (Double beam UV-visible spectrophotometer). Ascorbic acid & Butylated Hydroxytoluene (BHT) were used as reference standards and dissolved in distilled water to make the stock solution with the same concentration [20-23]. A control sample was prepared containing the same volume without any drug and reference standards. % scavenging of the DPPH free radical was measured using the following equation-

% DPPH radical-scavenging =

[(Absorbance of control - Absorbance of test Sample)/ (Absorbance of control)] x 100

2.5 Statistical Analysis

The results were expressed as mean ± SEM. The unpaired *t*-test was used for analyzing the data between the two groups. Statistical analysis of data among the groups was performed by using analysis of variance (ANOVA) followed by the Tukey test of significance.

3. RESULTS AND DISCUSSION

Concentration Vs % Inhibition (AA= Ascorbic acid, TT = *T. tricuspidata,* CM = *C. montana*). The results of present study revealed that, EtOH extracts of both of the plants caused significant ($p<0.01$) decrease in fasting sugar level in diabetic rats from day 7^{th} (252.5±1.87 and 253.0±1.03) to 21^{st} days (197.5±1.31** and 183.8±3.79**). The aqueous extracts of TT and CM were also exerting significant ($p<0.01$) antidiabetic properties in the diabetic wister rats from 7^{th} days (250.8±1.13 and 251.5±1.99) to 21^{st} days (202.8±1.53** and 193.3±2.04**) given in Table 1. However the aqueous extracts were more potent than the EtOH extract. The *in-vitro* antioxidant studies indictes that both of the plants have significant antioxidant properties (Fig. 1). The result was comparatively promising with standard (Glipizid) antidiabetic drugs and antioxidants (i.e. ascorbic acid and BHT).

At present the exact mechanism of action is not known but may be related to increase in the secretion of insulin, inhibition of α-Amylase or PTP-1B enzyme or other factors. This action may be due to the presence of saponins [23,24]. Where DPPH accepts an electron donated by an antioxidant compound and it is decolorized, which can be quantitatively measured from the changes in absorbance values [25]. Compound having the antidiabetic properties with slight antioxidant potency will enhance its antidiabetic properties, since diet is a strong factor in controling the atherosclerosis related to general vascular disease, coronary heart disease, and Stroke. The interrelated disorders in atherosclerosis of hyperinsulinemia, hyperlipidemia, and hypertension are strongly subject to dietary influence. The type of dietary protein, animal or plant, appears to be as important as the type of lipid, animal or plant, in atherosclerosis. Dietary protein type, with its differing amino acid ratios, appears to be a major secretagogue of insulin. Diabetes mellitus, or Type II diabetes, is a related disease in which diet is a possible causal or at least a strong contributing factor. Diet is the basis for the control of Type II diabetes. Interestingly, people with diabetes have a high incidence of atherosclerosis. It has been suggested that a high intake of fruit and vegetables, the main sources of antioxidants in the diet, could decrease the potential stress caused by reactive oxygen species [26,27].

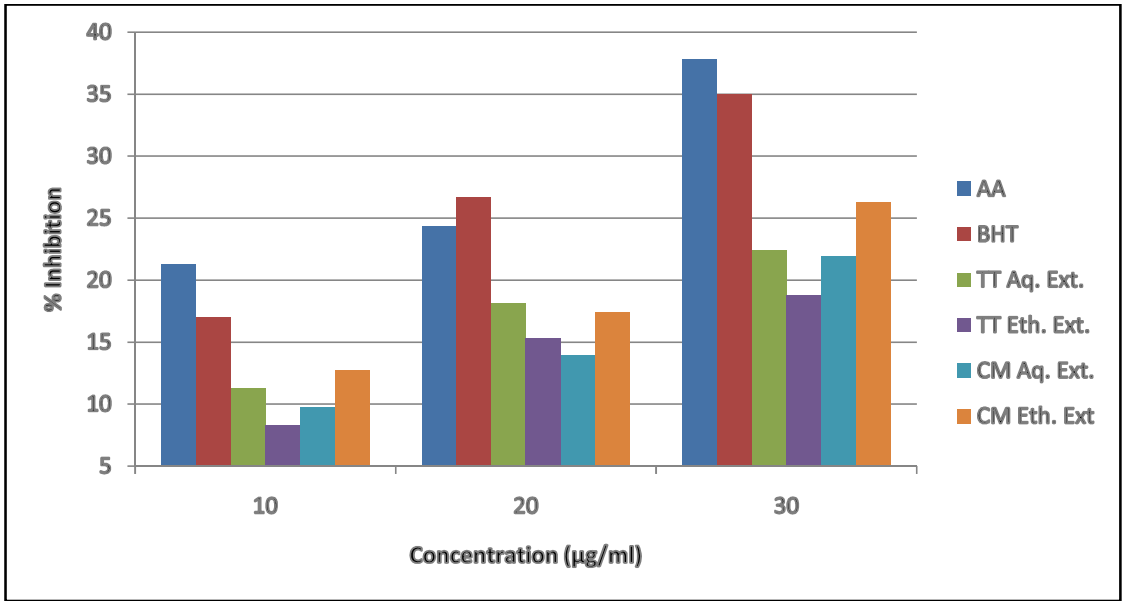

Fig. 1. *In-vitro* antioxidant activity of plant extracts

Table 1. Effect of extracts on blood glucose level (mg/dl) in STZ induced diabetes rats

Groups	Day 0 (Mean±SEM)	Day 7 (Mean±SEM)	Day 14 (Mean±SEM)	Day 21 (Mean±SEM)
Normal Control	89.2±1.10	88.2±0.87	88.3±0.71	89.0±1.03
Diabetes Control (STZ)	259.5±1.33	260.5±1.25	259.0±1.59	252.7±1.58
Standard	257.8±2.05#	251.7±2.21*	211.2±1.75***	151.8±1.59***
EtOH extract of TT	257.7±2.52#	252.5±1.87#	244.3±1.68#	197.5±1.31**
Aqueous extract of TT	257.8±2.44#	250.8±1.13#	242.8±0.94#	202.8±1.53**
EtOH extract of CM	262.5±2.17#	253.0±1.03#	236.5±1.68#	183.8±3.79**
Aqueous extract of CM	256.8±3.10#	251.5±1.99#	239.8±1.30#	193.3±2.04**

*Results are expressed as mean±SEM. ***P<0.001, **P<0.01, *P<0.05, #= Not Significant, compare with diabetic control. (TT= T. tricuspidata, CM=C. montana*)

4. CONCLUSION

The present study concludes that the EtOH and aqueous extracts of *T. tricuspidata* and *C. montana* have shown significant antidiabetic and antioxidant activity when compared with the standard. Further investigation into these studies indicated that there is a need to search for usage and pharmacological activities of bioactive compounds isolated from Indian medicinal plants having antidiabetic and antioxidant properties. Continuing research is essential to evaluate the pharmacological activities of these herbs or their active constituents that are being used for the treatments.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declares that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

5. REFERENCES

1. Padmanabha RA, Jamil K. Pharmacological evaluation of herbal extracts for their *in vitro* hypoglycemic activity. International Journal of Pharm and Biosciences. 2011;2(3):408-416.
2. Singh S, Ali S, Singh M. *In vitro* Antioxidant activity of alcoholic & aqueous root extract of *Tricosanthes Tricuspidata.* Inventi rapid. Planta Activa. 2011;4.
3. Gaur RD. Flora of district Garhwal North West Himalaya (with ethnobotanical notes). Transmedia, Srinagar Garhwal, Uttarakhand India; 1999.
4. Thapliyal RP, Bahuguna RP. Clemontanoside-C, a saponin from *Clematis montana* Phytochemistry. 1993;33(3):671-673.
5. Bahuguna RP, Jangwan JS, Kaiya T, Sakakibara J. Clemontanoside-A, a bisglycoside from Clematis montana. Phytochemistry. 1989;28(9):2511-2513.
6. Jangwan JS, Bahuguna RP. Clemontanoside B, A new saponin from *clematis montana*. Pharmaceutical Biology. 1990;28(1):39-42.
7. Bahuguna RP, Thapliyal RP, Murakami N. Saponins from Clematis Montana. Pharmaceutical Biology. 1990;28(2):125-127.
8. Peng H, Hui LV, Wang Y. Li CY, Meng L, Chen F, Bao JK. *Clematis montana* lectin, a novel mannose-binding lectin from traditional Chinese medicine with antiviral and apoptosis-inducing activities. Science Direct, Peptides. 2009;30:1805–1815.
9. Thapliyal RP, Bahuguna RP. An oleanolic acid based bisglycoside from *Clematis montana* roots. Phytochemistry. 1993;34(3):861-862.
10. Kulandaivel S, Bajpai P, Sivakumar T. Anti-hyperglycemic activity of

Trichosanthes tricuspidata root extract. Bangladesh J. Pharmacol. 2013;8:305-310.
11. Al-Ismail KM, Aburjai T. Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. J Sci. Food agric. 2004;84:173-178.
12. Chattopadhyay S, Ramanathan M, Das J, Bhattacharya SK. Animal models in experimental diabetes mellitus. Indian J. Exp. Biol. 1997;35:1141-1145.
13. Gerhard VH. Drug discovery and evaluation pharmacological assay. 2nd Ed. 2002; 947-951.
14. OECD. OECD guideline for testing of chemicals: Acute oral toxicity-fixed dose procedure. 2001;420. Available:http://www.oecd.org/document/22/0,2340,en_2649_34377_1916054_1_1_1_1,00.html
15. Sridhar M, Thirupathi K, Chaitanya G, Kumar BR, Mohan GK. Antidiabetic effect of leaves of *muntingia calabura*, in normal and alloxan induced diabetic rats. Pharmacologyonline. 2011;2:626- 632.
16. Gandhi GR, Sasikumar P. Antidiabetic effect of *Merremia emarginata Burm*. F. in streptozotocin induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2012;281-286.
17. Gupta MK, Lagarkha R, Sharma PK. Antioxidant activity of the successive extracts of *Grewia asiatica* leaves. Asian Journal of Chemistry. 2007;19(5):3417-3420.
18. Sharma A, Bhardwaj S, Mann AS, Jain A. Screening methods of antioxidant activity: An overview. Pharmacognosy Reviews. 2007;1(2):232-238.
19. Mruthunjaya K, Hukkeri VI. *In Vitro* Antioxidant and free radical scavenging potential of *Parkinsonia aculeata* Linn, Pharmacognosy Magazine. 2008;4(13):42-51.
20. Gupta MK, Lagarkha R, Sharma PK, Antioxidant activity of the successive extracts of *Grewia asiatica* leaves. Asian Journal of Chemistry. 2007;19(5):3417-3420.
21. Sharma A, Bhardwaj S, Mann AS, Jain A. Screening methods of antioxidant activity: An overview. Pharmacognosy Reviews. 2007;1(2):232- 238.
22. Gzella AG, Makuch MD, Matławska I. DPPH radical scavenging activity and phenolic compound content in different leaf extracts from selected blackberry species. Acta Biologica Cracoviensia Series Botanica. 2012;54(2):32–38.
23. Singh S, Farswan M, Ali S. Antidiabetic potential of triterpenoid saponin isolated from *Primula denticulate* Pharm. Biol. 2014;52(6):750-755.
24. Chen JC, Chiu MH, Nie RL. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. Nat. Prod. Rep. 2005;22:386-399.
25. Sharma US, Kumar A. *In vitro* antioxidant activity of *Rubus ellipticus* fruits. J. Adv. Pharm. Technol. Res. 2011;2(1):47-50.
26. Patil PS, Patel MM, Bhavsar CJ. Comparative antidiabetic activity of some herbal plants extracts. Pharma science Monitor. 2010;1(1):12-9.
27. Ebadi M. Pharmacodynamic basis of herbal medicine. Second edition. 2006;108.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=913&id=13&aid=7764