Effect of Ethanol Extract of *Coccinia grandis* Lin leaf on Glucose and Cholesterol lowering activity

Md. Mamun Al-Amin[1]*, Mir Muhammad Nasir Uddin[1], Ashfique Rizwan[2], Md. Siddiqul Islam[3]

[1]*Department of Pharmacy, North South University, Bashundhara, Dhaka 1229, Bangladesh*
[2]*Department of Pharmacy, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh*
[3]*Department of Pharmacy, Manarat International University, Dhaka 1212, Bangladesh*

ABSTRACT

Aims: To investigate the effect of ethanol extract of *Coccinia grandis* Lin leaf in glucose and cholesterol lowering activity in animal models.
Study design: Extraction, glucose and cholesterol lowering activity evaluation.
Place and Duration of Study: Department of Pharmacy, North South University, Dhaka between June 2012 and December 2013.
Methodology: The glucose and cholesterol lowering effect of the ethanol extract of *Coccinia grandis* Lin (Cucurbitaceae) leaf was evaluated using the alloxan-induced diabetic rat and compared the activity with control and Glibenclamide. Ethanol extract of **C. grandis** and Glibenclamide were administered to normal and experimental diabetic rats for 10 days.
Results: Phytochemical screening showed the presence of alkaloids, flavonoids, saponins, cardenolides and polyprenols in significant amounts. In the alloxan-induced diabetic rat model, *C. grandis* at 25 mg/kg significantly ($p<0.05$) lowered fasting blood glucose levels. *C. grandis* extract (25 mg/kg) also produced significant ($p<0.05$) total cholesterol lowering and HDL increasing ($p<0.05$) effects. In addition, body weight was increased significantly ($p<0.05$) in the *C. grandis* group.
Conclusion: These results suggest that the ethanol extract of *C. grandis* leaf possesses significant glucose and cholesterol lowering activity in animal models, thus supporting the usage of the plant in traditional medicine as an anti-diabetic medication.

Keywords: Antidiabetic activity, Glibenclamide, Coccinia grandis.

1. INTRODUCTION

High glucose and cholesterol level are known independent factors that accelerate the risk of cardiovascular disease [1,2]. Intolerance of glucose metabolism is commonly observed in diabetic patient. This intolerance is closely associated with the cholesterol level in the body. Insulin action is also influenced by the cholesterol metabolism [3]. Extensive research work has been carried out over the years for exploring the involvement of biochemical markers and the management of cardiac disease and associated complications like diabetes. Hence, the search for new chemical entities from plant source is contributing this research. A large number of medicinal plants have been studied over the years for lowering the blood glucose [4,5] and cholesterol [6] level.

* Tel.: +880192-7077102; fax: +88-2-8852016.
E-mail address: bd_pharmacy@yahoo.com.

Phytochemicals produced by the plants like *Coccinia grandis* is used in the treatment of diabetes in Asian countries as Ayurvedic remedies. *C. grandis* is also known as its synonyms *Coccinia indica* and *Coccinia cordifolia* [5]. *C. grandis* Linn is a medicinal plant under the family Cucurbitacea. Phytochemical screening of *C. grandis* reported the presence of triterpenoides [7], carotenoides [8], flavonoids [9], alkaloids [10] and fatty acids [11].

Extract of *C. grandis* leaves shows blood glucose lowering activity. Decreased phagocyte activities of macro-phages in alloxan-induced diabetic animal indicate its activity in reducing lipid peroxidation. Pectin of *C. indica* is responsible for providing this hypoglycemic action in diabetic model [12]. Human trials [5,13] confirm glucose lowering action [13]. In a double blind clinical trial 61 healthy volunteers were taken *C. grandis* (20 g leaves) and 61 healthy volunteers were taken placebo meals in their dinner. All of the volunteers were maintained a 10-hour fasting period. The authors reported that low postprandial blood glucose levels in the plant group than the control groups in both 1 hour and 2 hour postprandial period [5]. The authors reported that *C. grandis* reduces phosphorylase activity, increases liver glycogen and thus lower the blood glucose level [12]. Moreover, glucose lowering effect can be achieved by inhibiting key gluconeogenic enzyme, glucose-6-phosphatase in animal model [14].

Singh G et al., in 2007 reported that polyprenols of *C. grandis* lowers the plasma lipid level followed by a beneficial effect on HDL and its ratio with total cholesterol in dyslipidemic hamster model [15]. Previous research also stated that the action of polyprenols was mediated through peroxisome proliferators activated receptor-a (PPARa) by catabolizing triglycerides and improving HDLC/TC ratio for the maintenance of lipid–glucose homeostasis in hamster model [16,17].

Aims of the present study

We aimed to explore the glucose, total cholesterol lowering and HDL improving property of *C. grandis* and investigate the induced weight changes. Alloxan is used in our current study to induce diabetes in animal model. In rodent model alloxan selectively destroys pancreatic beta cell which in turn shut down the production of insulin. In the presence of thiols, alloxan generates reactive oxygen species (ROS) which in turn initiate toxic action in the beta cell by free radical formation.

Hypothesis

We hypothesized that *C. grandis* will be able to lower blood glucose and total cholesterol level while improve HDL level in the plasma. The extract of C. grandis leave might have a distinct mechanism to provide glucose and cholesterol lowering activity in animal model. We also thought that *C. grandis* will not affect the total body weight.

2. MATERIAL AND METHODS

2.1 Plant Material

The leaves of *C. grandis* were collected in December, 2012 from Jessore city in Bangladesh and authenticated by Bangladesh National Herbarium. The leaves were initially dried under shade and grinded.

2.2 Preparation of extract

The powder C. grandis leaves (1 kg) were mixed with ethanol in a 250mL flask with mild shaking. The flasks were closed with cotton plug and aluminum foil at 48 hours at room temperature. The extract was filtered through Whatman filter paper (No.1), concentrated using a rotary evaporator at low temperature (40-50°C). The extracts were preserved in airtight containers and kept at 4°C until further use.

2.3 Phytochemical Screening

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, terpenoids saponins, polyprenols and cardenolides were carried out for all the extracts by the method described by Harborne and Sazada [18,19]. In each test 10% (w/v) solution of the extract was taken. *Test for alkaloids:* Dragendroff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. After adding 1 ml of Dragendroff's reagent, orange brown precipitate indicated the presence of alkaloids. Test for cardenolides: The extract is to be dissolved in pryridine and a few drops of 2 per cent sodium nitorprusside together with a few drops of 20 per cent NaOH are to be added. A deep red colour which faded to a brownish yellow indicates the presence of cardenoloides. *Test for flavonoids*: A few drops of concentrated hydrochloric acid were added to a small amount of extract solution. Immediate appearance of a red color indicated the presence of flavonoids. *Test for saponins*: 1 ml solution of the extract was diluted to 20 ml with distilled water and shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam indicated the presence of saponins. *Test for polyprenols*: Polyprenols was identified by using the method described in Singh et al. in 2007 [20]. *Test for terpenoids*: Salkowski test: 5 ml of the extract solution was mixed in 2 ml of chloroform, and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.4 Test animals

Test animals were collected from International Cholera and Dysentery Disease Research, in Bangladesh (icddr,b). Albino rats (wistar strain) of both sexes weighing 175 g (average) were used for the study. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle.

2.5 Induction of diabetes and treatment

The solution of Alloxan monohydrate (10 mg/ml) was prepared in ice-cold citrate buffer 0.1 M pH 4.5 and kept in ice. Then the solution was administered intraperitonially to the animals within 5 minutes at a dose of 50 mg/kg body weight. Alloxan was chosen to induce diabetes due to its availability and widely reported in previous research [21]. After 48 hours of administration, diabetic model rats having glycosuria and hyperglycemia were taken for the experiment. Rats fasted over night were used for induction of diabetes. Rats were divided into two sets; diabetic and non-diabetic. Group I (n=12) received normal diet and served as normal control. Group II (n=12) consists of alloxan-induced rats receiving normal diet and serving as diabetic control. Group III (n=12) consists of alloxan-induced rats receiving Glibenclamide at 0.5 mg/kg body weight once a day orally for 10 days. Group IV (n=12) consists of alloxan-induced rats receiving *C. grandis* Linn (25 mg/kg) once a day orally for 10 days. Group V (n=12) consists of normal rats receiving *C. grandis* (25 mg/kg) once a day orally for 10 days. Blood samples were collected through the tail vein just prior to and on day 10 after drug administration. The blood glucose level was measured using Glucometer. Total cholesterol and HDL were measured using kinetic enzymatic method [22] and Gordon D.J. (1989) method [23] for all the samples.

2.6 Statistical Analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$ were considered to be statistically significant. All the statistical tests were carried out using SPSS statistical software.

3. RESULTS AND DISCUSSION

Phytochemical screening of the extract of *Coccinia grandis* revealed the presence of various bioactive components of which alkaloid, cardenolides, flavonoides and polyprenols were the most prominent (table 1).

Table 1: Phytochemical investigation of ethanol extract of *C. grandis leaf*.

Test	Alkaloids	Cardenolides	Flavonoids	Terpenoids	Saponins	Polyprenols
Observation	+	++	+++	+	+++	+

+++' indicates presence in high concentrations; ++' indicates presence in moderate concentrations; '+' indicates presence in trace concentration; '-' indicates absence

Blood glucose levels were measured for all the animals. The blood glucose and total Cholesterol level at day 10 in control group, alloxan-induced diabetic animal, glibenclamide treated diabetic groups, *C. grandis* extract treated diabetic and normal group is summarized in table 2. The differences were significant between control and diabetic control, diabetic control and glibenclamide treated diabetic group, diabetic control and *C. grandis* treated diabetic group. Total Cholesterol levels were also determined for all animals.

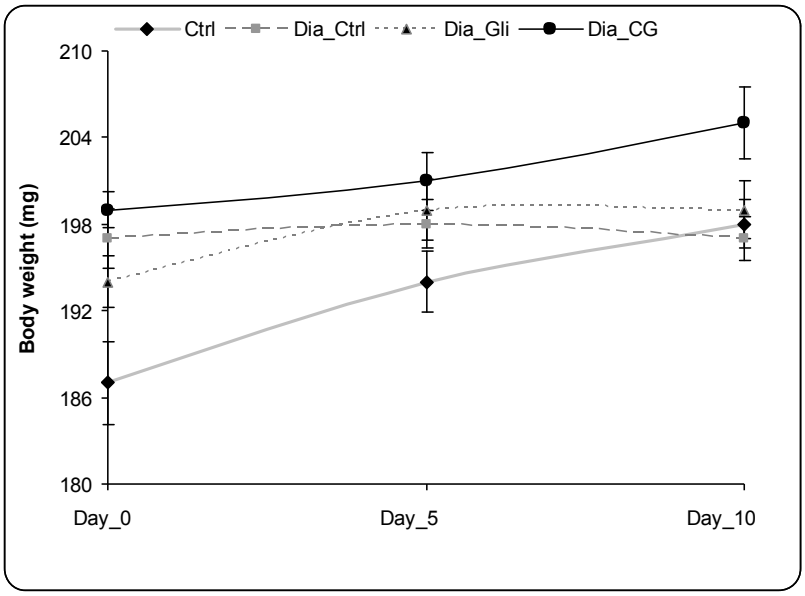
Table 2. Glucose and cholesterol level in serum between control and experimental animal groups.

Parameter (mg/dl)	Control	Diabetic Control	Diabetic + Glibenclamide (0.5 mg/kg)	Diabetic + *C. Grandis* Extract treated (25 mg/kg)	*C. Grandis* Extract treated (25 mg/kg)
Glucose	119.6±1.4	248.1±1.6***	101.11±1.7**	108.42±1.2*	107.06±2.8*
Total Cholesterol	124.7 ±1.5	238.1±1.2***	106.08±1.8**	111.78±11.2*	112.63±4.5*
HDL	42.23±2.7	37.51±2.87*	38.81±5.91*	49.67*	48.81±3.32*

Data were represented as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet's multiple comparison. The criterion for statistical significance was ***p< 0.001, **p< 0.01 and *p< 0.05.

The glucose level of diabetic group was significantly higher than diabetic with *C. grandis* extract group (p = .05). The total cholesterol level was lower in *C. grandis* extract group (p = .05) than diabetic group while HDL level was higher in *C. grandis* extract group (p = .05) than diabetic group.

Figure 1. Effect of *C. Grandis* extracts on the body weight of diabetic rats. Ctrl: Control; Dia_Ctrl: Diabetic Control; Dia_Gli: Diabetic animal treated with Glibenclamide; Dia_CG: Diabetic animal treated with *C. Grandis* extract (25 mg/kg).



Data were represented as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet's multiple comparison. The criterion for statistical significance was $p < 0.05$.

Total body weights were also measured for all animals on day zero (before administration of extract), day 5 and day 10. Surprisingly the body weight was raised for the diabetic animals that are treated with *C. grandis*. Average body weights of other animal groups were remaining unchanged (Figure 1).

The result of the present study demonstrated that ethanol extract of C. grandis leaf lowers serum glucose and cholesterol level in the alloxan-induced diabetic rats while improving HDL level in the serum.

Lowering of Blood glucose levels were consistent over long term treatment with ethanol extract of *C. grandis*. This result indicates that the extract was able to improve blood glucose tolerance. Blood glucose lowering activity might be achieved by the following mechanisms. The pythochemical constituents of *C. grandis* may reduce the phosphorylase activity by suppressing glucose 6-phosphatase enzyme. Therefore, glycogen in the liver will not be converted into glucose. Moreover, inhibition of posphorylase activity may produce an opportunity to increased glycogen synthesis in the liver. In result, glucose can enter into the liver as stored as glycogen form. The total mechanisms will ultimately help to bring excess glucose from the blood.

Previous findings are consistent with our present findings [13,14]. In the present study ethanol extract of *C. grandis* was able to reduce the cholesterol level during the 10 days treatment period. Singh G. et al., in 2007 isolate polyprenol from *C. grandis* leaf by ethanol extract. The authors reported that polyprenol might be responsible for cholesterol-lowering effects [20]. Krishnakumari, S. et al., in 2011 investigate the activity of methanol extract on lipid profile in streptozotocin induced diabetic rats. The authors reported that lipid profile

increased in diabetic group and after the treatment with the methanol extract the lipid profiles back to the normal level [24].

Body weight of the diabetic animals was increased after the administration of ethanol extract of *C. grandis*. This weight enhancing effect was not found in glibenclamide group. The mechanism of body weight changes possibly due to the deposition of fatty substance in the body. Further research is needed to explore the root cause of increased body weight.

One may think that why the extracts were administered for 10 days to the animal. We thought that 10 days are enough to simulate the diabetes in animal model. Moreover, several other researchers are also administered C. grandis and explore some other pharmacological action in animal model [25, 26].

4. CONCLUSION

In our present study ethanol extract of *Coccinia grandis* leaf showed blood glucose lowering effect in diabetic rodent model after oral administration. Thus, the uses of this plant extract as a traditional medicine in the treatment of diabetes is validated. The findings of the present study could contribute in the contemporary research by giving the idea that the pytochemical constituents of *C. grandis* have beneficial activities in diabetic model and in cardiac patient as well. Therefore, isolation of the lead compound(s) that is/are responsible for providing this action could be done in future studies.

COMPETING INTERESTS

No competing interests exist.

AUTHORS' CONTRIBUTIONS

Author MMNU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MSI performed the statistical analysis. Author MMAA and AR managed the literature searches. Author MMAA finalize the manuscript. All authors read and approved the final manuscript.

CONSENT (WHERE EVER APPLICABLE)

This section is not applicable in our paper.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Experimental protocol was approved by Institutional Ethics Committee of the Department of Pharmacy, North South University (approval no. NSU/DP/12/11). Animals were handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes for Health Publication, 1985).

REFERENCES

1. Cohen HW, Hailpern SM, Alderman MH. Glucose-cholesterol interaction magnifies coronary heart disease risk for hypertensive patients. Hypertension. 2004a;(43): 983-7.

2. Cohen HW, Sloop GD, Study P. Glucose interaction magnifies atherosclerotic risk from cholesterol. Atherosclerosis. 2004b;172:115-20.
3. Hallikainen M, Toppinen L, Mykkanen H, Agren JJ, Laaksonen DE, Miettinen TA,, Niskanen L, Poutanen KS, Gylling H. Interaction between cholesterol and glucose metabolism during dietary carbohydrate modification in subjects with the metabolic syndrome. Am J Clin Nutr. 2006;84:1385-92.
4. Bailey CJ, Day C. Traditional Plant Medicines as Treatments for Diabetes. Diabetes Care. 1989;12(8):553-564
5. Munasinghe M, Abeysena C, Yaddehige IS, Vidanapathirana T, Piyumal KPB. Blood Sugar Lowering Effect of Coccinia grandis (L.) J. Voigt: Path for a New Drug for Diabetes Mellitus. Exp Diabetes Res. 2011; 978762.
6. Doornbos AM, Meynen EM, Duchateau GS, Van Der Knaap HC, Trautwein EA. Intake occasion affects the serum cholesterol lowering of a plant sterol-enriched single-dose yoghurt drink in mildly hypercholesterolaemic subjects. Eur J Clin Nutr. 2006;60:325-33.
7. Vaishnav MM, Jain P, Jogi SR, Gupta KR. Coccinioside-K, triterpenoid saponin from Coccinia indica. Orient. J. Chem. 2001;17:465–468.
8. Barua AB, Goswami BC, Carotenoides of Cephalandra indica (Coccinia indica). Curr. Sci. 1979;48:630–632.
9. Vaishnav MM, Gupta KR. Ombuin 3-O-arabinofuranoside from Coccinia indica. Fitoterapia 1996;67:80.
10. Qudrat-i-Khuda M, Khaleque KA, Miah MA. Chemical investigations of Cephalandra indica. I. Constituents of dry aerial parts. Sci. Res. (Dacca) 1965;2:27–31.
11. Siddiqui IA, Osman SM, Subbaram MR, Achaya KT. Fatty acid components of seed fats from four plant families. J. Oil Technol. Assoc. India. 1973;5:8–9.
12. Azad Khan AK, Akhtar S, Mahtab H. Coccinia indica in the treatment of patients with diabetes mellitus. Bangladesh Med Res Counc Bull. 1979;5:60-6.
13. Kumar GP, Sudheesh S, Vijayalakshmi NR. Hypoglycaemic effect of Coccinia indica: mechanism of action. Planta Med. 1993;59:330-2.
14. Hossain MZ, Shibib BA, Rahman R. Hypoglycemic effects of Coccinia indica: inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. Indian journal of experimental biology. 1992;30(5):418-20.
15. Overtuff ML, Loose MDC. In vivo model system: the choice of the experimental model for the analysis of lipoproteins and atherosclerosis. Curr. Opin. Lipidol. 1992;3:179.
16. Owens DR, Luzio SD, Coates PA. Insulin secretion and sensitivity in newly diagnosed NIDDM Caucasians in UK. Diabetic Med. 1996; 13;9Suppl 6:S19–S24.
17. Hossain MZ, Shibib BA, Rahman, R. Hypoglycemic effects of Coccinia indica: inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. Indian J Exp Biol. 1992;30:418-20.
18. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London ISBN: 0341235727032, 1998;302.
19. Sazada S, Verma A, Rather AA, Jabeen F, Meghvansi MK. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res. 2009;3:1883195.
20. Singh GP, Gupta P, Rawat A, Puri BG, Maurya R. Antidyslipidemic activity of polyprenol from Coccinia grandis in high-fat diet-fed hamster model. Phytomedicine: international journal of phytotherapy and phytopharmacology. 2007;14(12):792-8.
21. Soto C, Muriel P, Reyes JL. Pancreatic lipid peroxidation in alloxan-induced diabetes mellitus. Archives of medical research. 1994;25(4):377-80.
22. Deeg R, Ziegenhorn J. Kinetic enzymic method for automated determination of total cholesterol in serum. Clinical chemistry. 1983;29(10):1798-802.

23. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79(1):8-15.
24. Krishnakumari SP, Bhuvaneswari, Rajeswari P. Ameliorative potential of Coccinia grandis extract on serum and liver marker enzymes and lipid profile in streptozotocin induced diabetic rats. Ancient science of life. 2011;31(1):26-30.
25. Moideen K, Sherief SH, Sengottuvelu S, Sivakumar T. Hepatoprotective and antioxidant activity of Coccinia grandis root extract against paracetamol induced hepatic oxidative stress in wistar albino rats. International Journal of Research in Ayurveda & Pharmacy. 2011;2(3):858-63.
26. Doss A, Dhanabalan R. Anti-hyperglycaemic and Insulin Release Effects of Coccinia grandis (L.) Voigt Leaves in Normal and Alloxan Diabetic Rats. Ethnobotanical Leaflets. 2008;12:1172-75.