Research paper

The Effect of Leaf Ethanol Extract of *Coccinia Grandis* Lin in glucose and cholesterol lowering activity

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ABSTRACT

Aims: To investigate the effect of the leaf extract of *Coccinia Grandis* Lin (Cucurbitaceae) 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 leaf extract of *Coccinia Grandis* Lin (Cucurbitaceae) was evaluated using the alloxan monohydrate induced diabetic rat and compared the activity with control and Glibenclamide. Graded doses of the ethanol extract 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 polyphenols in significant amounts. In the alloxan induced diabetic rat model, *C. grandis* at 2 ml significantly ($p<0.05$) lowered fasting blood glucose levels. The extract (2ml) also produced significant total cholesterol lowering ($p<0.05$) and HDL increasing ($p<0.05$) effects at 2ml. In addition, body weight was significantly ($p<0.05$) increased in the *C. grandis* group.
Conclusion: These results suggest that the aqueous leaf extract of *C. grandis* 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 and accelerate the risk of coronary heart disease [1,2]. The intolerance of glucose metabolism is linked with the cholesterol. Moreover, the actions of insulin and cholesterol metabolism are interrelated [3].

Extensive research work has been conducted so far for the management of heart disease and associated complications like diabetes. The search for new chemical entities from plant source is a common practice in south Asian research. A large number of medicinal plants have been studied over the years for lowering the blood glucose [4,5] and cholesterol [6] level.

Phytochemicals produced by the plants like *Coccinia grandis* is used in the treatment of diabetes in Asian countries as Ayurvedic remedies. *Coccinia 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. Kumar and his colleagues in 1993 reported that pectin of *C. indica* is responsible for providing this hypoglycemic action [12]. Human trials [5,13] confirm the glucose lowering action of *C. grandis* [13]. In a double blind clinical trial 61 healthy volunteers were taken 20 g of leaves of *C. grandis* 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 experimental group than the control groups in both 1 hour and 2 hour postprandial period [5]. *C. grandis* reduces phosphorylase activity, increases liver glycogen and thus lower the blood glucose level [12]. Moreover, glucose lowering effect is also 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

Many research works has been carried out so far to investigate the antidiabetic activity of *Coccinia grandis*. However, there are very limited research has been carried out to investigate the ethanol extract of the plant *C. grandis* in diabetic animal model. Hence, we aimed to explore the glucose and cholesterol lowering 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 since *Coccinia grandis* will be able to lower blood glucose level, its leave extract might have a distinct mechanism to provide glucose and cholesterol lowering activity in the animal model. We also thought that *C. grandis* will not affect the total body weight after administering the extract in the animal model. We also thought that, *C. grandis* might enhance HDL level in the plasma.

2. MATERIAL AND METHODS

2.1 Plant Material

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

2.2 Preparation of extract

The extract was prepared at the rate of 1g/5ml of solvent in a 250mL flasks with mild shaking. The flasks were closed with cotton plug and aluminum foil with mild shaking at 48 hours at room temperature, filtered through what man filter paper No.1 and then concentrated by 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, steroids, glycosides, saponins, tannin and terpenoids were carried out for all the extracts by the method described by Harborne and Sazada [18,19]. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner reagent, flavonoids with the use of conc. HCl, tannins with 5%, and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use ά-napthol and sulfuric acid and terpenoids with chloroform and concentrated HCl.

2.4 Test animals

Test animals were obtained from International Cholera and Dysentery Disease Research, in Bangladesh (icddr, b). Albino rats (wistar strain) of either sex weighing 150 - 200 g bred in Pharmacology research Lab at Jahangirnagar University in Bangladesh were used for the study. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions. Animals were given water and fed with rat pellet feed. 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).

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. After 48 hours of administration, diabetic model rats having glycosuria and hyperglycemia were taken for the experiment. Rats weighing 150 – 160 g fasted over night were used for induction of diabetes. Rats were divided into two sets; diabetic and non-diabetic. Group I received normal diet and served as normal control. Group II consists of alloxan-induced rats receiving normal diet and serving as diabetic control. Group III consists of alloxan- induced rats receiving Glibenclamide (synthetic antidiabetic drug) at 0.5 mg/kg body weight once a day orally for 10 days. Group IV consists of alloxan-induced rats receiving *C. grandis* Linn (2ml) once a day orally for 10 days. Group V consists of normal rats receiving *C. grandis* (2 ml) 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 and cholesterol levels were analyzed 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 ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, 0.001 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. The result of phytochemical test is summarized in the Table 1.

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

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

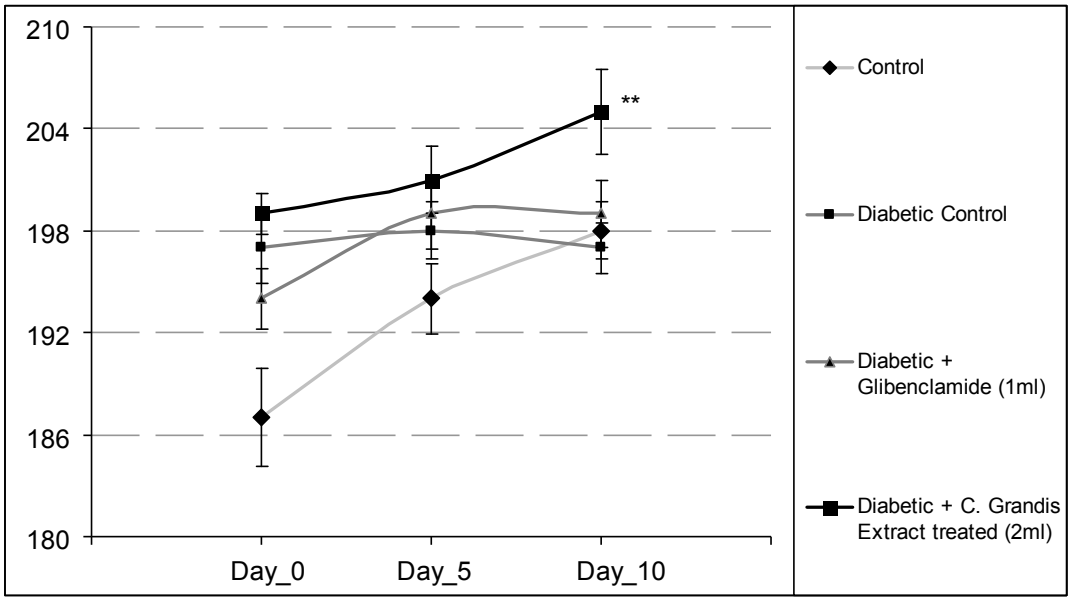
Blood glucose levels were measured for all the animals. The blood glucose level were 119.6 mg/dl in control group, 248.1 mg/dl in alloxan induced diabetic animal, 101.11 mg/dl in glibenclamide treated diabetic groups, 108.42 mg/dl in *C. grandis* treated diabetic group. The differences were significant between control and diabetic control, diabetic control and glibenclamide treated diabetic group, diabetic control and *C. grandis* treated diabetic group (Table 2). Total Cholesterol levels were also determined for all animals. The blood cholesterol level were 124.7 mg/dl, 238.1 mg/dl, 106.08 mg/dl, 111.78 mg/dl for control group, alloxan induced diabetic animal, glibenclamide treated diabetic groups and *C. grandis* treated diabetic group respectively. The differences were significant between control and diabetic control, diabetic control and glibenclamide treated diabetic groups, diabetic control and *C. grandis* treated diabetic groups (Table 2). The HDL level were 42.23 mg/dl in control group, 37.51 mg/dl in alloxan induced diabetic animal, 38.81 mg/dl in glibenclamide treated diabetic groups, 49.67 mg/dl in *C. grandis* treated diabetic group.

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

Parameter (mg/dl)	**Control**	**Diabetic Control**	**Diabetic + Glibenclamide (1ml)**	**Diabetic + *C. Grandis* Extract treated (2ml)**
Glucose	119.6±1.4	248.1±1.6***	101.11±1.7**	108.42±1.2*
Total Cholesterol	124.7 ±1.5	238.1±1.2***	106.08±1.8**	111.78±11.2*
HDL	42.23±2.7	37.51±2.87*	38.81±5.91*	49.67*

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.

Figure 1. Effect of *C. Grandis* extracts on the body weight of diabetic rats.



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 (just before administration of extract), day 5 and day 10. Average body weight was different before the administration of drug among the animal groups. Interestingly the body weight was increased for the diabetic animals that are treated with *C. grandis*. Average body weights of other animal groups were remaining unchanged (Figure 1).

Experimental result sowed that ethanol extracts of *C. grandis* lowers the serum glucose and total cholesterol level in the alloxan induced diabetic rats. Moreover, HDl level was also raised by the ethanol extract of *C. grandis*. Similar results were observed for the diabetic rats with Glibenclamide. Reduced Blood glucose levels were consistent over long term treatment with *C. grandis* extracts on alloxan-induced diabetes rats. Our findings indicates that ethanol extract of *C. grandis* improve the blood glucose tolerance. The lowering of blood glucose level may achieved by the increased glycogen synthesis and suppression of glucose 6-phosphatase enzyme. Other mechanisms might also be involved for glucose lowering action. Mechanisms including the reduced sugar absorption from the gut, rising of insulin production from the pancreas, reduction of insulin release from the liver or reduction of glycogen breakdown, increase the conversion of glucose to other substance such as fatty acid or amino acid can also be involved in reducing the glucose level in the blood. Previous research [13,14] findings are consistent with our present findings. Therefore, *C. grandis* can be recommended for diabetic patients.

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 [21].

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. Further investigations of its activity against a blood glucose tolerance, specific lipids such as LDL, VLDL, HDL and purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

4. CONCLUSION

In conclusion, ethanol extract of *Coccinia grandis* 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. Further studies should be conducted to isolate the lead compound(s) that is/are responsible for providing this action.

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 MMAA performed the statistical analysis of the study and finalize the manuscript. Author AR managed the literature searches. 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, H.W., Hailpern, S.M. & Alderman, M.H. Glucose-cholesterol interaction magnifies coronary heart disease risk for hypertensive patients. Hypertension, 2004a;43, 983-7.
2. Cohen, H.W., Sloop, G.D. & Study, P. Glucose interaction magnifies atherosclerotic risk from cholesterol. Findings from the PDAY Study. Atherosclerosis, 2004b;172, 115-20.

3. Hallikainen, M., Toppinen, L., Mykkanen, H., Agren, J.J., Laaksonen, D.E., Miettinen, T.A., Niskanen, L., Poutanen, K.S. & Gylling, H. Interaction between cholesterol and glucose metabolism during dietary carbohydrate modification in subjects with the metabolic syndrome. 2006; Am J Clin Nutr, 84, 1385-92.
4. Bailey, C.J. & C. Day, Traditional Plant Medicines as Treatments for Diabetes. Diabetes Care. 1989;12(8):553-564
5. Munasinghe, M., Abeysena, C., Yaddehige, I.S., Vidanapathirana, T. & Piyumal, K.P.B. 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, A.M., Meynen, E.M., Duchateau, G.S., Van Der Knaap, H.C. & Trautwein, E.A. 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, M.M., Jain, P., Jogi, S.R., Gupta, K.R., Coccinioside-K, triterpenoid saponin from Coccinia indica. Orient. J. Chem. 2001;17, 465–468.
8. Barua, A.B., Goswami, B.C., Carotenoides of Cephalandra indica (Coccinia indica). Curr. Sci. 1979;48, 630–632.
9. Vaishnav, M.M., Gupta, K.R., Ombuin 3-O-arabinofuranoside from Coccinia indica. Fitoterapia 1996;67, 80.
10. Qudrat-i-Khuda, M., Khaleque, K.A., Miah, M.A.W., Chemical investigations of Cephalandra indica. I. Constituents of dry aerial parts. Sci. Res. (Dacca) 1965;2, 27–31.
11. Siddiqui, I.A., Osman, S.M., Subbaram, M.R., Achaya, K.T., Fatty acid components of seed fats from four plant families. J. Oil Technol. Assoc. India 1973;5, 8–9.
12. Azad Khan, A.K., 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, G.P., Sudheesh, S. & Vijayalakshmi, N.R. Hypoglycaemic effect of Coccinia indica: mechanism of action. Planta Med, 1993;59, 330-2.
14. Hossain, M.Z., B.A. Shibib & R. Rahman, 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, M.L., Loose, M.D.C., 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, D.R., Luzio, S.D., Coates, P.A., Insulin secretion and sensitivity in newly diagnosed NIDDM Caucasians in UK. Diabetic Med. 13 (9 Suppl 6), 1996; S19–S24.
17. Hossain, M. Z., Shibib, B. A. & Rahman, R. Hypoglycemic effects of Coccinia indica: inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. Indian J Exp Biol, 1992; 30, 418-20.
18. J.B.Harborne (eds.). Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London ISBN: 0341235727032, (1998); 302.
19. S.Sazada, A.Verma, A.A.Rather, F.Jabeen and M.K. Meghvansi. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res., 2009; 3, 1883195.
20. Singh, G.P., Gupta, P. Rawat, A. Puri, Bhatia G. & 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. Krishnakumari, S., P. Bhuvaneswari & P. Rajeswari, 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.