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

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ABSTRACT

Aims: To investigate the effect of ethanol extract of *Coccinia grandis* Lin (Cucurbitaceae) leaf in glucose and cholesterol lowering activity in animal model.
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 2012.
Methodology: Glucose and cholesterol lowering effect of the ethanol extract of *C. grandis* leaf was evaluated using the alloxan-induced diabetic rat and compared the activity with diabetic control and antidiabetic drug (Glibenclamide). Ethanol extract (25mg/kg) of *C. grandis* and Glibenclamide were administered to normal and experimental diabetic rats for the duration of 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* (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. Surprisingly, body weight was increased significantly ($p<0.05$) in the *C. grandis* treated diabetic group.
Conclusion: These results suggest that the ethanol extract of *C. grandis* leaf possesses significant glucose and cholesterol lowering activity in animal model, 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 levels are two well-known independent major 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.

A large number of medicinal plants have been studied over the years to discover new chemical entities from plant source which possesses blood glucose [4,5] and cholesterol [6] lowering actions. In this context, here *C. granids* were investigated to discover its glucose and cholesterol lowering activity in animal model. Phytochemicals produced by the plants like *C. grandis* is used in the treatment of diabetes in Asian countries as Ayurvedic remedies.

C. grandis Linn is a medicinal plant under the family Cucurbitacea. *C. grandis* is also known as its synonyms *Coccinia indica* and *Coccinia cordifolia* [5]. Phytochemical screening of *C. grandis* reported the presence of triterpenoides [7], carotenoides [8], flavonoids [9], alkaloids [10] and fatty acids [11].

It has been found that extract of *C. grandis* leaves showed glucose lowering activity in the blood. In addition, 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 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]. The authors were 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 [15].

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 body weight changes. Alloxan is used in our current study to induce diabetes in animals. 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. Alloxan was administered for 10 days to simulate diabetes in the animals as this was supported by previous papers [16, 17].

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 were conducted for the identification of alkaloids, flavonoids, terpenoids, saponins, polyprenols and cardenolides in the *C. grandis* extract [18, 19, 20, 21]. *Test for alkaloids:* Dragendroff's test: Plant extract (2 ml) and dilute HCl (0.2 ml) were taken together in a test tube. Then 1 ml of Dragendroff'S reagent was added. Orange brown precipitate in the test tube indicates the presence of alkaloids in the extract sample. *Test for cardenolides*: At first, the extract was dissolved in pyridine. Then a few drops of 2% sodium nitroprusside and a few drops of 20 % NaOH were added. A deep red color which faded to a brownish yellow indicates the presence of cardenoloides. *Test for flavonoids*: A few drops of concentrated HCl were added to a small amount of extract solution. Immediate appearance of a red color indicates 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 indicates the presence of saponins. *Test for polyprenols*: Polyprenols was identified by using the method described in Singh et al. in 2007 [15]. *Test for terpenoids*: Salkowski test: 5 ml of the extract solution was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface 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. Munasinghe et al. in 2011 were also recruited both gender in their study [5]. 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 [17, 22]. 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 [23] and Gordon D.J. (1989) method [24] 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 *C. grandis* revealed the presence of various bioactive components of which alkaloid, cardenolides, flavonoids 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 concentration; '++'= indicates presence in moderate concentration; '+'= indicates presence in trace concentration;

Blood glucose levels were measured for all the animals. The blood glucose and total Cholesterol level at day 10 in control (group I), alloxan-induced diabetic animal (group II), glibenclamide treated diabetic group (group III), *C. grandis* extract treated diabetic (group IV) and *C. grandis* extract treated normal group (group V) 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.

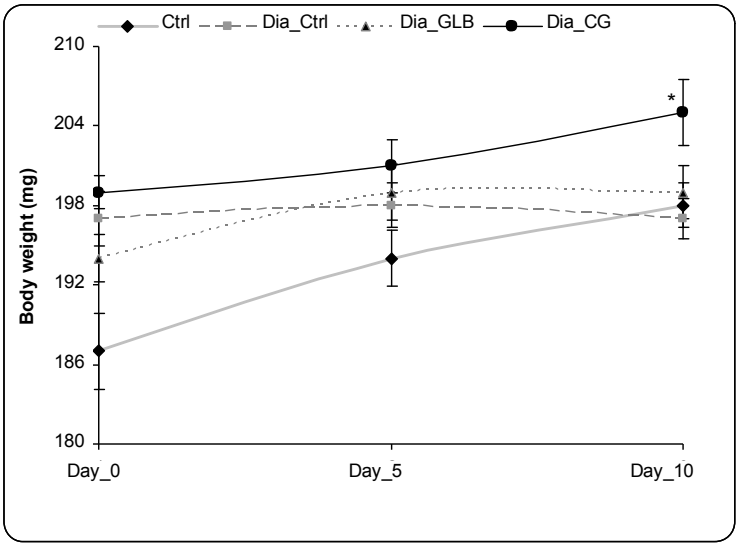
Table 2. Glucose and cholesterol level in serum between control and experimental animal groups.

Parameter (mg/dl)	Group-I: Control (n=12)	Group-II: Diabetic Control (n=12)	Group-III: Diabetic + GLB (0.5 mg/kg) (n=12)	Group-IV: Diabetic + CG treated (25 mg/kg) (n=12)	Group-V: CG treated (25 mg/kg) (n=12)
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±5.87**	48.81±3.32*

GLB= Glibenclamide; CG= *C. grandis* extract; 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 was significantly (p < .05) higher in alloxan-induced diabetic group (group II) than *C. grandis* treated diabetic group (group IV). The total cholesterol level was significantly (p < .05) lower in *C. grandis* treated diabetic group (group IV) than alloxan-induced diabetic group (group II) while HDL level was higher (p < .05) in *C. grandis* treated diabetic group (group IV) than alloxan-induced diabetic group (group II).

Figure 1. Effect of *C. Grandis* extracts on the body weight of diabetic rats. Ctrl: Control; Dia_Ctrl: Diabetic Control; Dia_GLB: 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 < .05 which is shown in Diabetic + *C. grandis* group on day 10.

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 in the alloxan-induced diabetic animals that are treated with *C. grandis* leaf extract (group IV). Average body weights of other animal groups were remaining unchanged (Figure 1).

The result of this study shows 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. One unanticipated finding was that body weight was increased in *C. grandis* treated diabetic control group.

As expected blood glucose lowering effect was found in this study. Even, the lowering of blood glucose levels was consistent over long term treatment with ethanol extract of *C. grandis*. This result indicates that the extract was able to improve blood glucose tolerance. There are so many mechanisms are involved in blood glucose lowering activity. Present study have not specifically designed to explore specific pathway involve in glucose lowering activity. However, previous research studies have reported the following mechanisms of *C.*

grandis by which it may achieve blood glucose lowering activity. In 1979, Azad et al. published a paper in which they studied *C. indica* in the treatment of diabetes. They demonstrated that *C. indica* reduces phosphorylase activity, increases liver glycogen and thus lower the blood glucose level [12]. In 1992, Hossain and coworkers published a paper in which they studied hypoglycemic effects of *C. indica*. They demonstrated that *C. indica* may inhibit key gluconeogenic enzyme, glucose-6-phosphatase in animal model [14]. In 2007, Rahul et al. *reported* a new mechanism in their review study. They demonstrated that *C. grandis* may diminish the phosphorylase activity by suppressing glucose 6-phosphatase enzyme [25]. Hence, through the various mechanisms as reported in the previous published paper, glycogen in the liver will not be converted into glucose. Moreover, inhibition of phosphorylase activity may produce an opportunity to increased glycogen synthesis in the liver. In result, glucose may enter into the liver and deposit there as glycogen form. The total mechanisms will ultimately help to remove excess glucose from the blood.

In the present study, we found that *C. grandis* reduces total cholesterol level during the 10 days treatment period which was expected in the hypothesis. Moreover, phytochemical screening showed the presence of polyprenol in *C. grandis* extract. Polyprenol has cholesterol lowering activity. In this context, the present findings seem to be consistent with earlier research [13, 14]. In 2007, Singh et al. isolate polyprenol from *C. grandis* leaf by ethanol extract. The authors reported that polyprenol might be responsible for cholesterol-lowering effects [15]. In 2011, Krishnakumari et al. studied the activity of methanol extract of *C. grandis* on lipid profile in streptozotocin induced diabetic rats. The authors reported that the treatment with methanol extract of *C. grandis* was able to reduce lipid profiles to the normal level [26]. We also found that methanol extract of *C. grandis* was able to improve HDL cholesterol level. This activity might be achieved due to the presence of polyprenol. This compound may increase the HDL level in the blood.

It was surprising that the body weight of the diabetic animals those are treated with *C. grandis* was increased. This weight enhancing effect was not found in glibenclamide group. Excess deposition of fatty acids, conversion of glucose into fatty acid and other mechanisms are might be responsible for this unwanted activity. Therefore, further research is needed to explore the root cause of increased body weight.

4. CONCLUSION

This study indicates that the methanol extract of *C. grandis* possesses significant glucose and total cholesterol lowering activity in the blood at 25mg/kg dose investigated on the experimental laboratory animals. This could provide rationale for its traditional uses in the management of diabetes and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

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. Animals were handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes for Health Publication, 1985).

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