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Antidiabetic and Antioxidant Activities of Decoctions of *Coccinia grandis* Linn. and *Centella asiatica* (L.) on Alloxan-induced Diabetic rats

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Abstract

The decoctions of *Coccinea grandis* (L.) and *Centella asiatica* (L.) leaves were evaluated for their antidiabetic and antioxidant potentials in alloxan-induced rat model and by DPPH free radical scavenging assay, respectively. Graded doses of the decoctions administered to normal and experimental diabetic rats for two weeks showed significant reduction in fasting blood glucose levels in the normal as well as in treated diabetic animals. The experiment showed 188.42 ± 1.2 mg/dl glucose and 117.78 ± 11.2 mg/dl cholesterol levels for *C. grandis*, while 187.23 ± 0.8 mg/dl glucose and 116.14 ± 1.5 mg/dl cholesterol for *C. asiatica*. Although, the decoctions produced significant effect on serum urea level, the total protein and liver enzymes (SGOT and SGPT) were found to be normal. Acute toxicity studies showed that both the decoctions were safe at 2-fold high dose when compared with the commonly used antidiabetic dose. Total phenolic contents in decoctions were found to be 12.45±0.52 and 8.32 ± 0.31 for *C. grandis* and *C. asiatica*, respectively while DPPH free radical scavenging assay showed the IC_{50} values of 0.14 ± 0.001 for *C. grandis* and 0.17 ± 0.001 for *C. asiatica*. Our current studies for the first time, justified the use of decoctions of the leaves of *C. grandis* and *C. asiatica* for treating diabeties, hyperlipaedemia and atherogenic lesion in the indigenous system of medicine.

Key words: Antidiabetic activity, serum cholesterol, antioxidant activities, *C. grandis*, *C. asiatica*.

Introduction

Dibetes mellitus (DM) is a metabolic disorder that affects carbohydrate, fat and protein metabolism. Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. In 2013, according to International Diabetes Federation, an estimated 381 million people had diabetes (Wild *et al*., 2004). The oral antihyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects (Pickup *et al*, 1991). This leads to increasing demand for herbal products with antidiabetic activity and less side effects (Vetrichelvan *et al.*, 2002). On the other hand, the search for naturally occurring antioxidants has been a demand of time in view to avoid the carcinogenic effect of synthetic antioxidants. Antioxidants inhibit the oxidation of fats and other bioactive lipids of our body and prevent diseases such as cancer, coronary heart disease, etc (Diaz *et al*., 1997).

C. grandis L. (Family: Cucurbitaceae, Bengli name: Telakucha, Synonyms: *Hydrocotyle asiatica* L., *Trisanthus cochinchinensis* Lour.) is an important herb having a lot of disease alleviating values like antibacterial and antifungal (Bhattacharya *et al.*, 2010), antioxidant (Deshpande *et al.*, 2011), hypoglycemic (Manish *et al.*, 2010) activities when different solvent extracts were used (Pekamwar *et al.*, 2013). It has been used in ayurvedic medicine to treat diabetes from ancient times (Munasinghe *et al*., 2011). It also proved to have antipyretic (Aggarwal *et al.*, 2011) and anti inflammatory (Deshpande *et al.*, 2011) effects on different animal models. On the other hand, *C. asiatica* L. (Family: Umbelliferare, Bengali name: Thankuni, Synonyms: *C. cordifolia, C. indica*) is another important medicinal herb used for various diseases and also used in Indian Ayurvedic medicine as a nerve tonic (Singh *et al.*, 2008). It has been known for many years in treating all kind of diseases such as gastrointestinal disease, gastric ulcer, asthma, wound,

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anxiety and eczema (Chen *et al*., 2006; Wijeweera *et al*., 2006). The use of Centella in food and beverages has increased over the years basically due to its health benefits such as antioxidant (Hamid *et al*., 2002; Ullah *et al*., 2009), as anti-inflammatory (Duke, 2001), wound healing (Kimura *et al*., 2008), memory enhancing (Subathra *et al*., 2005) properties and many others. The potential of *C. asiatica* L. as an alternative natural antioxidant of plant origin and its protection against age-related changes in brain antioxidant defense system, have notably increased in recent years (Subathra *et al*., 2005). Free radicals have been claimed to play an important role in ageing process and capable of damaging many cellular components. Various compounds like triterpenoids (James and Dubery, 2009), asiaticoside, asiatic acid which were found to have anticancer effect (Hsu *et al*., 2005).

Though different organic or aqueous soluble extracts of both herbs have been previously reported to have antidiabetic effect (Mustaffa *et al.*, 2011; Attanayake *et al.,* 2013) but no study have yet been done by using hot water extract of the leaves of both the plants. Since traditional healers usually prescribe the herbs to the patients to be taken with water, we for the first time, studied the antidiabetic activity of the decoctions of *C. grandis* (L.) and *C. asiatica* (L.) leaves on experimental diabetic rats. We have also evaluated the antioxidant activity and acute toxicities of the decoctions.

Materials and Methods

Plant material and decoction preparation: The leaves of *Coccinia grandis* L. and *Centella asiatica* L. were collected in December, 2012 from Jessore and Savar, Dhaka, respectively and authenticated by Bangladesh National Herbarium. The leaves were dried under shade and 100 g each of *C. grandis* and *C. asiatica* powdered leaves were boiled in one liter water for 10 minutes. This was allowed to stand for 30 min and filtered through clean cloth and then Whatman no. 1 filter paper. The filtrates so obtained were further reduced under vacuum to 100 ml for both the plants. Such procedure resulted in a decoction with a higher concentration than that produced by the method described by Teixeira *et al.* (1990). Both the concentrated decoctions contained 1 g equivalent powder per ml.

Test animals and treatment plan: Swiss Albino rats of Wister strain of either sex weighing 180-200 g used as test animals in the study and treatment plan were obtained from ICDDR, B, Dhaka. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions and were given water and rat pellet feed. Rats were divided into five groups (Each group contained 5 rats): Group I received normal diet and served as normal control. Group II consists of alloxan induced rats received normal diet and serving as diabetic control. Group III consists of alloxan-induced rats received Glibenclamide (synthetic antidiabetic drug) at 0.5 mg/kg body weight once a day orally for 14 days (Das *et al*., 2011). While, group IV and group V consist of alloxan-induced rats received concentrated decoction of *C. grandis* and *C. asiatica* leaves at a dose of 3 ml/kg body weight) once a day orally for 14 days each, respectively.

Alloxan monohydrate solution of 10 mg/ml was prepared in citrate buffer (0.1 M pH 4.5) kept in ice and was administered to the rats within 5 min at a dose of 50 mg/kg body weight intraperitonially. After 48 h of alloxan administration, rats with moderate diabetes having glycosuria and hyperglycemia were taken for the experiment.

Collection of blood sample and analysis: Blood samples were collected through the tail vein just prior to and on days 14 after drug administration. The blood glucose level was estimated by using glucometer (Bioland-423, Germany) (Khan *et al*., 2008). Urea, cholesterol, serum glutamate oxygenase transaminase (SGOT)/ Aspartate transaminase (AST) and serum glutamate pyruvate transaminase (SGPT)/ Alanine transaminase (ALT) were determined by UV spectrophotometric method (Shimadzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits according to manufacturer's protocol for all the samples (Khan *et al*., 2010).

Total phenolics content and free radical scavenging assay: Total phenolic contents in the decoctions of the leaves of *C. grandis* and *C. asiatica* were measured by using Folin-Ciocalteu reagent as described before (Amin *et al*., 2004) using 0.25 ml decoction for each reaction. Gallic acid (0.01-0.05 mg/ml) was used as standard.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity was assayed using the method described previously (Chan *et al*., 2006) using 0 ml, 0.25 ml and 0.5 ml decoctions in each test tubes for reactions.

The scavenging activities of the decoctions were calculated using the following equation:

Scavenging activity (%) = (1 - Absorbance of sample/ Absorbance of control) x 100

DPPH free radical scavenging activities were expressed as IC_{50} values.

Acute toxicity study: Acute toxicity studies of decoctions of both *C. grandis* and *C. asiatica* were also assessed to evaluate the safety by following the Organization for Economic Cooperation and Development (OCED) guidelines 425, fixed dose procedure. For screening the toxicity, healthy adult rats of either sex, starved overnight, were divided into five groups (each group containing five rats); control group was fed with hot water whereas the other groups were treated with concentrated decoction of *C. grandis* and *C. asiatica* at a dose of 3 ml and 6 ml (1 gm equivalent dry leaves powder/ml) per kg of body weight, orally. The treated rats were observed continuously for 2 h for behavioral, neurological and antunomic profiles. The lethality of the rats was observed after a period of 24 h and 72 h (Ghosh, 1984; Turner, 1965).

Statistical analysis: Experiments were carried out in triplicates and data were expressed as Mean ± SEM (standard error of mean). Unpaired *t-test* was carried out for statistical comparison. Statistical significance was indicated by $p < 0.05$ in all cases.

Results and Discussion

The hot water extracts or decoctions of the dried leaves of *C. grandis* and *C. asiatica* produced significant changes in the alloxan induced diabetic rats (Table 1). The decoction of both the plants reduced glucose level considerably in comparison to treatment of the diabetic rats and the results were comparable with that of Glibenclamide (10 mg/kg). The prolonged treatment also reduced urea, protein and total cholesterol (Tables 1 and 2) level in comparison to diabetic rats.

Table 1. Glaucose and cholesterol content of serum in control and experimental rats.

Group	Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)
Group-1	Control	118.6±1.4	124.7±1.5
Group-II	Diabetic control	248.1±1.6	238.1±1.2
Group-III	Diabetic + Glibenclamide	114.11±1.7*	118.08±1.8
Group-IV	Diabetic + *C. grandis* extract	188.42±1.2**	127.78±11.2
Group-V	Non diabetic + *C. grandis* extract	116.2±1.5	119.8±1.7
Group-VI	Diabetic + *C. asiatica* extract	187.23±0.8**	126.14±1.5
Group-VII	Non diabetic + *C. asiatica* extract	115.7±1.3	120.2±1.4

Values are expressed as Mean ± SEM (n= 5). **:p<0.05 significant compared to diabetic rats

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The blood glucose data obtained clearly indicate significant antihyperglycemic effect in alloxan induced diabetic rats. That means, both the herbs may potentiate pancreatic secretion or may reuptake glucose. Hypercholesterolemia, hypertriglyceridemia and hyperuricemia have been reported to occur in alloxan-induced diabetic rats (Resmi *et al*., 2001).

Increase in glycogen in liver can be brought about by an increase in glycogenesis and/or decrease in glycogenolysis. Therefore, decoctions of both the plants could have stimulated the process. Slight increase in total protein (Table 2) may be due to changes in circulating amino acids levels, hepatic amino acids uptake and muscle output of amino acid concentrations (Felig *et al*., 1977). The non-protein nitrogenous compound, urea and the liver enzymes level such as SGPT and SGOT were found to be increased in alloxan-induced diabetic rats. The serum level of urea and liver enzymes came to normal upon treatment with the decoctions of *C. grandis* and *C. asiatica*, remarkably. Our results support that the previous report that transaminase activity is increased in the serum of a diabetic rat which is active in absence of insulin due to the availability of amino acids in the blood (Ghosh *et al*., 2004). While on the other hand, we also found that the total cholesterol level as increased in diabetic rats was also significantly reduced upon treatment with the decoctions of both plants. These might be due to increased uptake of glucose by peripheral tissues or by depressed activities of lipogenic and cholesterogenic enzymes (Chi, 1982; Yeh, 2001).

Acute toxicological studies using 2-fold higher doses of the decoctions of both plants than that of the doses used in antidiabetic studies did not cause any beharioral, neurolocgical or antonomic changes (Table 3). There was no mortality or morbidity observed after three days of treatment by the decoctions. Morphological characteristic

Table 2. Concentration of urea, total protein, SGOT and SGPT in serum of control and experimental rats.

Group	Treatment	Urea (mg/dl)	SGOT (U/L)	SGPT (U/L)	Total protein
Group-I	Control	47.1±0.31	24.1±1.3	26.1±1.4	7.9±0.31
Group-II	Diabetic control	61.7±2.1	27.4±1.2	27.9±1.8	6.1±0.21
Group-III	Diabetic + Glibenclamide	53.6±1.9	30.9±2.1	34.2±11.5	3.8±0.8
Group-IV	Diabetic + *C. grandis* decoction	52.1±1.4	52.1±1.4	31.1±1.6	8.4±0.32
Group-V	Non diabetic + *C. grandis* decoction	48.1±1.5	25.7±1.4	27.8±1.1	8.5±1.4
Group-VI	Diabetic + *C. asiatica* decoction	51.9±1.6	28.2±2.8	30.2±1.3	8.1±0.41
Group-VII	Non diabetic + *C. asiatica* decoction	47.7±1.3	26.4±1.1	28.1±1.5	8.4±1.0

Values are taken as a mean of five individuals and expressed as Mean ± SEM.

Table 3. Toxicity study data of *C. grandis and C. asiatica* leaves on rat model.

Parameters observed	Treatment			
	C. grandis		*C. asiatica*	
	3 ml/kg	6 ml/kg	3 ml/kg	6 ml/kg
Stimulation				
Respiration	Normal	Normal	Normal	Normal
Agitation	Normal	Normal	Normal	Normal
Aggressiveness	Nil	Nil	Nil	Nil
Fur erection	Normal	Normal	Normal	Normal
Exophthalmia	Nil	Nil	Nil	Nil
Movement	Normal	Normal	Normal	Normal
Jaw movement	Normal	Normal	Normal	Normal
Convulsion	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil
Tail erection	Normal	Normal	Normal	Normal
Irritability	Nil	Nil	Nil	Nil
Depressor				
Static position	Normal	Normal	Normal	Normal
Dyspnea	Nil	Nil	Nil	Nil
Sleepiness	Nil	Nil	Nil	Nil
Prostration	Normal	Normal	Normal	Normal
Altered stride	Nil	Nil	Nil	Nil
Eye dullness	Nil	Nil	Nil	Nil
Others				
Fecal production	Normal	Normal	Normal	Normal
Diuresis	Normal	Normal	Normal	Increased
Spasms	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil
Regurgitation	Nil	Nil	Nil	Nil
Pallor	Nil	Nil	Nil	Nil
Abdominal distension	Nil	Nil	Nil	Nil
Spasticity	Nil	Nil	Nil	Nil
Cyanosis	Nil	Nil	Nil	Nil
Hemorrhagic spots	Nil	Nil	Nil	Nil

Nil: No sign was observed.

(fur, skin, eyes, and nose) appeared to be normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behavior were observed (Table 3). The acute toxicity study indicated that treatment with the decoctions at the selected doses was well tolerated by the test animals, suggesting its safety for further investigations.

Further attempt to the measurement of the total phenolic contents showed that the decoction of *C. grandis* and *C. asiatica* contained 12.45 ± 0.52 and 8.32 ± 0.31 mg of GAE/gm of dry powder of phenolic compounds, respectively, while *in vitro* DPPH free radical scavenging assay revealed the antioxidant activities with EC_{50} values of 0.14 ± 0.001 and 0.17 ± 0.001 for the decoction *C. grandis* and *C. asiatica*, respectively (Table 4). These data further provided primary evidence of the usefulness of decoction of leaves of both plants in the prevention of atherogenic lesion in addition to their uses as antidiabetic agents.

Table 4. Total phenolic content and IC_{50} values (in DPPH assay) of the decoctions of *C. grandis* and *C. asiatica.*

Samples	Total phenolic content (mg of GAE/gm of dry powder)	Free radical scavenging activity IC50 (mg/ml)
C. grandis	12.45 ± 0.52	0.14 ± 0.001
C. asiatica	8.32 ± 0.31	0.17 ± 0.001
Ascorbic acid (Standard)	-	0.004 ± 0.001
Trolox (Standard)	-	0.003 ± 0.001

Conclusion

In conclusion, the decoction of *C. grandis* and *C. asiatica* leaves showed significant anti-diabetic activity. For the first time we proved that the decoctions of the leaves of both the plants may also be used for the treatment of high cholesterol level. During the experiment, no acute toxicities were observed, which revealed their safety in animal model. Moreover, our preliminary measurement of phenolic contents also showed that both the decoctions of *C. grandis* and *C. asiatica* contained moderate amount of phenolics that could inhibit free radical related cellular damage. Thus, the decoctions might also be useful to prevent atherogenic disorders.

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