

Antidiabetic potential of the leaf extract of *Centella asiatica* in alloxan-induced diabetic rats

Talha Bin Emran, Mycal Dutta, Mir Muhammad Nasir Uddin¹, Aninda Kumar Nath and Md. Zia Uddin*

Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh

Abstract

The study was designed to evaluate the glucose and cholesterol lowering effect of the aqueous extract of *Centella asiatica* leaf using the alloxan-induced diabetic rats and compared the activity with diabetic control and antidiabetic drug (Glibenclamide). Leaf extract (50 mg/kg) of *C. asiatica* and Glibenclamide were administered to normal and experimental diabetic rats for the duration of 10 days. In the alloxan-induced diabetic rat model, *C. asiatica* extract (50 mg/kg) significantly ($p < 0.05$) lowered the fasting blood glucose level as well as the total cholesterol level. Serum insulin levels were not stimulated in the animals treated with the extract. In addition, changes in body weight, serum lipid profiles and liver glycogen levels assessed in the extract treated diabetic rats were compared with diabetic control and normal animals. Significant results ($p < 0.05$) were observed in the estimated parameters. Surprisingly, body weight was increased significantly ($p < 0.05$) in the *C. asiatica* treated diabetic group. Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, steroids and tannins in significant amounts.

Key words: Antidiabetic activity, anti-hyperglycemic activity, alloxan monohydrate, Glibenclamide, *Centella asiatica*.

INTRODUCTION

Diabetes mellitus (DM) is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin resulting in elevation of fasting and postprandial blood glucose levels. The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy and atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease (Davidson, 1981). According to recent estimation, the global population is approaching the midst of a diabetes pandemic. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (American Diabetes Association, 2005). Despite the great strides that have been made in the understanding and management of this disease, the graph of diabetes-related mortality is raising unabated (Dey *et al.*, 2002).

Generally, it is known that there are two basic types of diabetes; type 1 or insulin dependent diabetes mellitus (IDDM) characterized by a deficiency of insulin and type 2 diabetes or non insulin dependent diabetes mellitus (NIDDM) which is due to insulin resistance or reduced insulin sensitivity. The defective responsiveness of body tissues to

¹Department of Pharmacy, University of Chittagong, Chittagong, Bangladesh

* Corresponding author. Email: zia_ctg@live.com

insulin is believed to involve the insulin receptors of the cell membranes. Globally, type 2 diabetes mellitus is by far the commonest form of the disease and developing countries are the worst hit as far as this epidemic is concerned (Sharma *et al.*, 2007). Currently available therapies for diabetes include insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. (Kameswararao *et al.*, 2003). In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents (Pepato *et al.*, 2005). These drugs are used as monotherapy or in combination to achieve better glyceemic control. The oral anti-hyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects (Pickup & Williams, 1991). This leads to increasing demand for herbal products with antidiabetic activity and less side effects (Vetrichelvan *et al.*, 2002). *C. asiatica* is a mild adaptogen, is mildly antibacterial, anti-viral, anti-inflammatory, anti-ulcerogenic, anxiolytic, a cerebral tonic, a circulatory stimulant, a diuretic, nervine and vulnerary (Winston & Maimes, 2007; Jacques *et al.*, 2000). *C. asiatica* extract decreased the phagocyte activities of macrophages in alloxan-induced diabetes which indicate that it was effective reducing lipid per-oxidation in experimental diabetes. Therefore, the present study was carried out to evaluate the antidiabetic activity of *C. asiatica* in alloxan-induced diabetes rats and to probe into the mechanism of its antidiabetic property.

MATERIAL AND METHODS

Chemicals and reagents: The active drug, Glibenclamide was the generous gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. All other chemicals were of analytical grade.

Collection and identification of plant: The leaves of *C. asiatica* were collected from Chittagong University hilly areas during December 2013. A voucher specimen (Accession number: DACB36108) containing the identification characteristics of the plant has been preserved in Bangladesh National Herbarium for future reference.

Preparation of plant extract and decoction preparation: Fresh leaves of *C. asiatica* were washed immediately after collection and chopped into small pieces, air dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80 mesh, 355 g). The resulting powder was soaked in 2 liter of water for 5 min allowing the decoction to stand for 30 min and filtering through Whatman no.1 filter paper. Filtrate was concentrated under reduced pressure at the temperature below 50°C using rotatory evaporator (RE 200, Bibby Sterling Ltd., UK). The extracts (yield 4.4-5.6% w/w) were placed in glass petri-dishes (90 X 15 mm, Pyrex, Germany) to allow an air dry for complete evaporation of solvent.

Experimental animal: Albino Wistar rats (*Rattus norvigicus*) of either sex weighing 120-150 g were used for the present study. The rats were maintained under controlled conditions of temperature (23 ± 2)°C, humidity (50 ± 5%) and 12 h light-dark cycles. All the rats were acclimatized for seven days before the study. The rats were randomized into

experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. Rats were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress.

Experimental design: A total of 30 male Albino Wistar rats were utilized and randomly divided into 5 groups of 6 (n=6) rats in each group were as follows:

Group I: Normal control (treated with dimethylsulfoxide, 3 ml/kg).

Group II: Diabetic control (administered with alloxan).

Group III: Diabetic control + Glibenclamide (0.5 mg/kg body weight once a day orally for 10 days).

Group IV: Diabetic control + *C. asiatica* aqueous extract (2 ml once a day orally for 10 days).

Group V: Normal rats receiving *C. asiatica* aqueous extract (2 ml once a day orally for 10 days).

The extract was administered to the respective groups through oral route using intragastric tube for 45 days.

Phytochemical group tests of extract: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures described by Ghani (2003), Sofowara (1993), Trease & Evans (1989) and Harborne (1973). In each test 10% (w/v) solution of the extract was taken unless otherwise mentioned in the individual test.

Hypoglycemic activity test: The hypoglycemic effect of the extract was studied in alloxan-induced diabetic rats. The animals were fasted for 8 h but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) levels of the rats were determined using One touch® glucometer kit (Clever Check, Germany). Subsequently, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (70 mg/kg) (Aruna *et al.*, 1999) and normal feeding maintained thereafter. Five days later, blood was drawn from each rat by tail snipping and the blood glucose level measured to establish diabetes. Leaf extract (50 mg/kg) of *C. asiatica* (route: orally) and Glibenclamide (route: intraperitoneal injection) were administered and to normal and experimental diabetic rats for the duration of 10 days. Animals with blood glucose level ≥ 225 mg/dl were considered diabetic and used for the study.

Cholesterol estimation: 0.1 ml of the serum was added to 9.5 ml of ferric chloride acetic acid reagent and centrifuged. It was then stoppered and allowed to stand for 10 min to precipitate proteins. Then centrifuged and 5 ml of supernatant was used as test solution. Another set of tubes containing 0.5, 1.0, 1.5, 2.0 and 2.5 ml using ferric chloride acetic acid reagent were taken as a reference standard. In all tubes, 3 ml of concentrated H₂SO₄ was added and mixed by complete inversion. The tubes were allowed to stand for 30 minutes and the colour developed was read at 550 nm.

Serum glutamate oxygenate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) estimation: Three test tubes were taken and marked as test 1, test 2 and blank. In all the tubes, 1ml of substrate was added and kept for a few minutes at 37°C to attain the room temperature. In the tube marked as test 1 and 2; 0.2 ml of serum was added and incubated at 37°C for 30 minutes. After incubation, 0.2 ml serum was added to blank, 0.1 ml of phenyl hydrazine was added to test 1 and 4-dinitrophenyl hydrazine was added to test 2. All the tubes were allowed to stand for 20 minutes followed by the addition of 10 ml of 0.4N NaOH. The colour developed was read at 540 nm. The activity of SGOT and SGPT were expressed as IU/L.

Estimation of hepatic glycogen level and body weight: Hepatic glycogen level was measured according to the standard protocol (Babu *et al.*, 2003). Hepatic tissues were homogenized in hot ethanol (80%) at a tissue concentration of 100 mg/ml and centrifuged at 9500 rpm for 20 minutes. The residue was collected, dried over a water bath, and extracted at 0°C for 20 minutes by adding a mixture of 5 ml water and 6 ml of 52% perchloric acid. The collected material was centrifuged at 9500 rpm for 15 minutes for recovery of the supernatant. 0.2 ml supernatant was transferred in graduated test tube and made to 1 ml volume by distilled water. Anthrone reagent (4 ml) was added to all the test tubes and heated in a boiling water bath for 8 minutes, allowed to cool at room temperature, and the intensity of the green to dark green color of the solution was recorded at 630 nm. Glycogen content of the sample was determined from a standard curve prepared with standard glucose solution. Body weight was estimated on 0, 5 and 10 day.

Statistical analysis: All data are presented as mean \pm SEM. Data were analyzed by a statistical software statistical package for social science (SPSS, version 18.0, IBM Corporation, NY, USA) using one-way ANOVA followed by Dunnet's multiple comparisons and Tukey's multiple range *post hoc* tests.

RESULTS AND DISCUSSION

Phytochemical screening: The crude extract was subjected for chemical group tests and important chemical constituents.

i.e.alkaloids, flavonoids, glycosides, steroids and tannins were detected in the extract of *C. asiatica* leaf but saponins are absent. Further studies are suggested to isolate its active principles by using bioactivity guided approach.

Development of diabetes: Alloxan was used for the development of diabetes in the experimental rats. Due to destruction to β -cell, the glucose level increased rapidly and reached to diabetic level within 24 hours (Fig. 1).

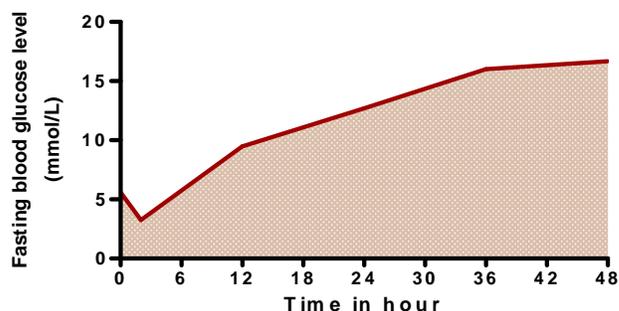


Fig. 1. Development of diabetes in Albino Wistar rats, *Rattus norvegicus* after hours of alloxan administration

Effect of the aqueous extract of *C. asiatica* leaf on FBG level in diabetic rats: The mean blood glucose concentration of control and *C. asiatica*-treated rats was estimated at the 2, 4, 8, 16 and 24 hours, respectively as shown in Figure 2.

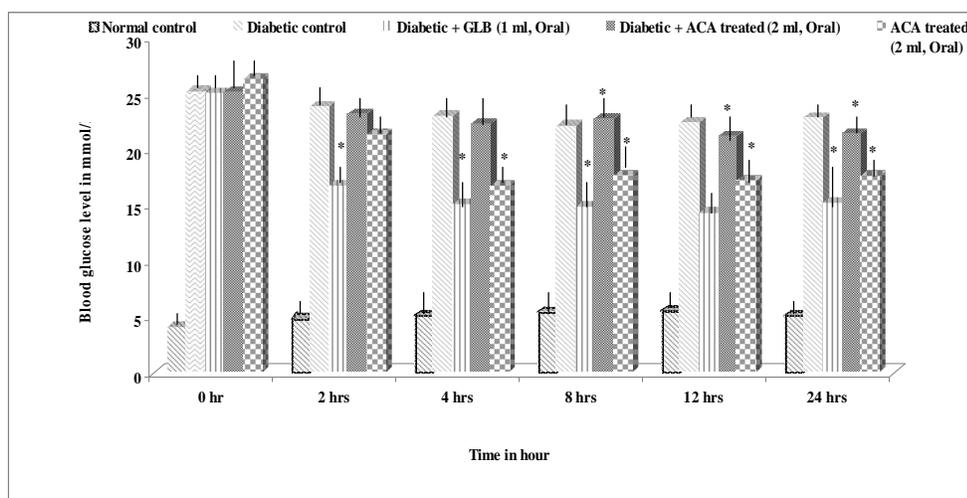


Fig. 2. Effect of *Centella asiatica* leaf extract on Fasting Blood Glucose (FBG) level of alloxan-induced diabetic rats

Here, GLB= Glibenclamide; ACA= Aqueous extract of *C. asiatica*;

Data were represented as the mean \pm SEM (n = number of animals in each group = 6). The criterion for statistical significance was $*p < 0.05$. In case of alloxan-induced diabetic rats, Glibenclamide reduced blood glucose level to 57.68%, 46.32%, 23.44%, 18.37% and 27.75% at 2, 4, 8, 16 and 24 hours, respectively. So, Glibenclamide caused maximum reduction of blood glucose level of 81.63% on 16 hours of the experiment.

Effect of the aqueous extract of *Centella asiatica* leaf on serum glucose and cholesterol: The extracts of *C. asiatica* produced significant changes in serum glucose and cholesterol level in the alloxan-induced diabetic rats (Table 1). The prolonged treatment of *C. asiatica* extracts produced consistent reduction in the blood glucose levels. The aqueous extract also reduced cholesterol and urea protein during the 10 days treatment period (Table 1 & 2).

Table 1. Glucose and cholesterol content of serum of control and experimental rat groups

Parameter	Control (Group I)	Diabetic Control (Group II)	Diabetic + GLB (1 ml) (Group III)	Diabetic + ACA treated (2 ml) (Group IV)	ACA treated (2 ml) (Group V)
Glucose (mg/dl)	119.8 ± 1.2	247.7 ± 1.3***	135.07 ± 1.1**	187.23 ± 0.8*	115.7 ± 1.3*
Cholesterol (mg/dl)	123.4 ± 1.7	237.2 ± 1.4**	117.24 ± 2.4*	116.14 ± 1.5**	120.2 ± 1.4*

NB: Here, GLB= Glibenclamide; ACA= Aqueous extract of *C. asiatica*; Data were represented as the mean ± SEM (n = number of animals in each group = 6). The criterion for statistical significance was ***p < 0.001, **p < 0.01 and *p < 0.05 on day 10.

The blood glucose data clearly indicate that the *C. asiatica* produced significant and consistent anti-hyperglycemic effect. The continuous treatment with *C. asiatica* for a period of 10 days produced a significant decrease in the blood glucose levels of the diabetic rats, but not in the normal rats. It is assumed that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Hypercholesterolemia, hypertriglyceridemia and hyper urea 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, the *C. asiatica* extracts could have stimulated glycogenesis and/or inhibited glycogenolysis in diabetic rat liver. Only plant extracts treated rats showed non-toxicity of the extract. Thus it indicates that unlike insulin and other common hypoglycemic agents, overdose of the drug may not result in hypoglycemia.

Effect of the aqueous extract of *Centella asiatica* leaf on liver enzymes (SGOT, SGPT) in alloxan-induced diabetic rats: In diabetic rats SGOT and SGPT levels were raised to 80.00% and 25.85% respectively in comparison to normal rats. Following intraperitoneally administration of *C. asiatica* extract, SGOT and SGPT levels were significantly reduced as shown in the Table 2.

Table 2. The concentrations of urea, total protein, SGOT and SGPT in the serum of control and experimental rat groups

Parameters	Control (Group I)	Diabetic Control (Group II)	Diabetic + GLB (1 ml) (Group III)	Diabetic + ACA treated (2 ml) (Group IV)	ACA Extract treated (2 ml) (Group V)
Urea (mg/dl)	46.2 ± 0.25	63.4 ± 2.7	54.4 ± 2.1	51.9 ± 1.6	47.7 ± 1.3
SGOT (IU/L)	23.8 ± 1.2	27.8 ± 1.7	31.2 ± 2.4	28.2 ± 2.8	26.4 ± 1.1
SGTP (IU/L)	25.9 ± 1.7	28.4 ± 2.3	33.4 ± 1.7	30.2 ± 1.3	28.1 ± 1.5
Total Protein	8.2 ± 0.38	5.3 ± 0.17	3.1 ± 1.8	8.1 ± 0.41	8.4 ± 1.0

NB: Here, GLB= Glibenclamide; ACA= Aqueous extract of *C. asiatica*; Data were represented as the mean ± SEM (n = number of animals in each group = 6). The criterion for statistical significance was ***p < 0.001, **p < 0.01 and *p < 0.05 on day 10.

Increase in total protein (Table 2) may be due to changes in circulating amino acid levels, hepatic amino acid uptake, and muscle output of amino acid concentrations (Felig *et al.*, 1977). The non-protein nitrogen compound urea is found to be increased when compared to plant extract treated rats. The level of SGPT and SGOT increased remarkably in the *C. asiatica* extract treated rats (Nagappa *et al.*, 2003). Present results support that of Ghosh *et al.*, 2004, who reported that transaminase activity is increased in diabetic rats. The increased levels of transaminases, which are active in absence of insulin, because of the availability of amino acids in blood, are responsible for the increased gluconeogenesis and ketogenesis. In diabetic animals, the changes in level of serum enzymes are directly related to changes in the metabolism in which these enzymes are involved (Scott *et al.*, 1984).

Table 3. Effect of the aqueous extract of *C. asiatica* leaf on the body weight and hepatic glycogen content in alloxan-induced diabetic rats

Groups	Body weight			Glycogen content (mg/g)
	0 day	5 days	10 days	28 days
Control (Group I)	180 ± 12.65 (100%)	172 ± 10.84 (96%)	183 ± 16.33 (102%)	7.54 ± 3.73
Diabetic Control (Group II)	190 ± 21.6 (100%)	187 ± 19.18 (99%)	201 ± 17.72 (106%)	6.54 ± 2.52
Diabetic + GLB (1 ml) (Group III)	192 ± 21.21 (100%)	191 ± 20.7 (100%)	203 ± 18.42 (106%)	20.39 ± 12.5
Diabetic + ACA treated (2 ml) (Group IV)	200 ± 23.90 (100%)	190 ± 19.27 (95%)	200 ± 17.73 (100%)	15.73 ± 4.75
ACA treated (2 ml) (Group V)	186 ± 10.33 (100%)	200 ± 23.88 (107%)	205 ± 17.18 (110%)	12.13 ± 8.42

NB: Here, GLB= Glibenclamide; ACA= Aqueous extract of *C. asiatica*; Data were represented as the mean ± SEM (n = number of animals in each group = 6).

Effect of the aqueous extract of *Centella asiatica* leaf on the body weight and hepatic glycogen content in alloxan-induced diabetic rats: 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 rats that are treated with *C. asiatica* leaf extract (group IV). Average body weights of other groups remained unchanged (Table 3).

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

In conclusion, *C. asiatica* leaf extract showed significant glucose and total cholesterol lowering activity at 50 mg/kg dose investigated on the experimental rats after oral administration. Hence the claim made by the Indian systems of medicine regarding the use of leaf extract of this plant in the treatment of diabetes may be justified. Present efforts are directed to isolate the active constituents from the water extract of *C. asiatica* leaf and elucidation of mechanism of action.

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