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ANTIDIABETIC ACTIVITY OF *CENTELLA ASIATICA* (L.) URBANA IN ALLOXAN INDUCED TYPE 1 DIABETIC MODEL RATS

Sonia Rahman, M A H Mostofa Jamal, Anzana Parvin, Md Mahfuz-Al-Mamun, M Rezuanul Islam*
Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh

Abstract

Context: Diabetes mellitus is becoming a major burden upon healthcare facilities in all affected countries. Current therapies used for diabetics have side effects, so the current shift to the use of herbal preparations may be more effective, relatively low cost, less side effect and low toxicity.

Objective: The present research was made to investigate the potential antidiabetic activity of *Centella asiatica* (L.) Urb. in alloxen induced diabetics.

Materials and Methods: Rats were divided into 6 groups and *C. asiatica* was administered containing 50, 100 and 200 mg/kgbwt powder, respectively in 1ml water orally in group A, B and C rats. Metformin (150 mg/kgbwt) used as a reference standard drug. Blood glucose (BG), triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated from the serum by using standard kits.

Results: *C. asiatica* juice had shown significant lowered the blood glucose levels in all groups. In addition, body weight, organ (liver, kidney, heart and pancreas) weight, food intake, water intake were also examined in all treated groups and compared against diabetic control group. After 22 days daily administration of *C. asiatica,* diabetic treated rats showed improvement in body weight, water intake as compared to diabetic control rats. In alloxan induced diabetic rats the maximum reduction in BG, TG, TC, HDL, LDL, SGOT and SGPT were observed at a dose level of 50 mg/kgbwt.

Conclusion: The present data indicates that *C. asiatica* juice possesses potential as an antidiabetic action

Keywords: *Diabetes mellitus*, Antidiabetic, *Centella asiatica*, Blood glucose, Triglycerides, Cholesterol.

Introduction

Diabetes mellitus is a continuously growing health problem, which causes substantial morbidity, mortality and long-term complications even in developed countries. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 (Haffner *et al.* 1998). In the past decade, the United States has recorded a 33% rise in the cases diabetes. About 150 million or 1.3% people are suffering from diabetes world wide which is almost five times more than the estimates ten years ago and this may double by the year 2030 (Ghosh *et al.* 2004)

Plants have been the major source of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world. The importance of antidiabetic plants in the development of economic and effective treatment for diabetes, currently estimated to affect over 30 million people worldwide (WHO 1985). Plant drugs (Bailey and Day 1989) and herbal formulation (Mitra *et al.* 1996, Annapurna *c.* 2001, Battacharya *et al.* 1997) are frequently considered to be less toxic and more free from side effects than synthetic one. Based on the WHO recommendations (WHO 1980) hypoglycemic agents of plant origin used in traditional medicine are important.

Centella asiatica (L.) Urbana or locally known as thankuni, is a weakly aromatic smelling herb of the family Umbelliferare. It has been used widely in folk medicine for hundreds of years to treat a wide range of illness (Brinkhaus *et al.* 2000). It is used in traditional and alternative medicine due to the wide spectrum of

*Corresponding author *Email:* rezwangbt@yahoo.com

pharmacological activities associated with these secondary metabolites (James and Dubery 2009). It accumulates large quantities of pentacyclic triterpenoid saponins, collectively known as centelloids.

In this present study, the effects of *C. asiatica* juice on blood glucose levels were evaluated in hyperglycemic and assessed by evaluating the comparative hypolipidemic (total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triglyceride (TG)) and hepatoprotective (serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)) activities in alloxan-induced diabetic rats and compared with those of metformin.

Materials and Methods

Plant extract and test animal: Fresh *C. asiatica* plants were collected from medicinal plant garden. The plant parts were shade dried completely in room and the dried plants were powdered with an electric grinder into fine powder and used for making juice. The authenticity of the *Centilla asiatica* was identified by the department of Botany, Rajshahi University, Rajshahi.

A total number of 23 male long evan rat (140-250 g) were used in the experiment. Rats were obtained from the International Centre for Diarrhoeal Diseases Research, Bangladesh. Animals were maintained under standard and ambient temperature under lights for twelve hours followed by 12 h of darkness environmental conditions having proper ventilation in the room, were fed with a standard pellet diet and water.

Induction of diabetes and experimental procedure: Male long evan rats were divided into 6 groups and allowed to fast overnight and injected single intraperitoneal injection with freshly prepared alloxan monohydrate (120 mg/kgbwt) in saline water to made diabetic by alloxan monohydrate and served as diabetic control, standard and treatment groups respectively. Rats exhibited in plasma glucose levels >10mmol/dl, 5day after administration of alloxan were included in the study and selected for drug treatments. Treatment for diabetes (*C. asiatica* extract 50,100 and 200 mg/kgbwt) was started from 6 th day of alloxan administration. Group A, B and C served as sample group were fed a sample (*C. asiatica*) containing 50mg/kgbwt, 100mg/kgbwt and 200mg/kgbwt powder respectively in 1 ml water orally (once daily), starting from the 6th day of alloxan administration, one diabetic control group (positive control group) which did not receive metformin or sample, one normal group (negative control group) which received only distilled water, and standard drug group (SD group) which received metformin as standerd drug in 1 ml water orally. The whole experiment was continued for 22 days.

Sample collection and estimation of biochemical parameter: Rats were sacrificed by ether anesthesia and about 3-5 ml of blood sample was collected directly from artery by syringes. Blood was collected in fresh centrifuge tube and centrifuged at 4000 rpm for 10 min and the serum was preserved to examine TG, TC, SGPT, SGOT, HDL and LDL level by semi-auto analyzer (Humalyzer 3000, Human, Germany) using wet reagent diagnostic kits according to manufacturer’s protocol.

Statistical analysis: Data from the experiments were analyzed using SPSS software for windows version 10. All results were expressed as the mean ± Standard Deviation. One-way analysis of variance (ANOVA) used and paired or unpaired t-test was done to see any difference between groups.

Results

Effect of C. asiatica on body weight (bwt) gain, food intake and water intake: In the course of investigation it was observed that, there is slightly increasing tendency of body weight in *C. asiatica* juice and Standard drug (SD) administered group at final day (22th day) as compared with initial day. Very significant weight loss, approaching 20%, occurred 48 h after administration of alloxan (120 mg/kgbwt). The final body weight of diabetic control group was found lower than that of normal group (Table 1). Among the treatment, group A gain highest body weight (117%).Where as group B and C gain 94% and 101%, respectively. However, there was no significant difference between *C. asiatica* and SD treatment group. It was observed that body weight was found to significantly related with food intake in all groups of rats. Among the three treatment groups the final (22nd day) body weight of group A increase 117% as food intake of this group was better when compared to diabetic control group. Moreover, improvement of body weight of treated animal further supports the antidiabetogenic effect of *C. asiatica* as diabetic condition is associated with loss of body weight.

Table 1. Effect of *Centella asiatica* on body weight (bwt) and growth parameter of alloxan induced type 1 diabetic rats for 22 days

Experimental Groups	bwt initial day (g)	bwt-8th day (g)	bwt-15th day (g)	bwt-22nd day (g)	Body Weight (g)	Food Intake (g/kgbwt)	Water Intake (ml/kgbwt)	Body Weight (g)
Normal	167.00 ± 13.78 (100%)	191.75 ± 38.96 (114%)	194.75 ± 38.39 (116%)	198.50 ± 43.37 (118%)	188.00 ± 14.26^{a}	59.92 ± 1.06^{a}	186.13 ± 22.67^{a}	188.00 ± 14.26^{a}
Diabetic control	166.50 ± 22.18 (100%)	158.25 ± 20.25 (95%)	149.75 ± 14.17 (90%)	105.75 ± 3.86 (64%)	145.06 ± 27.08^{b}	44.66 ± 1.06^{b}	242.00 ±17.17^{b}	145.06 ± 27.08^{b}
Diabetic+ Group A	144.50 ± 40.17 (100%)	163.25 ± 32.01 (113%)	164.50 ± 36.70 (114%)	169.00 ± 32.45 (117%)	160.31 ± 10.82^{b}	59.63 ± 1.41^{a}	188.00 ± 14.26^{a}	160.31 ± 10.82^{b}
Diabetic+ Group B	192.00 ± 5.44 (100%)	152.5 ± 26.66 (80%)	168.25 ± 27.02 (87%)	180.5 ± 38.71 (94%)	173.31 ±16.92^{NS}	59.25 ± 2.04^{a}	198.59 ± 17.44^{a}	173.31 ±16.92^{NS}
Diabetic+ Group C	154.75 ± 46.34 (100%)	156.50 ± 35.96 (102%)	166.25 ± 37.25 (108%)	166.75 ± 45.50 (108%)	161.06 ± 6.32^{bc}	59.60 ± 1.38^{a}	199.45 ± 1.79^{NS}	161.06 ± 6.32^{bc}
Standard drug (SD)	184.00 ± 43.71 (100%)	173.33 ± 51.05 (95%)	174.33 ± 47.98 (95%)	186.00 ± 48.28 (101%)	179.41 ± 6.51^{c}	59.10 ± 1.80^{a}	199.45 ± 1.79^{NS}	179.41 ± 6.51^{c}

Each value is the mean ± S.D. for 4 rats. $^{A,\,b,\,c}$ value with superscript letter means significant among the group at $p<0.05$. ^{NS} Not significant.Group A = 50 mg/kgbwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt.

Table 2. Effect of *Centella asiatica* on plasma triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein concentration in alloxan-induced type 1 diabetic rats.

Experimental Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	SGOT (mg/dl)	SGPT (mg/dl)
Normal	53.80± 5.29^{a}	59.92± 4.89^{a}	59.47± 2.97^{a}	16.27± 3.35^{a}	68.07± 12.01^{a}	24.62± 8.99^{a}
Diabetic control	91.12±14.00^{b}	91.15± 6.47^{b}	25.80± 2.94^{b}	40.50± 5.16^{b}	107.52± 3.97^{b}	62.55± 0.62^{b}
Diabetic+ Group A	57.02± 5.11^{ab}	64.65± 3.72^{ab}	58.27± 7.26^{b}	18.42± 7.42^{b}	72.07± 7.67^{b}	28.00 ±13.12^{b}
Diabetic+ Group B	68.47± 4.74^{ab}	62.32± 3.38^{b}	54.90± 4.66^{b}	18.95± 4.44^{b}	74.62± 4.98^{b}	34.60± 5.51^{ab}
Diabetic+ Group C	61.47± 7.09^{b}	64.12± 4.3^{NS}	57.37± 8.27^{b}	18.62± 3.41^{b}	82.72± 6.13^{b}	26.15± 3.68^{b}
Standard drug (SD)	57.33± 6.0^{NS}	62.90± 3.0^{NS}	58.40±7.46^{NS}	20.33±1.49^{NS}	83.23± 4.74^{NS}	32.73± 1.05^{NS}

Each value is the mean ± S.D. for 4 rats.$^{a\,,\,b}$ value with superscript letter in the same column significantly different among the group at $p<0.05$.^{NS} Not significant.Group A = 50 mg/kgbwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt. TG = Triglyceride, TC = Total cholesterol, HDL = High density lipoprotein, LDL = Low density lipoprotein.

Effect of C. asiatica on plasma glucose level, lipid profile, SGPT, SGOT and organ weight: Significant reduction in plasma glucose level in the A. B, C and SD administered groups were observed with different extent (Fig.1). As much as 39.40% reduction in plasma glucose level was observed in group A where only 22.43 % and 20.86% decrease in plasma glucose level could be noticed in group B and C, respectively. A significant reduction in TG, TC, HDL and LDL levels were noticed in all groups those administered *C. asiatica* and SD than to diabetic control (Table 2).

Table 3. Effect of *Centella asiatica* on various organ weight (g) in alloxan induced diabetic type 1 rats

Experimental Groups	Heart wt	Liver wt	Kidney wt	Pancreas wt
Normal	0.51± 3.10[a]	5.09± 0.41[a]	1.37± 0.15[a]	0.34± 2.50[a]
Diabetic control	0.26± 0.12[b]	4.00± 0.41[b]	1.09± 5.71[b]	0.26± 4.50[b]
Diabetic+ Gr A	0.55± 5.79[b]	5.15± 0.28[b]	1.21± 0.12[b]	0.32± 4.64[b]
Diabetic+ Gr B	0.44± 0.10[NS]	3.87± 0.96[NS]	1.15± 0.13[NS]	0.27± 6.48[NS]
Diabetic+ Gr C	0.52± 0.15[NS]	3.99± 1.11[NS]	1.21± 0.24[a]	0.24± 0.10[NS]
SD	0.50± 1.00[NS]	5.03± 0.79[NS]	1.22± 0.11[NS]	0.31± 5.13[NS]

Each value is the mean ± S.D. for 4 rats.[a, b,] value with superscript letter in the same column significantly different among the group at $p<0.05$.[NS] Not significant Group A = 50 mg/kgbwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt.

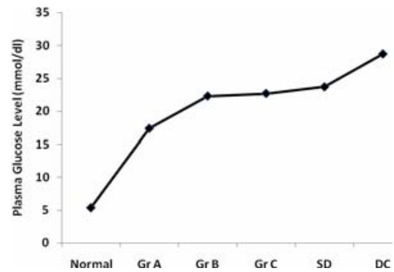

Fig. 1. Hypoglycemic effect of *Centella asiatica* in alloxan-induced type 1 rats by oral administration for 22 days. Each value is the mean ± S.D. for 4 rats.[a, b, c] value with superscript letter in the same column significantly different among the group at $p<0.05$.Group A = 50 mg/kgbwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt

It was found that, group A performed better, which decreased the TG level as much as 37.42% as compared to diabetic control group 69.36%. A substantial decrease in the plasma TC level 29.07% was found in group A, as compared to diabetic control group 52.11%. Increased level of HDL as much as 125% as compared to diabetic control group 56.61% and decreased LDL level as much as 54.51% as compared to diabetic control group 148.92% were also found in group A.

The values of SGOT and SGPT were shown to be reduce in all *C. asiatica* and SD administered groups which was increased by injection of Alloxan for inducing diabetes (Table 2). Both SGOT and SGPT level were found to significantly lower in group A 32.97% and 55.23%, respectively than the control group 57.19% and 154.06%. Our results indicate significant reduction of both SGOT and SGPT value was evidenced under treatment of *C. asiatica* which signifies their corrective role in liver function of the diabetic rats.

In this investigation the heart, liver, kidney and pancreas weight were shown to be significantly improved in all *C. asiatica* and SD administered groups as compared to diabetic control group (Table 3).

Discussion

C. asiatica could repair the damage of the pancreatic β-cells and promote insulin synthesis, thus may lowering the level of plasma glucose. The two glycosides, brahmoside and brahminoside, which are principle constituents of *C. asiatica* have been shown to exert sedative and hypoglycemic effects in experimental rats (Ramaswamy *et al.*, 1970). The triterpene in this plant had a significant effect on blood glucose, with initial glucose deviation of more than 30% and final deviation of more than 50%, similarly to tolbutamide (Mutayabarwa *et al.* 2003). Chauhan *et al.* (2010) observed that both ethanolic and methanolic extracts has reduced the glucose levels to 51% and 69% respectively. According to Ramaswamy (1970) two glycosides, brahmoside and brahminoside, which are principle constituents of *C. asiatica*, have been shown to exert sedative and hypoglycemic effects in experimental rats and this is in support of the of the present finding which showed that the extract.was effective against alloxan induced diabetic rats.

George *et al.* (2008) studied that the aqueous extract of *C. asiatica* exhibited anti-nociceptive activity in mice and anti-diabetic study indicated that the extract suppressed the elevated blood glucose, lipid levels didn't change in diabetic rats. Gayathri *et al.* (2011) induced diabetes in male Wister rats by streptozotocin (50mg/kg) injection and the dose of 200mg/kg of ethanol extract of *C. asiatica* showed significant anti-diabetic activity as judged from body weight, serum glucose, lipids. cholesterol and liver glycogen levels.

Many works have observed elevation in transaminase activity (SGOT and SGPT) in liver and serum in diabetic rat (Ghosh and Suryawanshi 2001). The restoration of SGOT and SGPT to their respective normal level after treatment with *C. asiatica* strengthens the antidiabetogenic effect in this plant. Moreover, SGOT and SGPT level also act as indicators of liver function and restoration of normal level of this parameters indicate normal function of liver (Kumar and Loganathan 2010). The present results are complementary with the findings of other workers (Somani and Singhai 2008) as *C. asiatica* juice treated rats showed improvement in organ (heart, liver, kidney and pancreas) weight as compared to diabetic control rats.

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