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Evaluation of the Anti-diabetic Effect of Ethanolic and Methanolic Extracts of *Centella asiatica* Leaves Extract on Alloxan Induced Diabetic Rats

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Abstract: The ethanolic and methanolic extracts of the leaves of *Centella asiatica* (Family-Umbelliferare) were tested for anti-diabetic activity, by glucose tolerance test in normal rats and alloxan induced diabetic rats. Ethanolic and methanolic extracts had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. In alloxan induced diabetic rats the maximum reduction in blood glucose was observed after 3h at a dose level of 250 mg/kg of body weight. The percentage protections by ethanolic and methanolic extracts were 30 and 48% respectively. In long term treatment of alloxan induced diabetic rats, the degree of protection was determined by measuring blood glucose, triglycerides, cholesterol and urea levels on 0, 3, 5, 7 and 10th day. Both the extracts showed a significant anti-diabetic activity comparable with that of glibenclamide. The histopathological studies during the long-term treatment have shown to ameliorate the biochemical damages caused by alloxan. These results indicate that the *Centella asiatica* leaves possess significant anti-diabetic activity.

Key words: *Centella asiatica* · Alloxan induced diabetes · Antidiabetic · Hypoglycemia

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. It is the most common of the serious metabolic diseases. Diabetes mellitus has been recognized as a clinical syndrome since ancient times, and remains a crippling global health problem today. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The possibility of its management by the oral administration of hypoglycemic agents has stimulated great interest in recent years. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is increasing demand by patients to use the herbal preparations with anti-diabetic activity. Current therapies seem to be insufficient to prevent diabetic complications, with a two-to four-fold likelihood for developing cardiovascular events [1]. Diabetes mellitus is a major cause of morbidity (such as blindness, kidney failure, lower-extremity amputation, and cardiovascular disease) and premature mortality [2]. The current shift to the use of herbal preparations may therefore be due to presumed effectiveness, relatively low cost, presumed less side effects and low toxicity even though the biologically active constituents are unknown most often. The use of herbs has more than tripled over the last 10 years [3]. In the indigenous Indian system of medicine (Ayurveda), a mention was made on good number of plants for the cure of diabetes or 'madhumeha' and some of them have been experimentally evaluated and the active principles were isolated [4]. However, search for new antidiabetic drugs continues. *Centella asiatica*, or locally known as pegaga, is a weekly 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 [5]. In India, it is described under the name of Mandukaparni and used in Ayurveda medicine. It has been used as a support for faster healing of small wounds. The plant extract has been incorporated into the Indian pharmacopoeia and recommended not only for wound healing but especially for the treatment of skin diseases such as eczema, leprosy and psoriasis [6]. In addition, it is also used in the treatment of burns, itching and insect bites. In contrast with other medicinal plants, this plant has been subjected to quite extensive experimental clinical investigations due to its ability to heal relieve and recover

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human being from various pain and sickness. In this present study, the effects of ethanolic and methanolic extracts of *Centella asiatica* on blood glucose levels were evaluated in hyperglycemic and alloxan-induced diabetic male wistar albino rats and compared with those of glibenclamide.

MATERIALS AND METHODS

Collection and Extraction of Plant Material: The plant material used was the dried leaves of *Centella asiatica* collected from village area of Dehradun and identified by Botanical Survey of India, Dehradun. For this purpose dried and powdered leaves of *Centella asiatica* were extracted with different solvents (ethanol, methanol) by Soxhlet apparatus. By removing the solvents in Rotary Evaporator (Butchi Type) at 70-80°C crude extract was obtained. A crude residue was obtained giving a yield of (75 g). When needed, the crude extract was suspended in distilled water and used in the study.

Test Animals: Male wistar albino rats (160-200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water.

Effect of *Centella Asiatica* Extracts on Glucose Tolerance in Rats: Fasted rats were divided into 3 groups of six rats each. Group l served as a control, received distilled water. Group II-III received ethanolic and methanolic extracts respectively at a dose of 250 mg/kg body weight as a fine aqueous suspension orally. The rats of all groups were given glucose (2 g/kg body weight, p.o) 30min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30 and 90 min after the glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose-oxidase method [7].

Effect of the *Centella Asiatica* Extracts on Alloxan-induced Diabetic Rats: Male wistar rats (180-200g) were made diabetic by a single i.p injection of 120mg/kg body weight of alloxan monohydrate in sterile normal saline. The rats were maintained on 5 % glucose solution for next 24h to prevent hypoglycemia [8]. Five days later blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes (350mg/dl). The diabetic rats were divided into four groups, each containing six animals. Controls rats (Group I) were given distilled water orally, while *Centella asiatica* ethanolic and methanolic extracts were given to groups II-III respectively, at a dose of 250 mg/kg, orally. Group IV received glibenclamide at dose of 10 mg/kg. Blood samples were collected from the tail vein just prior to and 1h, 3h and 5h after drug administration.

Histopathological Studies: Animals were sacrificed on 5th day after treatment. Pancreas, liver and kidney were removed, washed with cold saline and preserved in 10% formalin in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxilin and eosin for histomorphology evaluation.

Statistical Analysis: The results are expressed as mean S.E.M. the significant of various treatments was calculated using students t-test and were considered statistically significant when $P< 0.05$.

RESULTS

The extracts of *Centella asiatica* have shown significant ($P<0.001$) increase in glucose tolerance. The results are given in Table 1.

The blood glucose levels were reduced considerably within 60 minutes of the drug administration. The ethanolic and methanolic extracts reduced the glucose levels to normal. Maximum, effect was observed for methanolic extract. In alloxan-induced diabetic rats also, both extracts have shown considerable reduction in blood glucose levels. The results are shown in Table 2.

The reduction in glucose levels is significant ($p<0.001$) in the treated animals at 1h, 3h and 5h after drug administration. The maximum percentage reduction in blood glucose levels was found to be in methanolic extract (48.86%), while ethanolic extract showed (30%) blood glucose level. Treatment of the diabetic rats with glibenclamide (10 mg/kg) produced (29.77%) fall of blood glucose after 3h treatment. Both the extracts have shown significant ($p<0.001$) reduction of blood glucose, urea, total cholesterol and triglycerides. However the methanolic extract has shown maximum reduction (144.71 mg/dl on 5th day) and at a faster rate compared to ethanolic extract (223.51 mg/dl).

Table 1: Effects of *Centella asiatica* extracts on oral glucose tolerance in rats[a]

		Blood glucose (mg/dl)		
Group	Treatment (dose / kg body weight)	Fasting	30 min	90 min
I	Glucose (2 gm)	75.35±0.901	144.95±1.70	112.81±1.64
II	Ethanolicextract 250 mg +Glucose	76.95±0.80	101.34±1.36 *	83.77±1.10 *
III	Methanolic extract250 mg + Glucose	77.05±0.95	97.80±1.15*	81.50±1.05*

aValues are means±S.E.M, n = 6

*P<0.001 VS group I

Centella asiatica extracts were given orally 30 min before glucose loading.

Table 2: Effect of *Centella asiatica* extracts on blood glucose levels (mg /dl) of alloxan induced diabetic rats[a]

		Blood glucose at different hours after the treatment			
Group	Treatment	0h	1h	3h	5h
I	Diabetic -untreated	385.15±6.75	378.87±7.85	370.12±7.97	373.60±7.76
II	Diabetic rats treatedwith 250 mg/kg of ethanolic extract	363.16±8.45	264.67±6.96**	250.89±7.16**	261.69±7.58**
III	Diabetic rats treatedwith 250 mg/kg ofmethanolic extract	391.74±7.08	238.70±6.82**	198.96±5.64**	210.86±5.21**
IV	Diabetic rats treatedwith 10 mg/kg ofGliconclamide.	377.80±7.84	338.43±7.83*	258.41±7.34**	265.10±7.35**

[a] Values are mean±S.E.M, n = 6

*P<0.01

**P<0.001 compared with initial level of blood glucose of the rats (oh) in the respective group.

DISCUSSION

The present study was conducted to investigate the *in vivo* antidiabetic activity of *Centella asiatica* extracts. Results of anti-diabetic activity of *Centella asiatica* leaves extract established the scientific basis for the utility of this plant in the treatment of diabetes. The ethanolic and methanolic extracts have shown significant reduction in blood glucose levels in both glucose loaded and alloxan induced diabetic rats. The methanolic extract produced maximum anti-diabetic activity and is higher than the hypoglycemic activity of glibenclamide in the diabetic rats. Therefore it is obvious that the fractionation with methanol has enriched the active principles. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Both ethanolic and methanolic extracts has reduced the glucose levels to 51% and 69% respectively. Hypercholesterolemia, hypertriglyceridemia, hyperurea have been reported to occur in alloxan diabetic rats [9, 10] and a significant increased observed in our experiment was in accordance to these studies. According to Ramaswamy, AS *et al*., [11] the two glycosides, brahmoside and brahminoside, which are principle constituents of *Centella asiatica,* have been shown to exert sedative and hypoglycemic effects in experimental rats and This is in support of the present finding which showed that both the extracts of *Centella-asiatica* were effective against alloxan induced diabetic rats. Repeated administration of *Centella asiatica* extracts had decreased the blood glucose, urea, total cholesterol and triglycerides significantly. Histopathological examination of pancreas, liver and kidney showed the recovery of damaged tissues when section of treated groups compared with diabetic control. In conclusion, *Centella asiatica* butanolic extract showed significant anti-diabetic effect in diabetic rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of leaves juice of this plant in the treatment of diabetes stands confirms. Present efforts are directed to isolate the active constituents from butanolic extract of *Centella asiatica* leaves and elucidation of mechanism of action.

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