



## ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES EXTRACT OF *CENTELLA ASIATICA* 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 48% and 30% 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.

### KEYWORDS

*Centella asiatica*, alloxan, antidiabetic, glibenclamide, 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<sup>1</sup>. Diabetes mellitus is a major cause of morbidity (such as blindness, kidney failure, lower-extremity amputation, and cardiovascular disease) and premature mortality<sup>2</sup>. The current shift to the use of herbal preparations



## ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES EXTRACT OF *CENTELLA ASIATICA* ON ALLOXAN INDUCED DIABETIC RATS

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<sup>3</sup>. 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<sup>4</sup>. 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<sup>5</sup>. 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<sup>6</sup>. 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 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

#### (i). 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<sup>0</sup> 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.

#### (ii). 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.

#### (iii). Effect of *Centella asiatica* extracts on glucose tolerance in rats

Fasted rats were divided into 3 groups of six rats each. Group I 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<sup>7</sup>.

#### (iv). 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<sup>8</sup>. 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



## ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES EXTRACT OF *CENTELLA ASIATICA* ON ALLOXAN INDUCED DIABETIC RATS

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.

### (v). *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

hematoxylin and eosin for histomorphology evaluation.

### (vi). *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**.

**Table 1.**  
*Effects of Centella asiatica extracts on oral glucose tolerance in rats<sup>a</sup>*

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

<sup>a</sup>Values are means ± S.E.M, n = 6

\* $P < 0.001$  VS group I

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

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 ethanolic extract. In alloxan-induced diabetic rats also, both extracts have shown considerable reduction in blood glucose levels. The results are shown in **Table 2**.



**ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES EXTRACT OF *CENTELLA ASIATICA* ON ALLOXAN INDUCED DIABETIC RATS**

**Table 2.**  
*Effect of Centella asiatica extracts on blood glucose levels (mg /dl) of alloxan induced diabetic rats<sup>a</sup>*

Group	Treatment	Blood glucose at different hours after the 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 treated with 250 mg/kg of ethanolic extract	391.74 ± 7.08	238.70 ± 6.82**	198.96 ± 5.64**	210.86 ± 5.21**
III	Diabetic rats treated with 250 mg/kg of methanolic extract	363.16 ± 8.45	264.67 ± 6.96**	250.89 ± 7.16**	261.69 ± 7.58**
IV	Diabetic rats treated with 10 mg/kg of Gliconclamide	377.80 ± 7.84	338.43 ± 7.83*	258.41 ± 7.34**	265.10 ± 7.35**

<sup>a</sup> Values are mean ± S.E.M, n = 6

\*P<0.01

\*\*P<0.001 compared with initial level of blood glucose of the rats (0h) in the respective group.

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 ethanolic extract (48.86%), while methanolic 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 ethanolic extract has shown maximum reduction (223.51 mg/dl on 5th day) and at a faster rate compared to methanolic extract (144.71 mg/dl).

**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



## ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES EXTRACT OF *CENTELLA ASIATICA* ON ALLOXAN INDUCED DIABETIC RATS

diabetes. The ethanolic and methanolic extracts have shown significant reduction in blood glucose levels in both glucose loaded and alloxan induced diabetic rats. The ethanolic 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 ethanol 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 69% and 51% respectively. Hypercholesterolemia, hypertriglyceridemia, hyperurea have been reported to occur in alloxan diabetic rats<sup>9,10</sup> and a significant increased observed in our experiment was in accordance to these studies. According to Ramaswamy, AS et al., (1970)<sup>11</sup> 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* extracts 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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**ANTI-DIABETIC EFFECT OF ETHANOLIC AND METHANOLIC LEAVES  
EXTRACT OF *CENTELLA ASIATICA* ON ALLOXAN INDUCED DIABETIC RATS**

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