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Original Article

Antidiabetic activity of *Centella asiatica* on streptozotocin induced diabetic male albino rats

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ABSTRACT

The present study has been under taken to investigate the various phytochemicals present in *Centella asiatica* leaves and also to investigate its anti-diabetic property. In this study, rats were randomly divided into 6 groups containing 6 rats in each group. Group A and Group B served as positive (Normal) and negative (Diabetic) controls whereas Group C, D, E and F are the diabetic rats that are treated with methanol extract of *Centella asiatica* leaves (CALEt) at 100, 200, 300 and 400 mg /kg body weight (b.w) respectively. A marked raise in the fasting blood glucose level was observed in diabetic control rats (Group B) when compared to normal control rats (Group A). CALEt at 100, 200, 300 and 400 mg/kg b.w exhibited dose dependent anti-hyperglycemic activity on 4th 7th and 10th day of post treatment. The extract dose of 300 mg/kg b.w and 400mg/kg b.w caused the significant reduction in blood glucose. However the minimum dose of 300 mg/kg b.w was concluded as the effective dose against diabetes because it is the smallest dose with discernible useful effect or maximum dose beyond which no further beneficial effects is seen.

Key words: Diabetes mellitus, *Centella asiatica*, Streptozotocin (STZ).

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from defect in insulin secretion, insulin action, or both. WHO projects that diabetes will be the 7th leading cause of death in 2030 [1]. In 2014 the global prevalence of diabetes was estimated to be 9% among adults aged 18 years and above [2]. In 2012, an estimated 1.5 million deaths were directly caused by diabetes [3]. It is estimated that more than 80% of diabetes deaths occur in low and middle income countries [3]. There is no cure for diabetes mellitus. The management of diabetes mellitus is a global problem and successful treatment is yet to be discovered [4]. Traditional plant treatments for diabetes mellitus are used throughout the world. Management of diabetes without any side effect is still a challenge to the medical system [5]. This had led to increasing demand for natural products with antidiabetic activity with fewer side effects [6]. Plants are well known in traditional herbal medicine meant for their hypoglycemic activities, *Centella asiatica* is one such plant and it is locally known as Gotu kola. The present study was undertaken for preliminary phyto-chemical screening and to evaluate the effect of methanol extract of *Centella asiatica* leaves on the blood glucose levels in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Centella asiatica* were collected from Talakona forest. The taxonomic identification of *Centella asiatica* (L) plant was confirmed by a senior Botanist, Dr. Madhava Chetty, Department of Botany, S.V. University and a voucher specimen was deposited in the herbarium.

Wistar strain male albino rats, aged 3 months and weighing 160 ± 20 g, were purchased from the authorized dealer (M/S Raghavendra Enterprises, Bangalore, India). The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (25 ± 2°C) with a photo-period of 12 h light and 12 h dark cycle, humidity 50 ± 10%. The rats were fed with water and a standard rat pellet diet added libitum (purchased from Sai Durga Agencies,

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Bangalore, India). The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003) and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, India (Resolution No. 10/(i)/a/CPCSEA/IACE/SVU/PSR-MRA).

Plant Extract preparation: Fresh leaves of *Centella asiatica* were washed thoroughly, shade dried and powdered. The *Centella asiatica* leaves powder was subjected to successive solvent extraction. The extraction was carried out with the following solvents in increasing order of polarity: petroleum ether, chloroform, methanol, followed by water [7, 8]. *Centella asiatica* leaves powder was soaked in the above solvents for 48 hrs and filtered. The above filtrates were collected and evaporated in a rotavapour at 40-50°C under reduced pressure. A semisolid greenish material obtained was stored at 0-4°C until used.

Phytochemical Screening of the Plant: Phytochemical screening was done using color forming and precipitating chemical reagents to generate preliminary data on the constituents of the plant extract. Different extracts (petroleum ether, chloroform, methanol and aqueous) of *Centella asiatica* were subjected to following tests for identification of its various active constituents by standard methods. Carbohydrates were identified by molisch's test, proteins were identified by ninhydrin test, terpenoids and saponins were identified by libermann-burchard test, alkaloids were identified by draggendorf's test, tannins were identified by braemer's test, glycosides were identified by Benedict's test and flavonoids were identified by ferric chloride test.

Acute toxicity study: Acute toxicity studies were conducted according to the guidelines by the Organization for Economic Cooperation and Development 2001.

Induction of experimental diabetes mellitus: The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/kg b.w) [9, 10] and dissolved in 0.1 M cold citrate buffer, PH 4.5). After injection, rats had free access to food and water, and given a 15% glucose solution to drink overnight to counter hypoglycemic shock. The rats were considered as diabetic if their blood glucose values reached above 250 mg/dl on day 3 after STZ injection. The blood glucose levels were measured by using one touch glucometer (Johnson and Johnson). After diabetes confirmation, rats were allowed for 7 days to acclimatize to diabetic condition, and rats with hyperglycemia (blood glucose > 250 mg/dl) were chosen for the study. Treatment was started on 8th day after STZ injection which was also considered as the first day of treatment and continued further until end of the study period.

Experimental Design: In this study 36 rats of same age group (3 months old) were used. All the rats were randomly divided into 6 groups containing 6 rats in each group. Group A contains normal rats which served as the normal control group, which received only distilled water for equivalent handling; Group B served as diabetic control group (untreated) which received only distilled water for equivalent handling; Group C, D, E and F are the diabetic rats that are treated with CALEt at 100, 200, 300 and 400 mg per kg b.w. The rats were dosed orally once daily by gavage with either distilled water or extract for 10 days. Body weights and blood glucose levels of all rats (all six groups) were recorded during the study period (10 days). Blood samples were collected by tail snip and estimation of blood glucose was carried out by using dextrose strips (Glucose oxidase – peroxide method) with one touch glucometer (Manufacture: Johnson and Johnson).

Statistical analysis: Statistical analyses were necessary to determine the significance of the effect of the treatment on the experimental group of animals. The mean, standard deviation (SD) and probability test (Analysis of variance - ANOVA) were carried out according to Steel and Torrie (1960) using BASIC programming techniques on SPSS PC for different parameters. The p value of more than 0.05 was considered as not significant.

RESULTS AND DISCUSSION

Phyto-chemical screening: In this process compounds of different polarity from dried leaves of *Centella asiatica* were extracted by sequential extraction process using solvents such as petroleum ether, chloroform, methanol and water. These sequential extracts were subjected to phytochemical screening. The results of the preliminary phytochemical screening revealed the presence of different chemical groups, of all the extracts tested, methanol extract gave positive tests for carbohydrates, proteins, alkaloids, tannins, saponins, terpenoids, and flavonoids (Table 1.1). Therefore it is obvious that fractionation with methanol has enriched the active phytochemical components.

Table 1.1 Qualitative Phytochemical Analysis in Different Extracts of Leaves of *Centella asiatica*

Plant constituents	Extractive solvents			
	Petroleum ether	Chloroform	Methanol	Water
Carbohydrate	_	_	+	+
Protein	-	_	+	+
Steroids	+	+	_	_
Alkaloids	-	+	+	_
Tannins	-	-	+	+
Glycosides	-	-	_	+
Saponins	-	-	+	+
Flavonoids	-	-	+	-
Terpenoids	-	-	+	-

+ve and –ve symbol indicates the presence and absence respectively of plant constituents with respect to extractive solvents in the increasing order of polarity

Acute Toxicity Test: The extract was found to have no toxic effects when administered in doses up to 3000 mg/kg b.w. The blood glucose levels were determined on the 4th, 7th and 10th day after the administration of the CALEt, and the rat's body weights were also monitored on the same days. There was observable change in the body weights of treated and untreated diabetic rats. Treatment of diabetic rats with CALEt improved the weight gain compared to untreated diabetic rats (Fig 1.2). A marked raise in the fasting blood glucose level was observed in diabetic control rats when compared to normal control rats. CALEt at 100, 200, 300 & 400 mg/kg b.w exhibited dose dependent anti-hyperglycemic activity on 4th 7th and 10th day of post treatment (Fig 1.1). Antihyperglycemic response was observed in all the selected doses with no exception. The extract dose of 300mg/k.g b.w and 400mg/kg b.w caused the significant ($P < 0.001$) reduction in blood glucose. The extract dose of 100 mg/kg b.w and 200 mg/kg b.w also caused reduction in blood glucose but the results were found statistically insignificant ($P > 0.05$). The minimum dose of 300mg/kg b.w was concluded as the effective dose because both the doses (300mg/kg b.w and 400mg/kg b.w) were equally effective (Fig 1.1). So, the minimum dose of 300mg/kg b.w was concluded as the effective dose against diabetes.

Fig. 1.1: Effect of CALEt on blood glucose levels in dose finding experiment

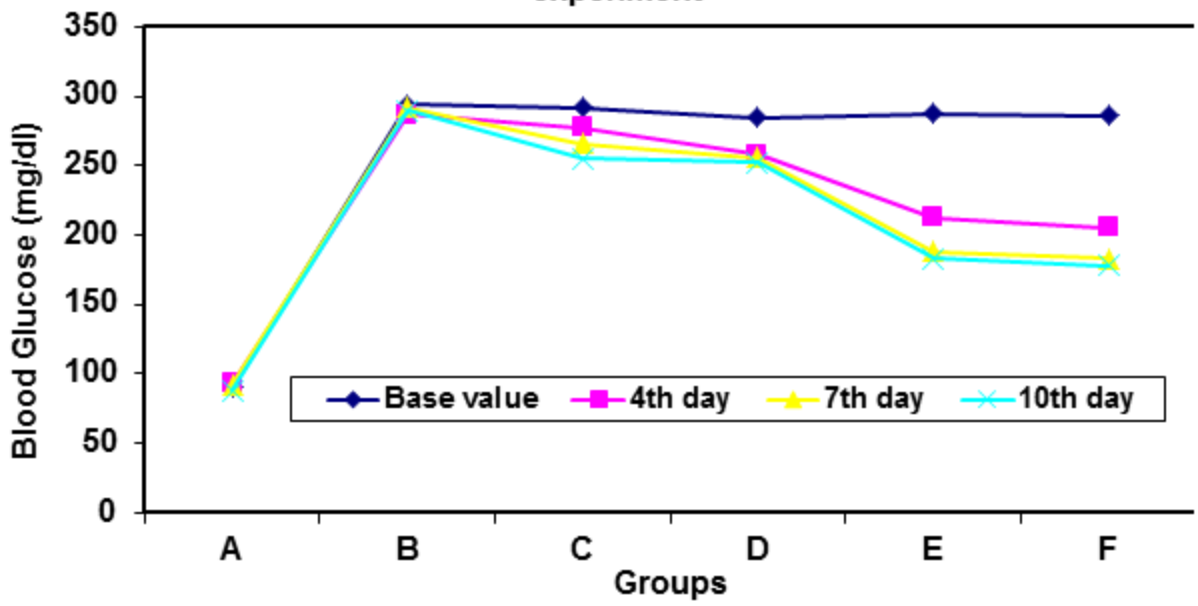
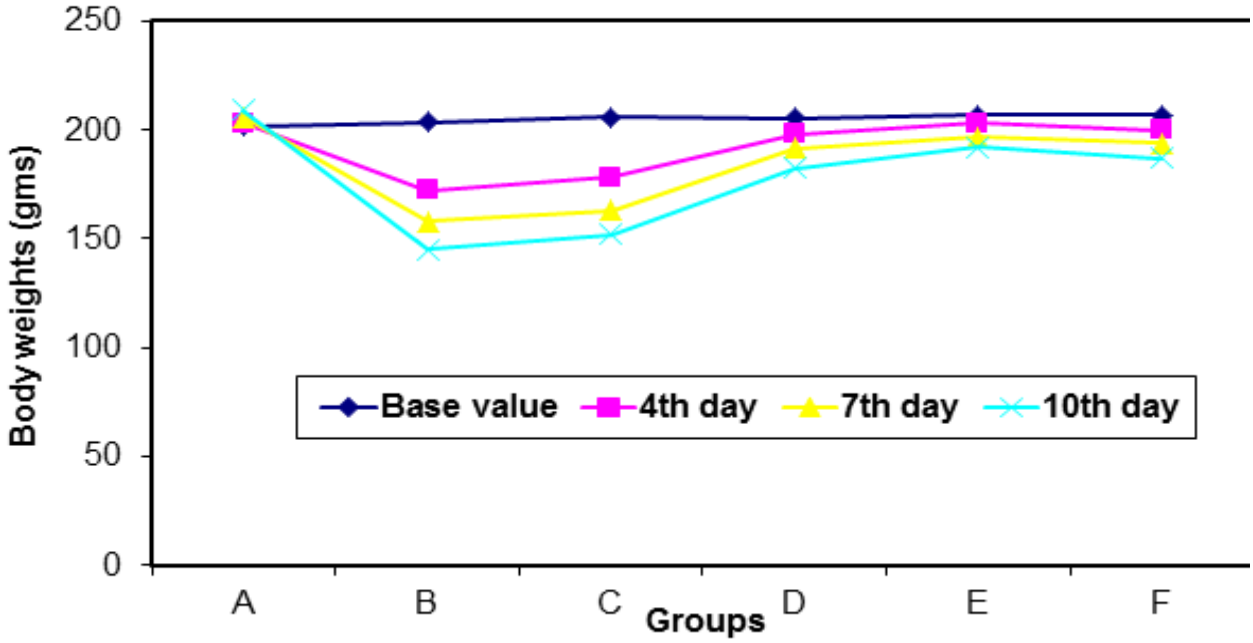


Fig. 1.2: Effect of CALEt on body weights in dose finding experiment



In the indigenous Indian system of medicine good numbers of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated [11]. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research [12, 13]. In the current study the preliminary phytochemical screening indicates the presence of tannins, saponins, flavonoids, alkaloids and terpenoids. Most plants with antidiabetic properties have been found to contain secondary metabolites such as tannins, saponins, alkaloids, and flavonoids [14]. The available literature reveals that flavonoids, terpenoids, alkaloids, saponins and tannins have been shown to possess hypoglycemic activity [15, 16]. Flavonoid and tannins isolated from other antidiabetic medicinal plants has been found to stimulate insulin secretion from pancreatic β-cells or possess insulin like effect [17]. It has been reported that some alkaloids possess antihyperglycemic activity which is mediated by the inhibition of α-glucosidase or stimulation of insulin secretion [15, 16]. Tannins are also known to stimulate insulin secretion from β-cells [15, 18]. These constituents may in part be responsible for the observed significant activity of the extract either singly or in synergy with one another [19].

In our investigation acute toxicity test of CALEt in rats produced no death or signs of toxicity even at the dose of 3000 mg/kg b.w which shows that the extract was well tolerated and the test doses are safe in the animals. Our results indicates that methanol extract of *Centella asiatica* leaves have good antidiabetic activity and from the observations, it was concluded that the reduction of blood glucose levels in diabetic rats was found to be dose dependent and the maximum effect was seen at 300 mg/ kg b.w and that effect seems to reach constant thereafter. The possible mechanism through which methanol extract of CALEt exerts anti-hyperglycemic effect may be due the increased release of the insulin from the existing β-cells of the pancreas [20] or it might be due to the increased release of insulin from regenerated β-cells. But some plants' extracts are reported to exert hypoglycemic action through extra pancreatic mechanisms by inhibition of hepatic glucose production or corrections of insulin resistance [21, 22]. The exact mechanism by which the plant extract lowered the blood glucose level is not yet clear but further studies should be performed to confirm this hypothesis.

CONCLUSIONS

In conclusion, the present study show that the methanol extract of *Centella asiatica* leaves has potential antidiabetic action in streptozotocin induced diabetic rats. Thus, the folk use of the leaves of *Centella asiatica* for the control of diabetes may be supported by this study.

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