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Research article

Antidiabetic potentiality of aqueous leaf extract of *Leucas zeylanica* in alloxan-induced diabetic rats

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Key words: Blood glucose lowering effect, alloxan-induced diabetic rats, Glibenclamide, *Leucas zeylanica.*

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Abstract

The study was designed to evaluate the blood glucose lowering effect of the aqueous extract of *Leucas zeylanica* leaf using the alloxan-induced diabetic rats and compared the activity with diabetic control and antidiabetic drug (Glibenclamide). Leaf extract (50 mg/kg) of *Leucas zeylanica* and Glibenclamide were administered to normal and experimental diabetic rats for the duration of 10 days. In the alloxan-induced diabetic rat model, *Leucas zeylanica* extract (50 mg/kg) significantly ($P < 0.05$) lowered the fasting blood glucose level. Serum insulin levels were not stimulated in the animals treated with the extract. In addition, changes in body weight and liver glycogen levels assessed in the extract treated to the diabetic rats were compared with diabetic control and normal rats. Significant results ($P < 0.05$) were observed in the estimated parameters. Surprisingly, body weight was increased significantly ($P < 0.05$) in *Leucas zeylanica* treated diabetic group.

Introduction

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD). The diagnostic criteria for diabetes are based on thresholds of glycemia that are associated with microvascular disease, especially retinopathy [1, 8].

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, insulin dependent diabetes mellitus (IDDM), the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes or non insulin dependent diabetes mellitus (NIDDM), the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. The faulty receptiveness of body tissues to insulin is believed to involve the insulin receptors of the cell surface membranes. Type 2 diabetes mellitus is by far the commonest form of the disease worldwide and developing countries are the worst hit as far as this epidemic is concerned [2, 9]. Presently practicing therapies for diabetes mellitus treatment directed to insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. [3, 10-11]. Traditionally, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents [4, 13, 15]. These drugs are used as mono-therapy or in combination to achieve better glycemic control [5, 14, 21]. The oral anti-hyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects [6, 20]. This leads to increasing demand for herbal products with antidiabetic activity and less side effects [7, 22]. *Leucas zeylanica* leaves are anthelmintic, diaphoretic, sedative, stimulant and vulnerary, applied topically to heal wounds. Itch, headaches and vertigo is treated by using the poultice of leaves of *Leucas zeylanica*. The entire plant is rubbed on the abdomen after childbirth. *Leucas zeylanica* extract decreased the phagocyte activities of macrophages in alloxan-induced diabetes which indicate that it was effective reducing lipid per-oxidation in experimental

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diabetes. Therefore, the current research job was carried out to assess the antidiabetic activity of *Leucas zeylanica* in alloxan-induced diabetes rat model and to explore into the mechanism of its antidiabetic activity.

Material and Methods

Chemicals and reagents

The Glibenclamide active drug was the bighearted gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. All other chemicals used in the study were of analytical grade.

Collection and identification of plant

The leaves of *Leucas zeylanica* were collected from the Chittagong University hilly forest region at November 2015. A voucher specimen containing the identification characteristics of the plant has been preserved in the Bangladesh National Herbarium for future reference.

Preparation of plant extract and decoction preparation

The fresh leaves of *Leucas zeylanica* 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 minutes allowing the decoction to stand for 30 minutes and filtering through Whatman no.1 filter paper. Filtrate obtained through cheese cloth and Whatman filter paper No. 1 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)ºC, humidity (50 ± 5%) and 12 hours 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 hours prior to experimental protocol to minimize if any of non-specific stress.

Experimental design

A total of 30 male Albino Wistar rats were used 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+*Leucas zeylanica* aqueous extract (2 ml once a day orally for 10 days).
Group-V: Normal rats receiving *Leucas zeylanica* 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.

Hypoglycemic activity test

The hypoglycemic effect of the extract was studied in alloxan-induced diabetic rats. The animals were fasted for 8h 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. Animals with blood glucose level ≥225 mg/dl were considered diabetic and used for the study.

Estimation of hepatic glycogen level and body weight

Hepatic glycogen level was measured according to the standard protocol of Babu *et al*., [9]. Hepatic tissues was 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 ± SEM. The 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

Development of diabetes

The blood glucose level of Alloxan treated rat animals was augmented. Due to destruction of β-cell, the glucose level increased rapidly and reached to diabetic level within 24 hours (Figure 1).

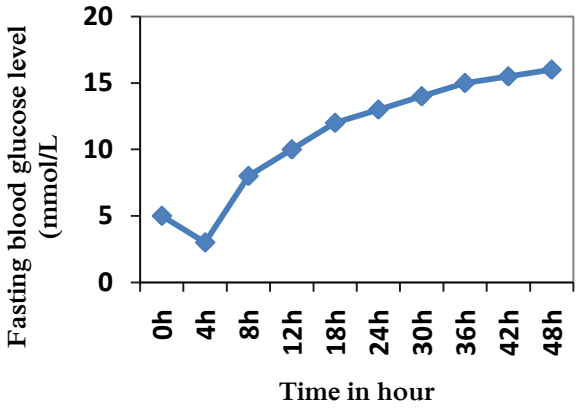


Figure 1. Development of diabetes

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

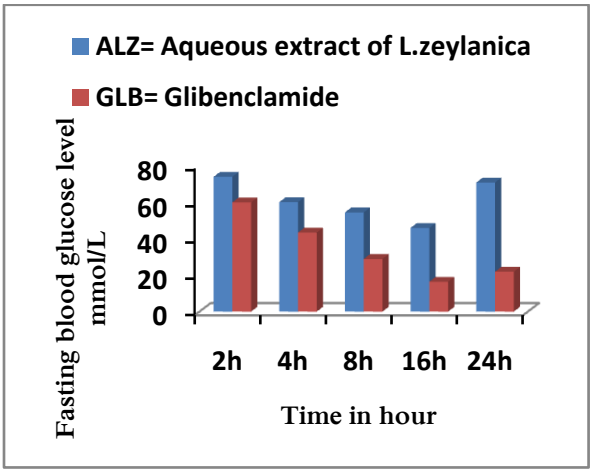


Figure 2. Effect of sample (*Leucas zeylanica*) on Fasting Blood Glucose (FBG) level of alloxan-induced diabetic rats. Here, GLB= Glibenclamide; ALZ= Aqueous extract of *L.zeylanica*; Data were represented as the mean ± SEM (n = number of animals in each group = 6). The criterion for statistical significance was *p < 0.05.

Their baseline glucose concentration was also measured. Aqueous *Leucas zeylanica* extract reduced blood glucose level to 73.88%, 60.01%, 54.38%, 45.88% and 70.77% at 2, 4, 8, 16 and 24 hours, respectively. Maximum reduction of blood glucose level of 45.88 % was observed on 16 hours during the 24 hours experimental period.

In case of alloxan-induced diabetic rats, Glibenclamide reduced blood glucose level to 59.88%, 43.32%, 28.84%, 16.37% and 21.85% at 2, 4, 8, 16 and 24 hours, respectively. So, Glibenclamide caused maximum reduction of blood glucose level of 16.37 % on 16 hours of the experiment.

Effect of the aqueous extract of *Leucas zeylanica* leaf on serum glucose content

The extracts of *Leucas zeylanica* produced significant changes in serum glucose and cholesterol level in the alloxan-induced diabetic rats (Table 2). The prolonged treatment of *Leucas zeylanica* extracts produced consistent reduction in the blood glucose levels.

Table 1. Serum glucose content of control and experimental rat groups

Parameter	Glucose (mg/dl)
Control (Group I)	121.7 ± 1.1
Diabetic Control (Group II)	230.8 ± 1.2***
Diabetic + GLB (1 ml) (Group III)	141.06 ± 1.3**
Diabetic + ALZ treated (2 ml) (Group IV)	192.24 ± 0.6*
ALZ treated (2 ml) (Group V)	119.8 ± 1.3*

NB: Here, GLB= Glibenclamide; ALZ= Aqueous extract of *Leucas zeylanica*; 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 *Leucas zeylanica* produced significant and consistent anti-hyperglycemic effect. The continuous treatment with *Leucas zeylanica* 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.

Effect of the aqueous extract of *Leucas zeylanica* leaf on the body weight and hepatic glycogen content in alloxan-induced diabetic rats

On day zero (before administration of extract), day 5 and day 10, total body weights were also measured for all rats. Surprisingly the body weight was raised in the alloxan-induced diabetic rats that are treated with *Leucas zeylanica* leaf extract (group IV). Average body weights of other groups were remained unaffected (Table 2).

It was surprising that the body weight of the diabetic rats treated with *Leucas zeylanica* was increased. This weight gaining 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 root cause of increased body weight.

Table 2. Effect of the aqueous extract of *Leucas zeylanica* leaf on the body weight and hepatic glycogen content in alloxan-induced diabetic rats

Groups	Body Weight		
	0 day	5 days	10 days
Control (Group I)	175 ± 23.63 (100%)	166 ± 14.84 (95%)	185 ± 16.34 (106%)
Diabetic Control (Group II)	180 ± 21.61 (100%)	177 ± 15.16 (111%)	200 ± 16.56 (106%)
Diabetic + GLB (1 ml) (Group III)	182 ± 22.11 (100%)	182 ± 21.13 (100%)	192 ± 17.42 (105%)
Diabetic + ALZ treated (2 ml) (Group IV)	190 ± 22.50 (100%)	180 ± 29.13 (95%)	190 ± 17.73 (100%)
ALZ treated (2 ml) (Group V)	186 ± 11.43 (100%)	195 ± 22.18 (105%)	203 ± 16.18 (109%)

Conclusion

This research work demonstrated that *Leucas zeylanica* leaf extract generated a significant glucose lowering activity at 50 mg/kg dose investigated on the experimental rats after oral administration. By considering the observations studied it will be justified the claim made by the Indian systems of medicine regarding the use of leaf extract of this plant in the treatment of diabetes. At hand efforts are directed to isolate the active constituents from the aqueous extract of *Leucas zeylanica* leaf and mechanistic battle clarification.

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