



BJP

Bangladesh Journal of Pharmacology
Research Article

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Hypoglycemic activity of aqueous leaf extract of *Limonia elephantum* in alloxan-induced diabetic rats

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Article Info

Received: 25 July 2014

Accepted: 21 August 2014

Available Online: 17 September 2014

DOI: 10.3329/bjp.v9i3.19705

Cite this article:

Kamalakannan K, Balakrishnan V. Hypoglycemic activity of aqueous leaf extract of *Limonia elephantum* in alloxan-induced diabetic rats. Bangladesh J Pharmacol. 2014; 9: 443-46.

Abstract

The hypoglycemic activity of the aqueous leaf extract of *Limonia elephantum* was evaluated. The extract was administered orally 1,000 mg/kg to normal and alloxan-induced (55 mg/kg body weight, i.v) diabetic rats. The hypoglycemic effect of the extract was statistically significant with an oral dose of 1,000 mg/kg and was comparable to that of the effect produced by standard antidiabetic agent, metformin 1.5 mg/kg. The extract increased the glucose tolerance using oral glucose tolerance test.

Introduction

Diabetes is a syndrome characterized by disordered metabolism of carbohydrate, protein and lipid with abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin's effect (Wells et al., 2009). Diabetes mellitus is a major public health problem worldwide. The prevalence of diabetes mellitus is expected to rise more rapidly in the future because of an increasing obesity and reduced activity levels (Sarah et al., 2004). The currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin are considered to have limitations of their own, hence herbal medicines have been recommended for the treatment of diabetes (Grover et al., 2002; Kamalakannan and Balakrishnan, 2009). Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Hamdan and Stephen, 1998). Antihyperglycemic effects of these are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or by inhibiting the intestinal absorption of

glucose or by facilitating the metabolites in insulin dependent processes. More than 400 plant species with hypoglycemic activity are available in literature for evaluation (Kar et al., 2003).

Limonia elephantum Corr. syn. *Limonia acidissima*, *Feronia elephantum* L. (Family:Rutaceae) is distributed throughout India at an elevation of 450 m. In traditional medicine, bark, leaves, fruits and gum of this plant are used (Kirtikar and Basu, 1995). Alkaloid, coumarins, flavanones, lignin, (2S)-5,3'-dihydroxy-4'-methoxy-6',6'-dimethylchromeno(7,8,2',3')-flavanone form the bark (Javed and Mohammad, 2009), fernolin, aurapten, marmesin, bergatpten and xanthotoxin from root (Kanhiya et al., 2008) and methyl chavicol as a major compounds has been reported from the essential oil (Garg, 2003) of *L. elephantum*. The oil extracted from the seeds has been reported to be useful in itching, skin diseases and leprosy (Patil et al., 2004). Chemical contents of the seeds of *L. elephantum* have been reported to be palmitic, oleic, linoleic, linolenic, palmitoleic, steric acids, β -sitosterol and β -amysin (Gupta et al., 1997). Ethanolic extract of the fruits of *L. elephantum* has been reported to significantly lower the blood glucose level in experi-



mental rats (Anurag et al., 2009). There is no scientific data available for hypoglycemic activity of the leaves of *L. elephantum*. Traditional healers widely used leaf decoction of *L. elephantum* to treat diabetes and therefore the present study was planned to evaluate the hypoglycemic activity of aqueous extract of *L. elephantum* both in euglycemic and alloxan-induced diabetic rats.

Materials and Methods

Collection and authentication of the plant materials

The leaves of *L. elephantum* were collected in the month of March 2010 from Tropical area of Western Ghats region of Coimbatore district. The plant was authenticated at the Botanical Survey of India (BSI) Coimbatore, where the voucher specimens were deposited (Herbarium voucher no BSI/SC/5/23/09-10/Tech-266). The samples were shade dried at room temperature.

Preparation of extract

The leaves of *L. elephantum* were shade dried and reduced to coarse powder by mechanical grinding. The powdered material was macerated in distilled water for seven days with 2 mL of chloroform to avoid any fungal or bacterial contamination. The mixture was sonicated in cold water for 30 min before filtration. The sonicated mixture was filtered using muslin cloth and the filtrate so obtained was lyophilized to get in powder form. The powdered *L. elephantum* extract was stored in deep freezer at -20°C for the experimental use.

Animals and housing condition

The healthy male Wistar rats (120-150 g body weight) used in the present study, were fed on commercial laboratory animal feed (Amrut brand, Sangli) and water *ad libitum*. The rats were housed in the laboratory for a week for acclimatization under 12:12 hours natural light/dark cycle. The animals were starved overnight with water *ad libitum* prior to the day of experimentation.

Dose determination

Based on the preliminary studies carried out on euglycemic animals, in the present study the dose of 1,000 mg/kg of the extract was selected. Rat equivalent dose 1.5 mg/kg of metformin, a standard hypoglycemic used in the present study was calculated using conversion table (Paget and Barnes, 1964).

Studies in euglycemic rats

The animals were fasted for 18 hours prior to the experiment and allowed to free access of water. Fasted rats were divided into three groups of six animals in each group. Group I served as vehicle control which

received saline p.o. The Group II received aqueous leaf extract orally 1,000 mg/kg and Group III received metformin 1.5 mg/kg. The glucose in the dose of 2 g/kg was administered orally after 30 min of various treatments. Blood samples were collected from tail vein at 0, 30, 60, 90 and 120 min post dose to estimate blood glucose using glucometer (Deore et al., 2008).

Studies in alloxan-induced hyperglycemic rats

The animals were fasted overnight and 55 mg/kg alloxan dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) was given intravenously in a volume of 1 mL/kg. After 6 hours 200 mg of glucose was given orally to protect from hypoglycemia. After a gap of one day the fasting blood glucose was estimated with the help of standard glucometer and those having blood glucose more than 300 mg/dL were included in the study. Selected animals were divided in three groups (n = 6, in each) to receive different treatments orally. Group I served as vehicle control which received saline p.o. The Group II received aqueous leaf extract orally 1,000 mg/kg and Group III received metformin 1.5 mg/kg. The glucose in the dose of 2 g/kg and blood samples were collected from tail vein at 0, 30, 60, 90 and 120 min after glucose challenge to estimate blood glucose (Prince et al., 2004).

Statistical analysis

The data were expressed as mean \pm SEM and analyzed by ANOVA followed by Dunnett's post hoc test and $p \leq 0.05$ was considered significant.

Results

There was no significant change blood glucose levels of animals treated with *L. elephantum* extract as well as with metformin as compared to those of vehicle treated controls using oral glucose tolerance test (Figure 1). In alloxan-induced hyperglycemic rats, both the treatments lowered blood glucose as compared to that of control group, the reduction was not significant.

As expected saline treatment did not alter the glucose level significantly, while metformin in therapeutic equivalent dose decreased the blood glucose significantly at 3rd and 5th hour. *L. elephantum* extract treatment with the higher (1,000 mg/kg) dose significantly lowered blood glucose at 3rd hour as compared to the 0 hour reading, indicating its hypoglycemic activity begins within 3rd hour after administration and disappears by 5th hour (Table I).

Discussion

Finding of the present preliminary investigation of the aqueous extract of *L. elephantum* in alloxan-induced

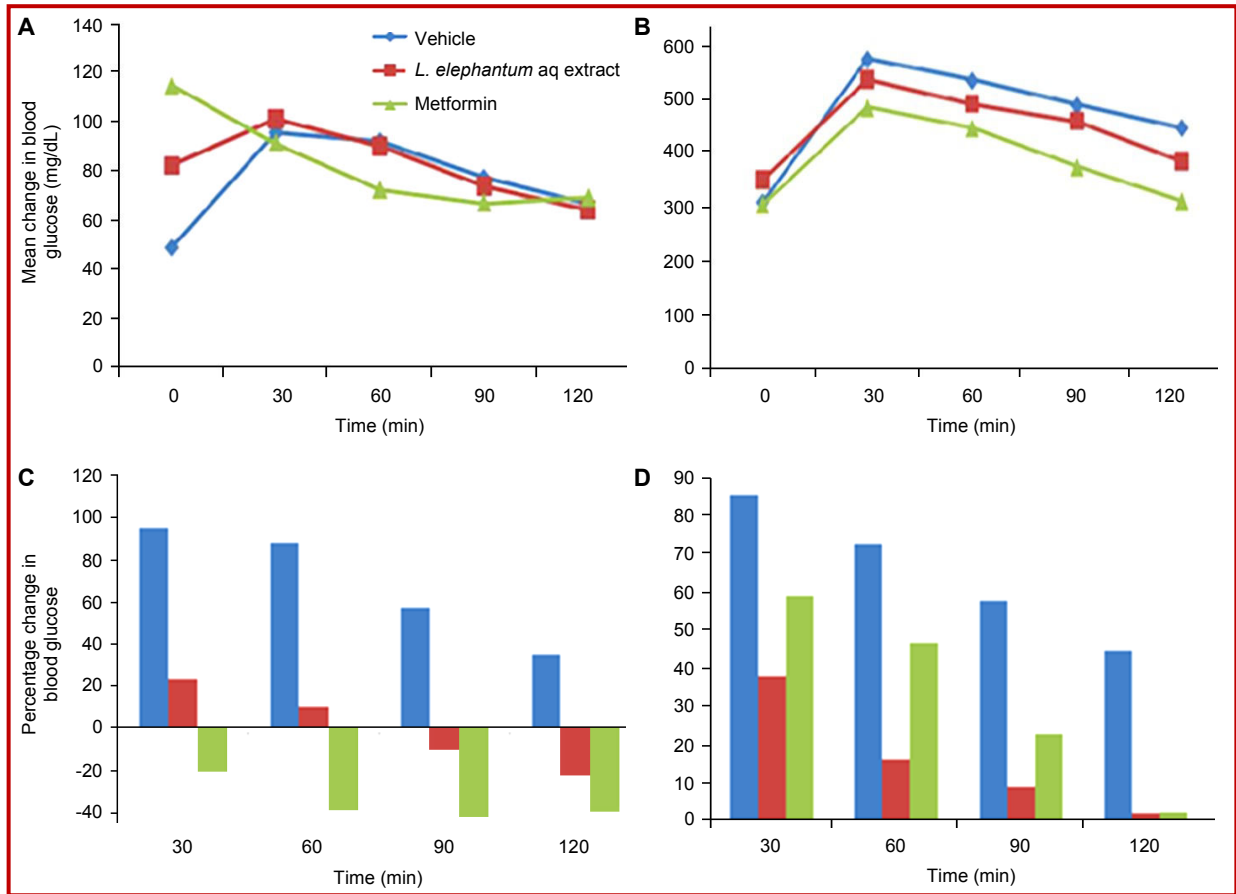


Figure 1: Oral glucose tolerance test in euglycemic (A) and diabetic (B) rats treated with *L. elephantum* aqueous extract. Percentage change in blood glucose level are shown in euglycemic (C) and diabetic (D) rats

Table I

Effect of *L. elephantum* aqueous extract on blood glucose of alloxan-induced diabetic rats

Treatment group	Blood glucose mg/dL (Mean \pm SEM)			
	0 hour	1 hour	3 hours	5 hours
Vehicle	312.9 \pm 26.0	320.3 \pm 6.2	322.1 \pm 2.7	323.8 \pm 4.6
<i>L. elephantum</i> 1,000 mg/kg	450.8 \pm 26.7	412.7 \pm 35.5	362.4 \pm 27.6 ^b	372.6 \pm 32.7
Metformin 1.5 mg/kg	332.9 \pm 2.7	315.7 \pm 3.8	302.8 \pm 2.6 ^b	274.8 \pm 6.7 ^a

Values are given as mean \pm SEM; ^ap \leq 0.01, ^bp \leq 0.05

hyperglycemic rats showed a clear hypoglycemic activity, which is in accordance with traditional use of the plants leaves to the reduced blood glucose level, though it was of short duration as compared to that of metformin. Kamalakannan and Balakrishnan (2014) stated that *L. elephantum* have anti-oxidant potential. This finding is in accordance with traditional use of the plant leaves in the form of decoction for diabetes. Probably repeated administration in higher dose of *L. elephantum* extract for a prolonged period may produce sustained hypoglycemic effect. To identify the main hypoglycemic constituents in the extract, to elicit its possible mechanism of hypoglycemic activity and to

evaluate its efficacy on chronic administration, further studies are desirable.

Ethical Issue

Ethical clearance was obtained from Institutional Animal Ethical Committee (NCP/IAEC/Ph.D/2010-001) constituted as per CPCSEA guidelines.

Acknowledgements

The authors are grateful to the management, Principal for

providing necessary laboratory facilities. The author acknowledge to Mr. M. Suresh, Lab Assistant of Nandha College of Pharmacy, Erode for providing the invaluable critique and support for carrying out this research work. The authors are acknowledging to the experts for review the manuscript for improvement.

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