EVALUATION OF THE EFFECT OF *LEUCAS ASPERA* ALCOHOLIC EXTRACT ON BLOOD GLUCOSE LEVEL IN NORMOGLYCEMIC AND DIABETIC RATS

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Summary

The Leucas plant is used as a folk remedy to treat diabetes in India. The objective of our study was to evaluate the effect of the leaves and stem of *Leucas aspera* on blood glucose level in normoglycemic rats and diabetic rats. Animals were divided into eight groups of six rats each.4 of the groups (n=24) were induced diabetes with streptozotocin. The group treated with gum acacia served as control.Rats were treated with metformin (200mg/kg), and Leucas alcoholic extract at doses of 200mg/kg and 400mg/kg respectively. All the animals were treated with drugs orally as suspensions in gum acacia, daily for 15 days. Blood glucose levels were measured on day 0, just prior to initiation of any treatment i.e. fasting and at time intervals of 30,60 and 120 mins after drug administration Fasting blood samples were collected 24 hrs after drug administration on day 7 and day 15.
Administration of the plant extract did not show any reduction in blood glucose level in normal and diabetic rats with single dose administration study on day 0.There was an increase in levels at intervals of 30 and 60 min. Upon chronic administration the extract showed significantly reduced FBS levels, and was comparable to the reference drug metformin. The alcoholic extract of leucas aspera has shown hypoglycemic activity in chronically treated normoglycemic and diabetic rats which could merit its role in treatment of diabetes mellitus.

Key Words: Diabetic rats, hypoglycemic effect, *Leucas aspera*

Introduction

Traditional medicine often use plants in the treatment of diabetes mellitus.*Leucas aspera* belonging to family Lamiaceae commonly called Thumbe grows as an herbaceous, much branched,erect or diffuse annual, 30 to 60 cm high as a weed on waste lands and road sides throughout India. The leaves and flowers are carminative, digestive, antiinflamatory antipyretic[1]. It is used in the treatment of respiratory tract disorders, gastrointestinal disorders.In Indian traditional medicine the juice of leaves of *Leucas aspera* is used to treat psoriasis,chronic skin erruptions, and rheumatism[2,3]. Compounds isolated from the plant include long chain aliphatic compounds,triterpene leucolactone,reducing sugars-galactose,

sterols.lignans, flavonoids and diterpenes[4,5]. The plant is used as a folk remedy to treat diabetes in India and Bangladesh [6]. The objective of our study was to evaluate the effect of the leaves of *Leucas aspera* on blood glucose level in normoglycemic and diabetic rats.

Materials and methods

Preparation of alcoholic extract:

The herb in full bloom was collected and authentified. The shade dried leaves and stem of the plant (2 kg) was reduced to fine powder .The coarse powder was packed in thimble made of whatmann filter paper and extracted in soxhlet extraction column with 95% alcohol obtained by double distillation. The dried residue (22g) was stored in a dessicator.

Animals:

Adult albino rats of wistar strain weighing 180 -250 g of either sex raised in central animal house were used. They were fed on standard chow and given tap water ad libitum. The study was performed with due permission from institutional animal ethics committee. The effect of the extract was studied in the normoglycemic and hyperglycemic rats.

Studies in normoglycemic rats:

Animals were divided into four groups of six rats each. Group I treated with gum acacia served as control, group II was treated with metformin (200mg/kg), group III&IV received Leucas extract at doses of 200mg/kg and 400mg/kg respectively based on the safety reports in earlier studies[7,8]. All the animals were treated with drugs orally as suspensions in gum acacia, daily for 15 days. Blood glucose levels were measured on day 0, just prior to initiation of any treatment i.e. fasting and at time intervals of 30, 60 and 120 min. after drug administration Blood was collected from the tail vein and glucose was measured using high precision electronic glucometer. Fasting blood samples were collected 24 hrs after drug administration on day 7 and day 15.The body weight of rats was also noted.

Studies in hyperglycemic rats:

A solution of STZ was prepared by dissolving the weighed quantity of Streptozotocin (STZ) obtained from sigma chemicals in 0.1 M freshly prepared ice-cold citrate buffer (pH 4.5) solution. The solution of STZ so prepared was administered 30mg/kg [9]. intraperitoneally. A period of 12 days was allowed to confirm stable hyperglycemia.

Diabetic rats (n=24) were randomly divided into four groups of six rats each. Group I treated with gum acacia served as control, group II was treated with optimal dose of metformin (200mg/kg), group III &IV received Leucas extract at doses of 200mg/kg and 400mg/kg respectively. Single dose of drug was given to overnight fasted rats and blood collected at 30,60,120 min after administration for estimating blood sugar.The rats continued to recieve the drug for 15 days. Fasting blood samples were collected on day 7and 15 for glucose estimation.

Statistical analysis

All values are expressed as as mean ± standard error of mean(SEM).The data was analysed by one way ANOVA followed by Tukeys post hoc test using SPSS computer package. $p<0.05$ was considerd to be statistically significant.

Results

Administration of the plant alcoholic extract did not show any reduction in blood glucose level in normal rats with single dose administration study on day '0' (Table1).There was a significant increase in levels at intervals of 30 and 60min. Upon chronic administration the extract showed significantly reduced FBS levels,similar to the reference drug metformin when compared with control rats(Table 2).

Table 1: Effect of single dose administration of drugs in normoglycemic rats

Treatment groups (mg/kg)	Fasting blood sugar (mg/dl)			
	0 min	30 min	60 min	120 min
Control(5ml of 2% gum acacia)	86.16±3.85	79.5±2.20	78.66±2.84	80.5±2.07
Metformin(200)	90.66±3.80	75.33±4.73	65.66±2.51*	70.0±1.87
***L.aspera* (200)**	94.33±4.66	99.66±3.37*	91.66±4.47*	90.33±5.21
***L.aspera* (400)**	88.83±1.87	95.67±2.43*	99.17±3.87*	92.66±3.21

n=6, values expressed as Mean ± S.E.M
p <0.05 significant as compared to control

Table 2: Effect of repeated administration of drugs on blood glucose in normoglycemic rats

Treatment groups (mg/kg)	Fasting blood sugar (mg/dl)		
	0 day	7th day	15th day
Control(5ml of 2% gum acacia)	86.16±3.85	79.0±3.48	74.33±1.74
Metformin(200)	90.66±3.80	71.33±3.48	62.33±1.74*
***L.aspera* (200)**	94.33±4.66	61.5±1.49*	58.5±0.95*
***L.aspera* (400)**	88.83±1.87	66.5±1.49*	63.5±0.76*

n=6, values expressed as Mean ± S.E.M,
* p <0.05 significant as compared to control

In diabetic rats the fasting blood glucose on the initial day of treatment showed a rise in levels at intervals of 30 and 60 min, and returned to pretreatment values at 120 min (Table 3). The fasting blood glucose of the extract treated groups following repeated administration for 15 days showed a significant reduction when measured on day 7 and day 15 as compared to control and and is parallel to metformin (Table 4). At a dose of 200 mg/kg the extract significantly lowerd the day '0' blood glucose concentration by 30% and 46% on day 7 and 15 respectively. A similar observation i.e. 29% and 47% reduction of blood glucose on 7th and 15 th day was noted with higher dose(400mg/kg). A reduction by 33% on day 7 and 53% by day 15 was observed with metformin treated group.

Table: 3 Effect of single dose administration of drugs in diabetic rats

Treatment groups (mg/kg)	Fasting blood sugar (mg/dl)			
	0 min	30 min	60 min	120 min
Control(5ml of 2% gum acacia)	262.67±6.81	256.83±4.57	253.67±2.37	242.67±3.82
Metformin(200)	216.0±6.70	184.0±5.34	171.11±3.33*	162.33±2.88*
***L.aspera* (200)**	277.17±1.21	290.1±78.3	304.33±1.25	275.5±1.02
***L.aspera* (400)**	238.0±8.68	257.5±5.34	268.50±2.74	243.17±7.10

n=6, values expressed as Mean ± S.E.M
* p <0.05 significant as compared to control

Table 4: Effect of repeated administration of drugs on blood glucose in diabetic rats

Treatment groups (mg/kg)	Fasting blood sugar (mg/dl)		
	0 day	7th day	15th day
Control(5ml of 2% gum acacia)	260.67±6.81	236.50±3.93	230.50±2.72
Metformin(200)	216.0±6.70	143.83±5.17*	100.50±2.61*
***L.aspera* (200)**	277.17±1.21	192.67±1.0*	147.0±4.48*
***L.aspera* (400)**	238.0±8.68	169.0±6.08*	126.5±5.73*

n=6, values expressed as Mean ± S.E.M
* p <0.05 significant as compared to control

Discussion

Streptozotocin induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents.Streptozotocin enhances free radical formation or produces defects in antioxidant defence system resulting in destruction of beta cells of pancreas which may lead to hyperglycemia[10]. Both type 1 and type 2 diabetes comprise abnormalities of insulin action, including deficiency and insulin resistance [11].

In our study it is seen that continuous treatment with leucas aspera produced hypoglycemic effect in rats, but not in acute study. Rather a rise in blood glucose at 1hr is observed after single dose of extract administration. This is contradictory to the literature reported in other studies [12,13].A possible reason could be the activity of certain phytoconstituents of the plant [5].

In diabetes, elevation of blood glucose is a consequence of increased hepatic glucose output along with reduced peripheral glucose utilization. Insulin deficiency is clearly associated with a change in hepatic metabolism [14]. Moreover, a reduction in insulin-mediated glucose uptake caused by decreasing gene expression of GLUT-4 has been reported in skeletal muscle, a major site for glucose disposal, in diabetic rats [15]. Biguanides are known to produce hypoglycaemia in diabetic animals by extrapancreatic mechanism. The plasma glucose–lowering activity of metformin is said to be associated with an attenuation of the raised hepatic PEPCK gene expression that is responsible for hepatic gluconeogenesis in STZ-induced diabetic rats. An increase in the gene expression of GLUT-4 is also claimed to contribute to plasma glucose regulation in metformin-treated STZ-induced diabetic rats [16].

Because long-term exposure is required for the gene expression associated with glucose regulation in STZ-induced diabetic rats, a similar mechanism could be attributed to the beneficial effect of leucas aspera plant on chronic use, which could merit its role in treatment of diabetes mellitus.

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