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Hypoglycemic Effect of *Leucas lavandulaefolia* Willd in Alloxan-Induced Diabetic Rats

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ABSTRACT

Leucas lavandulaefolia Willd. is mainly used in Indian folk medicine for the treatment of diabetes mellitus. The oral administration of 0.15, 0.20 and 0.25 g/kg of chloroform extract of the *Leucas lavandulaefolia* flowers (LLFEt) for 30 days resulted in a significant reduction in blood glucose, glycosylated hemoglobin and an increase in total hemoglobin, and the effect was highly significant in the case of 0.25 g/kg. It also prevents decrease in body weight. Oral glucose tolerance test was also performed in experimental diabetic rats in which there was a significant improvement in glucose tolerance in animals treated with LLFEt and the effect was compared with glibenclamide. Thus, the study shows that LLFEt has hypoglycemic action.

Key words: Diabetes, glucose, hypoglycemic, *Leucas lavandulaefolia*

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INTRODUCTION

Leucas lavandulaefolia Willd. is a herbaceous annual weed found in pastures and waste land throughout India. It is erect, slightly pubescent or tomentose, 0.3 to 0.75 m in height, usually branched; branches are quadrangular, pubescent. Flowers are sub sessile or shortly pedicellate, in axillary and terminal whorls 1.3 to 2 cm diameter 1.3 to 2 cm diameter, toward the end of the branches. It has been used as an antidiabetic agent in traditional system of medicine in India.[1] It has strong flavors and is reputed for its use as sedative, vermifuge, stomachic dermatosis and is also useful in the treatment of migraine.[2] It has also been extensively used by rural people of Mithila region (Bihar) in human and cattle ailments, such as cough, cold skin diseases, headache and snake bite.[3] Isolation of flavonoid glycoside from the flowers of *Leucas lavandulaefolia* using chloroform extract was reported earlier.[4] Hypoglycemic activity of *Leucas lavandulaefolia* in streptozotocin-induced diabetic rats has been investigated.[5] Synthetic hypoglycemic agents can produce serious side effects including hematological, coma and disturbances of liver and kidney. In addition, they are not suitable for use during pregnancy.[6] Various parts of *Leucas lavandulaefolia* Willd. (family: Labiatae) have been used for various medicinal purposes including the treatment of diabetes mellitus. The present investigation was undertaken to study the effect of chloroform extract on *Leucas lavandulaefolia* flowers (LLFEt) on blood glucose, glycosylated hemoglobin and oral glucose tolerance in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant materials

Leucas lavandulaefolia Willd. flowers were collected

freshly from Manipal, Udupi District, Karnataka, India. The plant was identified and authenticated by Dr. Gopalkrishna Bhat, Department of Botany, Poornaprajna College, Udupi. A voucher specimen (NGSM 45) was deposited in Pharmacognosy Department of N.G.S.M. Institute of Pharmaceutical Sciences, Mangalore.

Preparation of chloroform extract of *Leucas lavandulaefolia* flowers

Almost 250 g of *Leucas lavandulaefolia* fresh flowers were extracted using 1.5 l of chloroform by the method of continuous hot extraction.[7] Extract was evaporated in a container to a constant weight. Almost 15.5 g of extract was obtained after complete removal of the solvent. The above residual extract was dissolved in sterile water and investigated.

Selection of dose

The albino rats were administered orally a single dose of 2.5, 5 or 10 times of effective dose of chloroform extract of *L.lavandulaefolia*. The rats were observed for gross behavioral, neurological, autonomic and toxic effect at regular intervals. Food consumption, faeces and urine were also examined at 2 and then at 6-h intervals for 24 h.

Experimental induction of diabetes in rats

Male albino Wister rats of body weight 180-200 g bred in Central Animal House, N.G.S.M. Institute of Pharmaceutical Sciences, Paneer, Mangalore were used in this study. The animals were fed on a pellet diet (Hindustan Liver, India) and water ad libitum. The rats were injected with Alloxan monohydrate dissolved in sterile normal saline in a dose of 150 mg/kg, intraperitoneally. As Alloxan is capable of producing total hypoglycemic as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15-20 ml) intraperiatoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia.[8] After two weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hypoglycemia with blood glucose range of 200-260 mg/100 ml were used for the experiment. Blood was collected from the eyes (venous pool).

Determination of blood glucose and hemoglobin

- Fasting blood glucose was estimated by o-toluidine method.[9]
- Hemoglobin was estimated by cyanmethaemoglobin method.[10]

Determination of glycosylated hemoglobin

Glycosylated hemoglobin (GHb) was estimated by the method of Sudhakar Nayak and Pattabiraman.[11]

Effect on oral glucose tolerance in rats

After overnight fasting, a 0-min blood sample (0.2 ml) was taken from the rats in different groups viz., normal, diabetic control, diabetic + LLFEt (0.25 g/kg) and diabetic + Glibenclamide (600µg/kg) by the orbital sinus puncture[12] without delay a glucose solution (2 g/ml per kg) was administered by gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration.[13] All blood samples were collected with potassium oxalate and sodium fluoride solution for the estimation of blood glucose.

Experimental design

A total of 36 rats (30 diabetic surviving rats, six normal rats) were used for the experiment. Diabetes was induced in rats two weeks before starting the experiment. The rats were divided into six groups after the induction of Alloxan diabetes. In the experiment, six rats were used in each group:
Group 1 Normal untreated rats;
Group 2 Diabetic rats;
Group 3 Diabetic rats that were given *Leucas lavandulaefolia* flower extract (LLFEt) (0.15 g/kg) in aqueous solution daily using an intragastric tube for 30 days;
Group 4 Diabetic rats that were given LLFEt (0.20 g/kg) in aqueous solution daily using an intragastric tube for 30 days.
Group 5 Diabetic rats that were given LLFEt (0.25 g/ kg) in aqueous solution daily using an intragastric tube for 30 days; and
Group 6 Diabetic rats that were given given Glibenclamide orally (600 µg/kg) in aqueous solution daily using an intragastric tube for 30 days.

After 30 days, the rats were sacrificed by decapitation. Blood was collected in a tube containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose. The experiments were repeated in same number of rats.

Statistical analysis

All the group data were statistically evaluated and the significance of various treatments was calculated using Student's t-test. All the results were expressed as mean ± S.D.

RESULTS

No toxic effect was reported up to 5 and 10 times of effective

Table 1: Blood glucose, total haemoglobin, change in body weight and urine sugar of normal and experimental rats[a]

Groups	Body weight (g)		Fasting blood glucose	Haemoglobin	Glycosylated	Urine
	Initial	Final	(mg/100 ml)	(g/100 ml)	haemoglobin (mg/g Hb)	sugar[b]
Normal	180.1 ± 9.5	189.8 ± 10.1	70.32 ± 3.51	11.8 ± 2.2	0.22 ± 0.01	Nil
Diabetic control	171.5 ± 10.3	153.6 ± 10.8c	212.55 ± 14.2c	5.77 ± 0.6c	0.88 ± 0.11c	+++
Diabetic + LLFEt (0.15 g/kg)	161.6 ± 10.5	165.6 ± 11.1d	200.1 ± 12.3d	5.98 ± 0.4d	0.84 ± 0.45d	++
Diabetic + LLFEt (0.20 g/kg)	178.1 ± 10.9	183.7 ± 11.0c	132.5 ± 11.4c	7.88 ± 0.8c	0.33 ± 0.03c	+
Diabetic + LLFEt (0.25 g/kg)	180.1 ± 10.8	186.5 ± 12.2c	81.1 ± 4.4c	12.1 ± 1.5c	0.21 ± 0.03c	Nil
Diabetic + glibenclamide	182.8 ± 10.9	183.3 ± 11.4c	90.4 ± 3.71c	8.5 ± 0.7c	0.3 ± 0.02c	Trace

[a]Values are given as mean ± S.D. for six rats in each group. Diabetic control was compared with normal. Experimental groups were compared with diabetic control. [b]0.25% sugar and (+++) indicate more than 2% sugar. [c]Values are statistically significant at P < 0.001 as compared with diabetic control. [d]Not significant as compared with diabetic control.

Table 2: Glucose tolerance test in normal and experimental animal group[a]

Groups	Blood glucose levels (mg/100 ml)				
	0 min	+ 30 min	+ 60 min	+ 90 min	+ 120 min
Normal	71.1 ± 7.7	162.2 ± 12.1	141.1 ± 11.9	101.5 ± 12.0	70.2 ± 7.7
Diabetic control	215.3± 15.7[b]	311 ± 12.5[b]	371.3 ± 20.5[b]	335.5 ± 22.1[b]	301.4 ± 12.3[b]
Diabetic + LLFEt (0.25 g/kg)	85.5 ± 7.2[b]	170.3 ± 12.3[b]	151.2 ± 13.7[b]	111 ± 11.6[b]	81.3 ± 7.3[b]
Diabetic + glibenclamide	83.3 ± 7.7[b]	181.7 ± 15.5[b]	161.5 ± 14.1[b]	115.4 ± 11.1[b]	98.4 ± 3.2[b]

[a]Values are given as mean ± S.D. for six rats in each group. Diabetic control was compared with normal. Experimental groups were compared with diabetic control. [b]Values are statistically significant at $P < 0.001$ as compared with diabetic control.

dose of the chloroform extract. Table 1 demonstrates the blood glucose, total hemoglobin, glycosylated hemoglobin, change in body weight and urine sugar of normal and experimental animals. There was a significant elevation in blood glucose ($P < 0.001$), glycosylated hemoglobin ($P < 0.001$) while the level of total hemoglobin ($P < 0.001$) decreased during diabetes when compared with the corresponding groups. Administration of LLFEt at 0.02 and values near normal LLFEt at a dose of 0.25 g/kg shows highly significant effect, when compared with glibenclamide. Table 2 gives the blood glucose of control, diabetic control LLFEt and glibenclamide treated animals after oral administration of glucose (2 g/kg per ml). In LLFEt and glybenclamide treated animals more significantly decreased blood glucose concentration after 1 and 2 h. LLFEt treated animals tend to bring the values near normal. LLFEt (0.25 g/kg) were more effective than glybenclamide.

DISCUSSION AND CONCLUSION

The blood glucose data obtained using Alloxan hyperglycemic rats [Tables 1 and 2] clearly shows that the chloroform extract of LLFEt can produce significant and consistent hypoglycemic effects. It is generally accepted that the sulfonyl areas, including glybenclamide produce hypoglycemia in normal animals by stimulating the pancreatic β cells to release more insulin. These drugs, however, do not decrease blood glucose in Alloxan diabetic animals.[14] In contrast to the oral antidiabetic agents, the exogenous administration of insulin is well known to produce hypoglycemia in both normal and Alloxan induced subjects.[6] It is, therefore, conceivable that the hypoglycemia principal(s) in the extract of *Leucas lavandulaefolia* plants exert a direct effect in diabetic rats. In diabetic rats, LLFEt cannot act indirectly by stimulating the release of insulin since Alloxan treatment causes permanent destruction of β cells. The anti-hyperglycemic effect in the alloxan - diabetic rats suggest, that its main mechanism may not be due to potentiation of insulin release from pancreatic cells and thus the drug may be effective in insulin independent diabetes also, as the significant and consistent hypoglycemic effect LLFEt in diabetic rats after 30 days indicates that the plant extract acts by stimulating glucose utilization by peripheral tissues. These results confirm the earlier findings.[15,16] Glycosylated hemoglobin was found to increase in patients with diabetes mellitus to about 16%[17] and the amount of this increase is directly proportional to the fasting blood glucose level,[18,19] we have observed a decrease in total hemoglobin during diabetes and this may be due to the increased formation of glycosylated hemoglobin. Our study also gave a clear view that LLFEt prevents a significant elevation in glycosylated hemoglobin level in diabetic rats that were fed daily for 30 days with LLFEt. We have also observed that LLFEt was administered to animals given Alloxan, the weight loss was reversed and the animals returned to near normal. The ability of the LLFEt to protect body weight loss seems to be due to its ability to reduce hyperglycemia. It is interesting to note that in glucose fed rats, the chloroform extract of *Leucas lavandulaefolia* (0.25 g/kg) effectively prevented increase in blood glucose levels without inducing a hypoglycemic

state. In conclusion, the chloroform extract of *Leucas lavandulaefolia* flowers, which was found to exhibit a hypoglycemic activity in Alloxan induced diabetic rat, was more effective than glybenclamide. Studies are in progress in our laboratory to elucidate in detail the mechanism of action of these drugs at the cellular and molecular levels. Flavonoids are known to have bioactive antidiabetic principles.[20] Flavonoid glycoside is assumed to be the compound responsible for the lowering of glucose.

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