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A COMPARATIVE ANTIDIABETIC AND HYPOGLYCAEMIC ACTIVITY OF THE CRUDE ALCOHOLIC EXTRACTS OF THE PLANT *LEUCAS ASPERA* AND SEEDS OF *PITHECELLOBIUM BIGEMINUM* IN RATS

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ABSTRACT

This study indicates that *Leucas aspera* and *Pithecellobium bigeminum* alcoholic extracts have good antidiabetic activity. Ethanolic extracts of *Leucas aspera* and *Pithecellobium bigeminum* at a dose level of 200 mg/kg/p.o exhibited significant anti-hyperglycemic activities in alloxan induced as well as streptazotocin induced hyperglycemic rats. They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels. The extracts of the plant *Leucas aspera* and *Pithecellobium bigeminum* were tested for oral hypoglycaemic and anti-diabetic activity, by glucose oral tolerance test, alloxan induced and streptozotocin induced diabetic rats respectively. The extracts of *Leucas aspera* and *Pithecellobium bigeminum* have shown significant ($P<0.001$) increase in glucose tolerance, the maximum effect was given by *Leucas aspera* extract. In alloxan-induced diabetic rats also the maximum percentage reduction in blood glucose levels was found to be in *Leucas aspera* ethanolic extract. Animals, which received STZ, also showed a significant reduction in body weight, and increase in water and food intake as compared to vehicle control, which is significantly reversed by ethanolic extracts of *Leucas aspera* and *Pithecellobium bigeminum* after 21 days of treatment. These results indicate that the plant *Leucas aspera* and *Pithecellobium bigeminum* possess significant anti – diabetic activity, but extract of *Leucas aspera* showed more activity then the extract of *Pithecellobium bigeminum*.

KEY WORDS: *Leucas aspera*, *Pithecellobium bigeminum*, hypoglycemicactivity, antidiabetic activity, streptozotocin, alloxan

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INTRODUCTION

Diabetes Mellitus is the name given to a group of disorders characterised by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action.[1] In the recent past many hypoglycaemic agents are introduced, still diabetes and related complications continue to be a major medical problem not only in developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes.[2,3]

Leucas aspera belonging to the family Labiatae is used as anti-inflammatory, stimulant, in jaundice, cough, asthma, conjunctivitis, diabetes, malaria, headache, otalgia, skin diseases, snake bite, toothache, and wound healing etc.[4] The seeds of kachlora or *Pithecellobium bigeminum* L (Family : Fabaceae) is a supplement and also used as antioxidant and used in hyperlipidemia and hyperglycemic condition[5]. The present study was undertaken to verify the claim and evaluate the anti-diabetic activity of the plant *Leucas aspera* and *Pithecellobium bigeminum*.

MATERIALS AND METHODS

Plant Material

The seeds of *Pithecellobium bigeminum* and plants of *Leucas aspera* were collected from the himalyan valley near Deharadun and local areas around the Mangalore, India, respectively and after authentification by botanist,

voucher specimens (NIMS/2010/NPB, NIMS/2010/NLA) are being maintained in the laboratory of Phytochemistry and Pharmacognosy, NIMS Institute of Pharmacy, Shobha Nagar, Jaipur, India. The seeds of *Pithecellobium bigeminum* and whole plant of *Leucas aspera* then, including root, stem, leaves and flower were shade dried and chopped into small pieces.

Preparation of extracts

The seeds of *Pithecellobium bigeminum* and shade dried plants of *Leucas aspera* were powdered (300g) separately and extracted with ethanol (99.99%), in two different soxhlet extractors exhaustively for 20-24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50^0 C) using flash evaporator.

Test animals

Male wistar albino rats (160 – 200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water. Institutional animal Ethics Committee's permission was obtained before starting the experiments on animal.

Effect of *Leucas aspera* extracts on oral glucose tolerance in rats

Male wistar albino rats (160 – 200 g), fasted for 16h before experimentation but allowed free access to water and were divided into 3 groups of 6 rats each. Group 1 control and received distilled water, Group II received ethanol extract of plant *Leucas aspera*, and *Pithecellobium bigeminum* ethanol extracts were given to group III at a dose of 200 mg/kg body weight as a fine aqueous suspension orally. All groups were given glucose (2 g/kg body weight, p.o) 30 min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30 and 90 min after the glucose loading. Serum was separated and glucose levels were measured immediately by glucose-oxidase method.[6]

Effect of the *Leucas aspera* extracts on alloxan-induced diabetic rats

Male wistar rats (180-200g) were made diabetic by a single i.p. injection of 120mg/kg body weight of alloxan monohydrate in sterile normal saline (Insulin dependent diabetes mellitus)[7], The rats were maintained on 5 % glucose solution for next 24h to prevent hypoglycaemia. Five days later blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes.

The diabetic rats were divided into four groups, each containing six animals. Controls rats (Group I) were given distilled water orally, *Leucas aspera* ethanol extracts were given to groups II, *Pithecellobium bigeminum* ethanol extracts were given to groups III, at a dose of 200 mg/kg, orally. Group IV received glibenclamide at dose of 10 mg/kg. Blood samples were collected from the tail vein just prior to and of 1h, 3h and 5h after drug administration.[8,9]

Effect of the *Leucas aspera* extracts on streptozotocin induced diabetes

Sprague Dawley rats were used, to induce Non-insulin dependent diabetes mellitus, a single dose of injection of streptozotocin (90 mg/kg: i.p.) was given to the 2 days old pups. Another group of pups received only saline. The animals were weaned at 30 days and after a period of 3 months; they were checked for fasting glucose levels to confirm the status of NIDDM. The animals showing fasting glucose levels > 140 mg/dl were considered as diabetic. The pups that received saline were considered as control animals.

The experimental animals were divided in five groups, six animals in each group. Group I vehicle control, Group II, and Group III NIDDM treated with *Leucas aspera* ethanolic extract and *Pithecellobium bigeminum* ethanolic extract 200 mg/kg respectively, and group IV treated with standard drug glibenclamide (10 mg/kg per day p.o), Treatment was given daily for 3 weeks. At the end of 3 weeks treatment, the animals were kept on 12 h fasting and the blood samples were collected from the tail vein. Serum samples were analysed spectrophotometrically for glucose, cholesterol and triglycerides. Body weight, Water intake (ml/rat/day) and food intake (grams/rat/day) were also recorded.[10,11]

Histopathological studies

For the histopathological studies, animals were sacrificed at the end of 3 weeks treatment; after the collection of blood samples, pancreas, liver and kidney were removed, washed with cold saline and preserved in 10% formalin in buffered form, processed and stained with hematoxylin and eosin.

Statistical analysis

The results are expressed as mean ± S.E.M. the significant of various treatments was calculated using students t-test.

RESULTS

The extracts of *Leucas aspera* and *Pithecellobium bigeminum* have shown significant ($P<0.001$) increase in glucose tolerance (**table 1**). Both the extracts reduced the glucose levels approximately near to normal within 90 minutes of the drug administration but the maximum

effect was given by ethanolic extract of the plant *Leucas aspera*.

In alloxan-induced diabetic rats also, both extracts have shown considerable reduction in blood glucose levels. The maximum percentage reduction in blood glucose levels was found to be in ethanolic extract of plant *Leucas aspera*. The results are graphically represented in **figure 1**.

Administration of the STZ (90 mg/kg: i.p.) led to significantly elevated levels of Serum glucose, cholesterol and triglycerides in experimental animals in comparison to vehicle control. Treatment with *Leucas aspera, Pithecellobium bigeminum* ethanolic extracts and standard drug has shown significant reduction in levels of these parameters as compared to diabetic control rats (**table 2**).

Animals, which received STZ, also showed a significant reduction in body weight, and increase in water and food intake as compared to vehicle control, which is significantly reversed by alcoholic extracts of *Leucas aspera* and *Pithecellobium bigeminum* after 21 days of treatment (**Table 3**).

DISCUSSION

This study indicates that *Leucas aspera* and *Pithecellobium bigeminum* ethanolic extracts exhibit significant anti-hyperglycemic activities in alloxan induced as well as streptazotocin induced hyperglycemic rats. They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels.

The number of functionally intact β-cells in the islet organ is of decisive importance for the development course and outcome of diabetes. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug.[12] In alloxan-induced diabetes, (-)Epicatechin[13] and Vinca rosea extracts[14] has also been shown to act by -cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic [15], ephedrine[16], and Gymnema sylvestre leaf extracts[17]. In the current studies, the damage of pancreas in streptazotocin-treated diabetic control rats and regeneration of β-cells by glibenclamide was observed. The comparable regeneration was also shown by ethanolic extracts of *Leucas aspera* and *Pithecellobium bigeminum*, but the prior one is more effective.

CONCLUSION

The data obtained from this study indicates that the ethanolic extracts of the plant *Leucas aspera* and *Pithecellobium bigeminum* are capable of exhibiting significant antihyperglycemic activities in diabetic rats. The extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas and so might be valuable in diabetes treatment.

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Table 1: Effect of *Leucas aspera* and *Pithecellobium bigeminum* extracts on oral glucose tolerance in rats

Gp	Treatment (dose/kg body weight)	Blood glucose (mg/dl)		
		Fasting	30 minutes	90 minutes
I	Glucose; 2g	77.25 ± 0.907	146.90 ± 1.76	114.71± 1.60
II	PBEE 200 mg + Glucose	76.91 ± 1.31	98.68± 0.78*	84.71±0.92*
III	LAEE 200 mg + Glucose	79.03±0.91	93.81±1.17*	81.52±1.02*

Values are means ± S.E.M., n = 6,

* P<0.001 vs group I, *L. aspera* and *P. bigeminum* extracts were given orally 30 min before glucose loading to their respective groups

PBEE: Ethanolic extract of seeds of *Pithecellobium bigeminum* and

LAEE: Ethanolic extract of plant *Leucas aspera*

Table 2: Effect of extracts of *Leucas aspera* and *Pithecellobium bigeminum* on serum profile in diabetic rats (STZ) after treatment of 21 days

Gp	Treatment	S. glucose	S. cholesterol	S. triglycerides
I	Vehicle control	74.3 ± 1.37	47.08 ± 2.2	46.83 ± 5.5
II	Diabetic untreated	192 ± 6.58*	79.33 ± 1.5*	122.83 ± 7.1*
III	Diabetic treated with PBEE 200 mg	119.7 ± 0.84**	59.61 ± 0.96**	65.33 ± 5.49**
IV	Diabetic treated with LAEE 200 mg	112.3 ± 4.17**	56.83 ± 2.9**	60.26 ± 7.9**
V	Diabetic treated with 10 mg/kg of glibenclamide	94.6 ± 2.13**	51.83 ± 2.1**	54.00 ± 6.1**

Each value is mean±SEM (n=6).

*significantly different from vehicle control (P < 0.05)

**significantly different from diabetic untreated (P < 0.05)

PBEE:- Ethanolic extract of seeds of Pithecellobium bigeminum and

LAEE :- Ethanolic extract of plant Leucas aspera

Table 3: Effect of various extracts of *L. aspera* and *P. bigeminum* in streptozotocin induced diabetic albino rats after 21 days of treatment

Gp	Treatment	Average Body weight (grams/rat)	Water intake (ml/rat/day)	Food intake (grams/rat/day)
I	Vehicle control	204.2 ± 3.01	37.91 ± 1.22	16.75 ± 0.45
II	Diabetic untreated	151.6 ± 3.84*	51.42 ± 1.51*	33.83 ± 0.71*
III	Diabetic treated with PBEE 200 mg	178.2 ± 2.76**	39.31 ± 0.62**	20.41 ± 0.62**
IV	Diabetic rats treated with LAEE 200 mg	182.3 ± 4.17**	41.15 ± 2.9**	21.34 ± 0.92**
VI	Diabetic rats treated with 10 mg/kg of glibenclamide	194.6 ± 2.13**	40.83 ± 2.1**	18.13 ± 1.31**

18. Each value is mean±SEM (n=6).
19. *significantly different from vehicle control (P < 0.05)
20. **significantly different from diabetic untreated (P < 0.05)
21. PBEE:- Ethanolic extract of seeds of Pithecellobium bigeminum and
22. LAEE :- Ethanolic extract of plant Leucas aspera

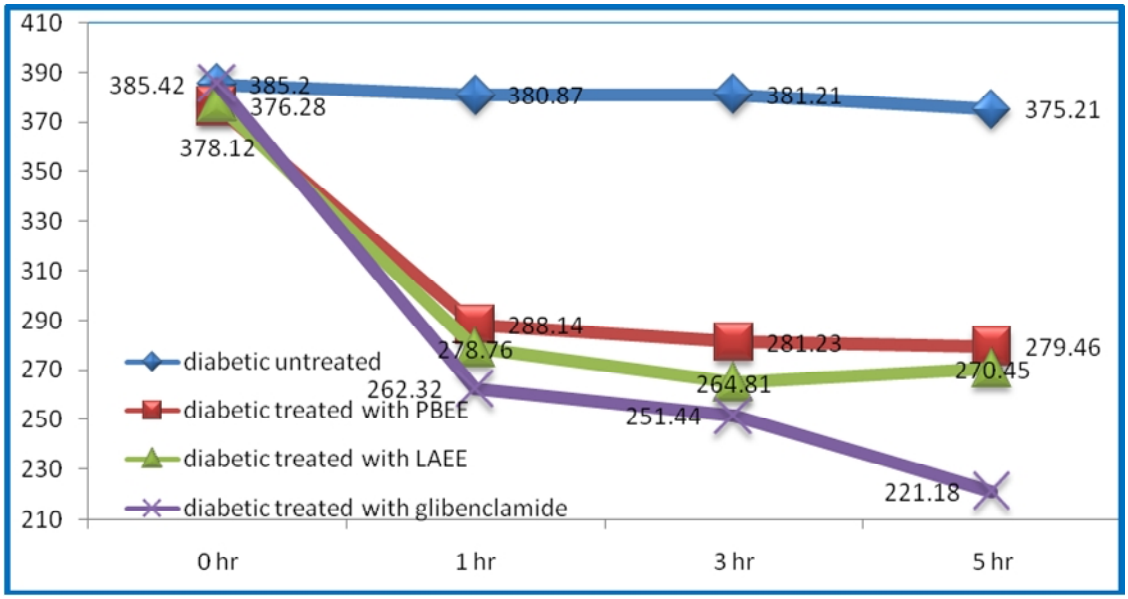
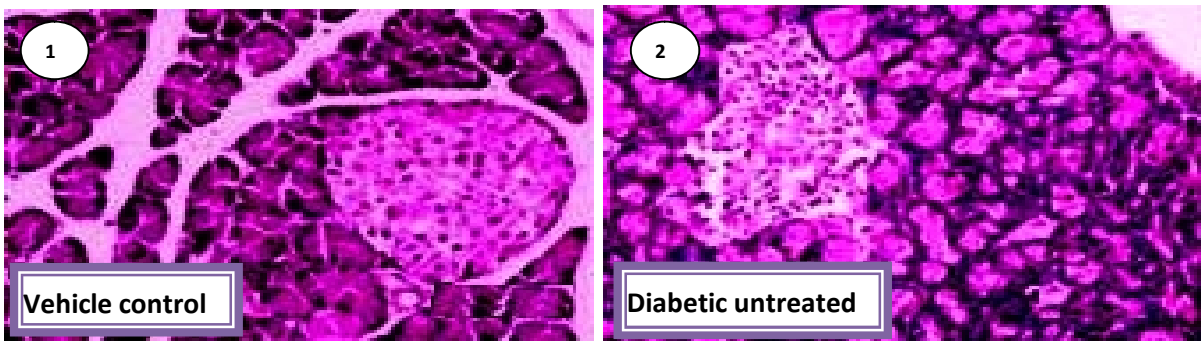
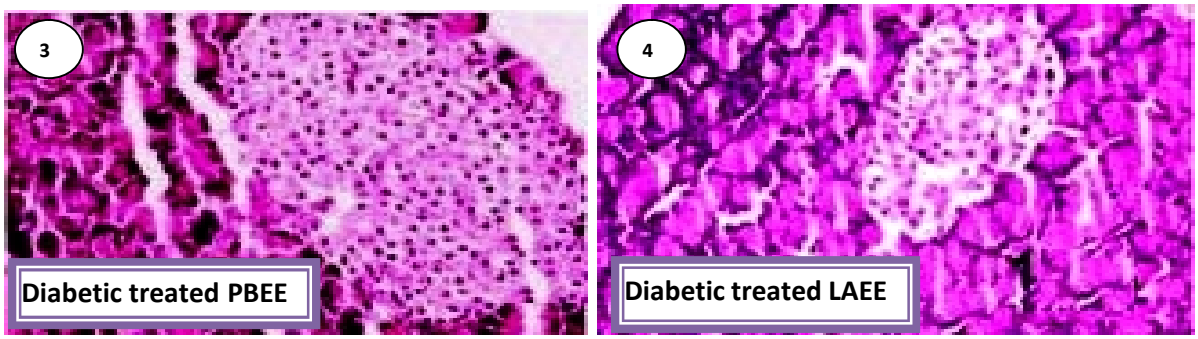


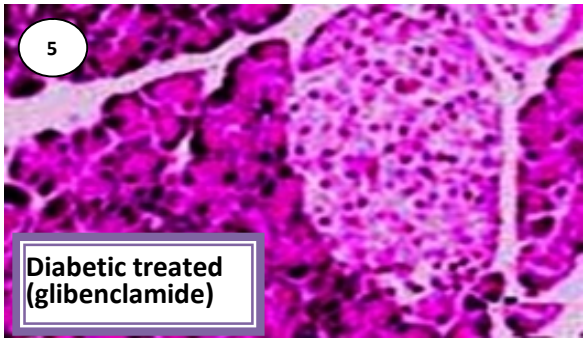
Figure 1: Effect of extracts of Leucas aspera and Pithecellobium bigeminum on blood glucose levels in Alloxan induced diabetic rats.
Each value is mean±SEM (n=6)
PBEE:- Ethanolic extract of seeds of Pithecellobium bigeminum and
LAEE :- Ethanolic extract of plant Leucas aspera



1. Vehicle control islets with normal round and structural intactness with their nucleus
2. Diabetic untreated rat's islets damaged and shrunken in size.



3. Diabetic rats treated with Pithecellobium bigeminum ethanolic extract orally at a dose of 200mg/kg, islets resemble normal rat islets but islets enlarged in size.
4. Diabetic rats treated with Leucas aspera ethanolic extract orally at a dose of 200mg/kg, islets resemble normal rat islets and glibenclamide treated group.



5. Diabetic rats treated with glibenclamide orally at a dose of 10 mg/kg, islets resemble vehicle control rat islets with normal round and structural intactness with their nucleus

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