

ANTIHYPERGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT OF *LEUCAS ASPERA* WILD WHOLE PLANT ON BLOOD GLUCOSE LEVELS OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Leucas aspera (Lamiaceae) is commonly known as Thumbai widely distributed in Africa, China, Malaysia and some places of India. Decoction of *Leucas aspera* Wild whole plant is used to alleviate the symptoms of Psoriasis, chronic skin eruption, painful swellings and diabetes in folk medicine. In the present study, the antihyperglycemic potential of *Leucas aspera* Wild was evaluated by using *in-vivo* methods in streptozotocin-induced diabetic rats. Preliminary phytochemical screening of the methanol extract of whole plant *Leucas aspera* Wild revealed the presence of steroids, triterpenes, alkaloids, carbohydrates, flavonoids. The acute toxicity studies of selected medicinal plant extract was performed on rats by using standard procedure. It was observed that there was no mortality with selected plant extract at 2000 mg/kg body weight dose. The antihyperglycemic activity of methanol extract of *Leucas aspera* Wild was investigated in streptozotocin-induced diabetic rats. The oral administration of the extract at the doses of 100, 200, and 400mg/kg b. w. The doses 100, 200, 400mg/kg b.w were showed significant decrease in (P<0.05) blood glucose levels. The dose of 400mg/kg b. w was more effective with the highest glycemic change of 34.45% at 8th hour of extract administered. The methanolic whole plant extract of *Leucas aspera* Wild possess antihyperglycemic activity in streptozotocin-induced diabetic rats.

Key words: *Leucas aspera* Wild, Antihyperglycemia, Streptozotocin, Diabetes.

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrate, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in *diabetes mellitus*. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems[1]. Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Although, herbal medicines have long been used effectively in treating diseases in Asian communities and through out the world. The mechanism of most of the herbals used has not been defined. Many traditional plants treatments for their beneficial effects is anecdotal[2]. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines. India is well known for its herbal wealth. Medicinal plants like *Trigonella foenum graecum*, *Allium sativum*, *Gymnema sylvestris* and *Syzgium cumini* have been studied for treatment of *diabetes mellitus*[3]. However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed.

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world[4]. Diabetes is one of the leading causes of death in humans and animals. In animals it occurs most frequently in the dog with an incidence of approximately 0.2%. In the indigenous Indian system of medicine good number of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated[5]. WHO (1980) has also recommended the evaluation of the effective of plants in conditions where there are no safe modern drugs[6]. The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential[7]. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research[8].

The plant *Leucas aspera* Wild, belongs to family Lamiaceae. *L.aspera* is distributed throughout India from Himalayas down. It grows as a weed on waste lands and road sides all over India and is widely distributed in and around forest areas of East Godavari District, Andhra Pradesh, India. It also found in Africa, Nepal, Pakistan, Sri Lanka, Malaysia, Myanmar and Thailand. The past

pharmacological work stated that the aerial parts of *Leucas aspera* Wild for antiinflammatory and hepatoprotective[9], roots for antinociceptive and cytotoxic activity[10] and whole plant for Prostaglandin inhibiting activity[11].

The present study was designed to test the antihyperglycemic effect of methanolic extract of whole plant *Leucas aspera* Wild in streptozotocin -Induced diabetic rats.

MATERIALS AND METHODS

Chemicals used

All chemicals and solvents used were of the analytical grade obtained from S.D Fine chemicals Pvt .Ltd. Mumbai and Loba chem Pvt. Ltd., Mumbai.

Blood glucose kit: Span diagnostics Ltd, Sachin 394230 (Surat) India.

Streptozotocin: Sigma Aldrich, St.Louis, USA.

Glibenclamide: Orchid chemicals and pharmaceuticals Pvt.Ltd, Chennai.

Plant Material

Whole plant of *Leucas aspera* Wild collected from the regions in and around forest areas of Maredimilli, East Godavari District, A.P (India). The plants were authenticated and certified by Dr. M.Venkaiah, Associate Professor, Department of Botany, Andhra University, Visakhapatnam. Voucher of plant was deposited in the department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam. The voucher number was TSN/DOP/LA/IND/2461.

Extract Preparation

The plant parts under investigation were coarsely powdered using a mechanical grinder, sieved(60-80 mesh size) and stored in a air tight container until further use . The dried powdered drug material was weighed and packed in a soxhlet apparatus. The powdered drug material was extracted with methanol in soxhlet apparatus for 72 hrs. The extract thus obtained was concentrated under vacuum in a rotary evaporator, dried completely and weighed.

Animals

Healthy adult albino rats of wistar strain weighing 150 to 200g were from Mahaveer Enterprises Pvt . Ltd., Hyderabad, India. The animal

house was well ventilated and animals had 12 hr dark/12hr light cycle with temperature between 25±2°C and relative humidity 50±15%. The animals were housed in a large spacious hygienic polypropylene cage during the course of the experimental period. The animals were fed with rat pellet feed supplied by Rayons biotechnologies Pvt. Ltd., India and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the animal Regulatory body of government (Regd.No.516/01/A/CPCSEA).

Phytochemical Screening

The methanol extract of *Leucas aspera* Wild were subjected to preliminary phytochemical screening, to identify the chemical constituents. The method of analysis employed were those described by brain and turner[12] (1975).

Acute Toxicity Study [13]

Toxicity studies conducted as per Internationally accepted protocol drawn under OECD guidelines in Wistar albino rats at a dose level of extract upto 2000mg/kg b.w.

In order to study any possible toxic effects of the selected plant *Leucas aspera* Wild, adult healthy rats weighing 150-200 gm were used. Rats were randomly divided into 4 groups of 6 animals each. The rats of group 1 are served as control. These animals received orally 1% CMC only. The animals of groups 2 to 4 received 2000mg/kg b.w of methanolic extract of *Leucas aspera* Wild whole plant. The number of animals which died within 7 days after a single dose was recorded. The animals were also closely examined for signs of intoxication, lethargy, behavioral modification and morbidity[14]. All the rats remain alive and did not show any visible symptoms of toxicity at this high dose. The treated rats showed no restlessness, respiratory distress, convulsions, coma, death, etc.

Induction of Diabetes [15,16,17]

Diabetes was induced by a single intraperitoneal dose of 60mg/kg of b.w of streptozotocin dissolved in 0.1M fresh cold buffer (pH 4.5) into 12 hr fasted rats. On the third day of the STZ injection of the rats, blood samples were taken from retro-orbital plexus of the rats for the estimation of blood glucose levels by using auto analyzer. Rats with *diabetes* having hyperglycemia were taken for the experiment. The diabetic rats were then randomly divided into different groups.

Experimental Design

In this experiment, 30 rats were used which were randomly divided into 05 groups of 6 animals each. The different doses of extracts were administered orally to the STZ induced diabetic rats. The extract was suspended in 1%CMC suspension. In these 05 groups, one group served as untreated control as they received orally 1% CMC suspension only and one group received standard drug Glibenclamide (3mg/kg b.w).

Group 1: vehicle control (1%CMC suspension)

Group 2: received extract of *L.aspera*(100mg/kg)

Group 3: received extract of *L.aspera* (200mg/kg)

Group 4: received extract of *L.aspera*(400mg/kg)

Group 5: standard (Glibenclamide 3mg/kg)

Determination of Blood Glucose Level

The blood samples were collected from the retro-orbital plexus of rats[18]. After collection of blood sample, the serum was separated by centrifuge at 3000rpm for 10 min as well as serum glucose level were estimated at 0,2,4,8,12,18,and 24 hr. The percent blood glucose reduction was also calculated for each group.

Statistical Analysis [19,20,21]

The values were expressed as mean±sem. The data was subjected to the analysis of variation (one way ANOVA) to determine the significance of changes followed by students "t"- test. The statistical significance of difference between two independent groups was calculated for the determination of blood glucose levels.

RESULTS AND DISCUSSION

Acute toxicity studies revealed no visible signs and symptoms of toxicity. None of the rats had died within 7 days, even at a high dose like 2000 mg/kg b.w.p.o

Table No 1 showed the results of phytochemical constituents of the crude methanolic extracts of whole plant of *Leucas aspera* Wild.

Table 1: Qualitative Chemical tests on extracts of *Leucas aspera*

Name of the test	Methanolic extract of <i>L.aspera</i>
Steroids	+
Triterpenes	+
Saponins	+
Alkaloids	-
Carbohydrates	-
Flavanoids	+
Tannins	+
Glycosides	-

+ Present -Absent

As shown in Table No.2, despite the results of the three doses (100, 200, and 400 mg/kg. b.w) of the extracts of *L.aspera* and Glibenclamide (3mg/kg.b.w) on streptozotocin-induced diabetes rats. The data obtained from the test carried out on STZ-induced diabetic rats clearly showed that the methanolic extract of *L.aspera* whole plant at dose levels of 100, 200 and 400 mg/kg b.w produced a significant reduction of blood glucose level. The reduction of blood glucose levels in diabetic rats was found to be dose dependent manner. Comparing the results of 100,200 and400 mg/kg b.w extracts of *L.aspera* whole plant in diabetic rats, it was found that the extract at 400 mg/kg b.w. showed highly significant (P<0.001) decrease in blood glucose levels when compared to control with percentage glycemic change of 34.45% in STZ-induced diabetic animals and was comparable with the standard Glibenclamide (3mg/kg.b.w).

Table 2: Effect of methanol extract of *Leucas aspera* on percent decrease in blood glucose levels in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours					
		2	4	8	12	18	24
1	Control	2.30±2.02	2.48±2.83	2.90±2.61	4.54±2.92	2.91±2.55	3.29±2.34
2	Glibenclamide (3 mg/kg b.w)	25.47±4.23**	42.41±2.94***	21.65±5.62*	13.81±3.41**	7.59±4.60	4.87±4.18
3	LS 100	6.34±4.36	11.98±4.97	18.63±4.55**	8.34±4.27	5.87±3.76	1.18±3.88
4	LS 200	8.32±1.82**	13.13±1.63***	14.41±3.92*	22.03±2.19***	15.82±6.52	16.05±7.42
5	LS 400	10.96±3.51*	18.01±1.87***	34.45±1.31***	15.33±4.75*	9.75±3.80*	7.22±3.30

N.S: No significant difference as compared to control (P>0.05); *: significant decrease as compared to control (P< 0.05); **: More significant decrease as compared to control (P<0.01); ***: Highly significant decrease as compared to control (P< 0.001).

The *L.aspera* whole plant seems to have a promising value for the development of potent phytomedicine for diabetes. Results obtained

from the present study are very much promising and comparable with Glibenclamide, a standard drug used to treat *diabetes mellitus*.

The reported antidiabetic activity of *Aloe vera* in streptozotocin induced diabetic rats[22] was mentioned that there are two possible explanations for this finding. First *A. vera* may exert its effect by preventing the death of β -cells and/or second, it may permit recovery of partially destroyed β -cells[22]. Herbal plants may produced hypoglycemic action in diabetic rats by possible through the insulinomimetic action[23]. Other probable mechanisms by which the plant drugs lowered blood glucose levels in diabetic rats may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver or inhibiting the absorption of glucose from the intestine and stimulation of glucose uptaken by peripheral tissue. The antihyperglycemic activity of the studied plant may be due to any of the above mechanism of actions. But further studies needs to carryout to establish their exact mechanism of action.

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