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HYPOGLYCEMIC EFFECTS OF *LUFFA ACUTANGULA* (L.) ROXB. FRUIT EXTRACT IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT: *Luffa acutangula* (L.) Roxb. (Family: Cucurbitaceae) has been used in the folkloric Indian medicinal system to treat numerous health conditions. The fruits of the plant have been used in dysentery, jaundice, diabetes, hemorrhoids, leprosy, and ringworm infection. The present study aimed to establish the hypoglycemic effect of the aqueous fruit extracts of *Luffa acutangula* was investigated in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. In normal rats, the aqueous extract of the fruit of *L. acutangula* (50 and 100 mg/kg/p.o.) significantly ($P < 0.001$) reduced the blood glucose levels from 65.2–49.4 and 68–48 mg% 2 h after oral administration of fruit extract and also significantly lowered the blood glucose in STZ diabetic rats from 67–101 and 64–89.2 mg% 21 days after daily oral administration of the extract ($P < 0.001$). The results suggested that the aqueous fruit extract of *L. acutangula* has a potential hypoglycemic effect in diabetic rats and authenticates the folkloric use of the plant for the management and treatment of diabetes.

INTRODUCTION: It has been projected that about 371 million people globally have diabetes, and the number is aggregating at a frightening rate, which is caused due to unhealthy lifestyles and emerging urbanization [1]. It is anticipated to be the most prevalent non-communicable disease by 20252.

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Additionally to short-range symptoms, there are long-term macro and microvascular difficulties, including cardiovascular disease, cerebrovascular disease, renal failure, and diabetic foot disease, which leads to gangrene, amputation, neuropathy, and blindness [3].

The available commercial drugs for diabetes mellitus are connected with numerous adversative impacts and, not being cheap, and often unaffordable to buy in developing and undeveloped nations [4]. Thus, the continuing search for potential alternative anti-diabetic drugs from plants, as recommended by the WHO, seems formerly rational [5].

Medicinal plants have often been used in India as well as in certain Asian and African countries that still rely solely on medicinal herbs as a key source of medication for the treatment of various illnesses [6]. Using experimental trials of diabetic rats, numerous hot studies have been attempted to validate hypoglycemic and anti-diabetic effects of various medicinal herbs conventionally used for the control of diabetes [7-13]. The normal fasting blood glucose of humans is 80-120 mg/dl, which may upsurge over 350 mg/dl in cases of diabetic individuals.

The normal functioning of the body organs can be affected, and the body functions for the regulation of homeostasis ensures long-lasting alterations due to the escalation of the glucose quantity in the blood. This has negative impacts on the tissues, which are dependent on insulin for glucose transport (namely adipose, liver and muscle) and independent on the brain, RBC, and renal tissues in different ways [14]. A variety of herbal preparations has been recommended in Ayurveda and other indigenous systems of medicine, which are useful in diabetes [15]. Various types of plant preparations, extracts, and individual compounds derived from these medicinal herbs have been identified to possess a broad spectrum of pharmacological effects on diabetes [16]. In our lab, the hypoglycemic activity of many of these bioactive compounds is experimentally well-documented [11, 17-20].

Luffa acutangula (L.) Roxb. is belonging to the family Cucurbitaceae, which has been used widely by diverse ethnic groups in India for treatment of various ailments such as jaundice, diabetes, bacterial and fungal infections, dysentery, headache, renal stone, splenitis, hemorrhoids, granular conjunctivitis, ringworm infection, laxative, and leprosy [21-23]. In addition, the fruit possesses demulcent and diuretic properties while the seeds have purgative, diuretic, emetic, and anthelmintic properties [24]. Various phytochemical studies have been studied and identified about 50 compounds, *viz.*, flavonoids, anthraquinones, alkaloids, proteins, saponins, triterpene, volatile components, and other phytoconstituents [24-26]. The extracts from different parts of the plant demonstrated potent hepatoprotective, anti-diabetic, anti-hyperlipidemic, anticancer, anti-bacterial, CNS depressant, immunomodulatory and antiulcer activity [27-31]. The number of persons with type 2 diabetes mellitus is aggregating with a rate of three new cases every ten seconds, and it is being diagnosed and treating at an earlier age of the adolescents [13]. The administration of exogenous insulin is extensively acknowledged as the best choice of medication for diabetic therapy. Still, the connected mission and their impact, especially on insulin resistance individuals in DM, are poor. Hence, the present study was planned to investigate the fruit of *L. acutangula* on blood glucose levels of glucose-fed hyperglycemic, STZ induced diabetic and normal rats and to compare it with glibenclamide as a reference standard.

MATERIALS AND METHODS:

Plant Material: The fruit of *Luffa acutangula* (L) Roxb was collected from Erumapatti, Namakkal District, Tamil Nadu, India, and authenticated by a taxonomist, and the Voucher Herbarium specimens were deposited in the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirapalli, Tamil Nadu, India for future references.

Animals: The study was carried out on either sex of Wistar albino rats (160-200g). The rats were bred in Animal house, Muthayammal Arts and Science College, Namakkal was used. They were acclimatized for a week at laboratory conditions and provided a standard pellet diet and water *ad libitum*. Animal studies were carried out based on the guidance of the National Institute of Health guide [32].

The normal and diabetic rats were divided into 5 groups of 6 animals each. Group I served as vehicle alone, control. Group II received streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO, USA (60 mg/kg/i.p) dissolved in 0.1M-citrate buffer. Group III and Group IV received the aqueous extract of fruit (50 mg, 100 mg/kg/p.o) suspended in a vehicle followed by single intraperitoneal administration of STZ. Group V received glibenclamide (600 µg/kg/p.o) followed by a single intraperitoneal administration of STZ. Blood samples were collected at 30 m, 60 m, and 90m for the estimation of blood glucose, and the experiment was conducted further to study the effect of fruit extract on plasma antioxidant status in STZ diabetic rats.

Preparation of Fruit Extract: Dried raw deseeded fruits of *L. acutangula* (1 Kg) were peeled, washed, cut into small pieces, and homogenized in a warring blender with 2 liters of distilled water. The extraction was carried out in a cold room (20 ± 1 °C) with constant stirring overnight. The homogenate was filtered using cheesecloth and spinned at 3000 rpm at 4 °C for 15 min. The supernatant being the *L. acutangula* fruit extract (yield 21% w/w) was collected and kept at 4 °C until use.

Induction of Diabetes: Rats were prepared diabetic by the administration of a single dose of STZ (60 mg/kg/i.p), which dissolved in citrate buffer (0.1 M, pH 4.5). Forty-eight hours later, blood samples were obtained, and glucose levels were analyzed to endorse the development of diabetes. Only those animals, which exhibited high glucose levels> 260 mg/dl) were utilized in further study.

Experiment Design:

Evaluation of the Hypoglycemic Effects of Fruit Extracts of *L. acutangulain* Normal Rats: The hypoglycemic effect was calculated by oral gavage of fruit extract in 24 normal rats, earlier fasted for at least 12 h and randomly allocated into four groups: (n = 6 per group). Group I: administered distilled water and served as a control group.

Group II: administered with aqueous extract of the fruit of *L. acutangula* at a dose of 50 mg/kg/p.o. Group III: administered with aqueous extract of the fruit of L. acutangula at a dose of 100 mg/kg/p.o. Group IV: administered with glibenclamide at a dose of 600 µg/kg, and functioned as a reference control. The blood samples were obtained by tail pinching before the treatment of the glucose (10 g/kg) and at 30, 60 and 120 m later.

Evaluation of Fruit Extracts of *L. acutangula* on Glucose Tolerance: Subsequent overnight fasting, the animals were provided the standard reference control and fruit extracts orally, and after 30 min, glucose (10 g/kg/p.o.) was given. Blood samples were obtained before the treatment of the glucose and at 30, 60, and 120 m after.

Assessment of Fruit Extracts of *L. acutangula* on STZ Diabetic Rats: The diabetic rats were allocated into 5 groups of 6 rats each. Group, I served as the control, received vehicle alone. Group II received STZ (60 mg/kg/i.p.). Groups III and IV received the aqueous fruit extract of L. *acutangula* (50 and 100 mg/kg/p.o.) suspended in a vehicle followed by a single treatment of STZ (60 mg/kg/i.p.). Group V received glibenclamide (600 µg/kg/p.o.) followed by treatment of STZ (60 mg/kg/i.p.). Blood samples were collected at weekly intervals until the completion of the investigation (*i.e.,* 3 weeks). Blood glucose and oral glucose tolerance test were quantified by the glucose oxidase method [33] and oral glucose tolerance test [34,] respectively.

Acute Toxicity Study on Fruit Extracts of *L. acutangula*: A toxicity study was conducted on the aqueous fruit extracts of *L. acutangula* in animals based on the following ordered doses (12.5, 25, 50, 100200, and 400 mg/kg b.w.). Besides, the general rat behavior was monitored constantly at least for 2 weeks and analyzed any ensuing death of the animals.

Using those fixed doses, the aqueous fruit extracts did not display any death or any notable signs of toxicity and/or any significant alterations in normal rat behavior.

Statistical Analysis: Values were presented as means ± SD. Data were analyzed using analysis of variance (ANOVA), and group means were compared with Duncan's multiple range test (DMRT) using Statistical Package for Social Science.

RESULTS:

Evaluation of the Acute hypoglycemic Activity of *L. acutangula*: The effect of fruit extract of *L. acutangula* on fasting blood sugar was determined in normal rats, as shown in **Fig. 1A**. The percentage reduction in glucose levels after 2 h in the *L. acutangula* (50 and 100 mg/kg/p.o.) treated groups were 24.8 and 31.2%, respectively.

Glibenclamide caused a significant ($P < 0.001$) reduction of 26.8% in glucose levels 2 h after its administration, while control rats did not exhibit any significant alteration in their glucose levels through the duration of the experiment. The hypoglycemic effect of aqueous fruit extract of *L. acutangula* (100 mg/kg/p.o.) was higher than that seen in the glibenclamide treated rats.

Evaluation of *L. acutangula* Fruit Extracts on Glucose Tolerance: Results of the glucose tolerance test conducted on normal rats fed with aqueous fruit extract of *L. acutangula* (50 and be 15.68% and 20.74%, respectively, after 1 h of oral administration. However, after 2 h BGL rises slightly as compared to that of 2 h. 100 mg/kg) are shown in **Fig. 1B**. Thirty minutes after feeding glucose, the blood sugar rose by 143.5% in normal controls, while the same rise was only 128.4% in rats treated with glibenclamide. Administration of aqueous fruit extract of *L. acutangula* produced a maximum dose-dependent reduction in blood sugar at 60 and 120 m in comparison to normal controls (16.4; 20.3% and 21.5; 29.5%). The effect was less noticeable at 30 m as it was representing a late onset of effect. The percentage fall in glibenclamide treated rats as compared to normal controls at 60 and 120 m was 16.4 and 26.5%, respectively.

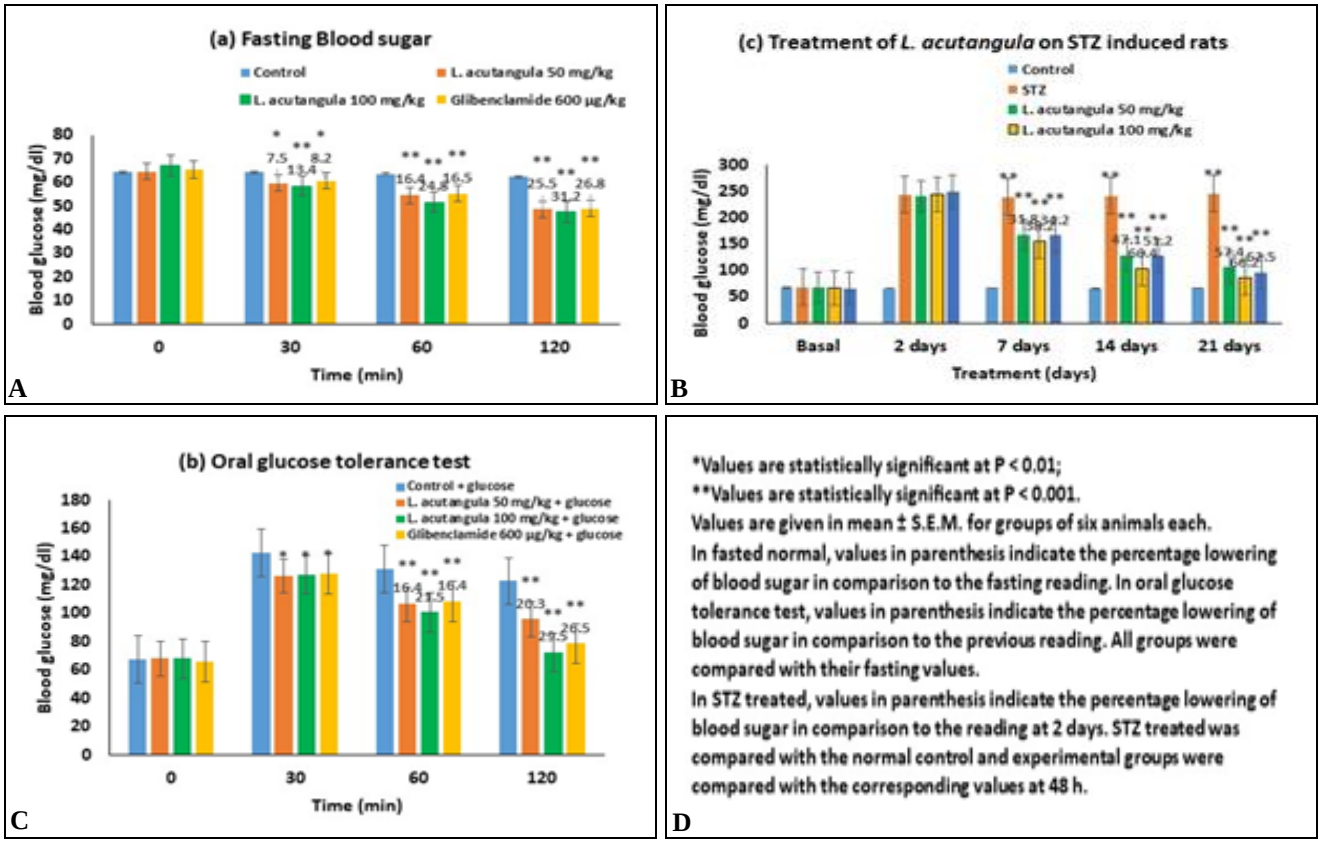

FIG. 1: EVALUATION OF *L. ACUTANGULA* FRUIT EXTRACTS (A) FASTING BLOOD SUGAR (B) ORAL GLUCOSE TOLERANCE TEST (C) ON STZ INDUCED DIABETIC RATS

Effect on STZ Induced Diabetic Rats: The effect of aqueous bark extract of *L. acutangula* in STZ induced diabetes in rats is given in **Fig. 1C**. Treatment of STZ (60 mg/kg/i.p.) often provided a three-fold elevation of blood glucose levels that were kept throughout the study period.

After 3 weeks of daily treatment with aqueous bark extract of *L. acutangula* (50 and 100 mg/kg), there was a dose-dependent fall in blood sugars by 57.4 and 66.2%, respectively.

Glibenclamide caused a significant ($P < 0.001$) reduction of 62.5% in plasma glucose levels 3 weeks after its oral treatment.

DISCUSSION: Since STZ, induced diabetic model and destroying β-cells as well as impairing renal function have been well documented [35-37]. The present study of the fruit extracts of *L. acutangula* and glibenclamide exhibited significant hypo-glycemic effects in the STZ diabetic rats. In the absence of plasma insulin concentration, blood glucose levels have been considerably reduced, which suggests that the *L. acutangula* administration may involve an insulin-dependent mechanism.

The underlying mechanisms behind the hypoglycemic effects of fruit extract of *L. acutangula* include an extra-pancreatic action

feasibly through triggering glucose utilization in peripheral tissues [38] or an elevation of glycolytic/ glycogenic enzymes activity in peripheral tissues [39, 40] or reduce the synthesis of the counter-regulatory hormones such as glucagon, cortisol and growth hormones [41-45]. Additional studies are mandatory to confirm this underlying mechanism. In addition, lowering blood glucose was noticed in the treatment of the aqueous fruit extract of *L. acutangula* in STZ diabetic rats and fasted normal rats. This outcome might be due to the inhibition of the reabsorption mechanism for glucose in the renal tubules, if any, that can also influence to reduce the glucose levels in the blood46.

From the above results, we noticed that the aqueous fruit extract of *L. acutangula* made a noteworthy reduction of blood glucose levels, and this impact was often found in repeated treatment than a single dose of administration. These outcomes generally propose that the reduction of blood glucose levels is due to the presence of bioactive compounds in the fruit, possibly, on the accumulation of active principles in the blood or tissues [47].

From the fruits of *L. acutangula*, various bioactive compounds such as flavonoids, anthraquinones, alkaloids, proteins, saponins, triterpene, volatile components, fibers, and other phytoconstituents have been identified and characterized [24-26]. It is interesting to note that in many medicinal herbs, flavonoids have been reported to exhibit hypoglycemic effects [48-52]. Nevertheless, the possible mechanism of flavonoids as the hypo-glycemic agent can only be recognized after the validation of pharmacological trials. The LD50 value of the aqueous fruit extract of *L. acutangula* (4 g/kg) [53, 54] was greater than the treatment effective dose. We can recommend, thus, that aqueous fruit extract of *L. acutangula* has low acute toxicity and may be considered comparatively toxic-free effects.

CONCLUSION: Based on the present study, we conclude that *L. acutangula* demonstrates as a promising plant concerning its hypoglycemic effect and may be prescribed as an adjunct to dietary therapy and drug treatment for controlling diabetes. A further complete pharmacological investigation is required to elucidate the exact mechanism of action of this extract.

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CONFLICTS OF INTEREST: The authors declare that there is no conflicts of interest

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