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STUDIES ON THE METHANOL EXTRACT OF *LEPIDAGATHIS FASCICULATA* AND ITS FRACTIONS FOR THE TREATMENT OF DIABETES AND FOR *IN-VITRO* ENZYME INHIBITION

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Keywords:

α-amylase, α-glucosidase, *In-vitro*, Antidiabetic, *Lepidagathis fasciculata*

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ABSTRACT: The present research focused to decide the premier dosage of methanol extract of *Lepidagathis fasciculata* whole plant extract and examining the α-amylase and α-glucosidase inhibitors of diabetic rats utilized to execute stronger command of hyperglycemia allowed for the blood glucose to be lowered in these animals. In streptozotocin-induced rats, the anti-hyperglycemic effects of oral administration of various doses of methanol extract revealed that the highest dose considerably lowered blood glucose levels analyzed to further dosage. Additionally, ethyl acetate and n-butanol fractions showed a better anti hyperglycaemic impact than n-hexane and chloroform fractions, according to the results of the repeated administration of methanol fractions. In addition, the *in-vitro* studies results demonstrated that the consequences of methanol extract and its fraction onα-amylase and α-glucosidase enzyme exercises could be controlled with low concentrations of ethyl acetate and n-butanol measured to different fractions and Acarbose, which was utilized as a positive control. These results suggested that the presence of polyphenolic active ingredients allowed the mobile coarse extract (methanol) and its vital fractions (ethyl acetate and n-butanol) to significantly lower blood sugar levels. The discovery of novel drugs for the treatment of diabetes and its consequences may be facilitated by the separation of the active components of *Lepidagathis fasciculata*.

INTRODUCTION: The International Diabetic Federation projects that there will be 643 million individuals worldwide have diabetes by 2030 and 784 million by 2045. In the world's population, a person is diagnosed with diabetes every five seconds and 10 people die from it. Therefore, diabetes is constricting the scope of the biological cosmos [4].

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Despite the fact that there are many different types of anti-diabetic medications available, it is still required to look for novel therapeutic agents due to the negative side effects of current medications and the lessened response that results from their continued usage [7, 10].

Numerous plants from various parts of the world have been researched for their potential to treat diabetes [8]. Recently, certain medicinal plants have been utilised ephemerally as antidiabetic therapies and notified to be beneficial for diabetes across the globe [13]. The plants with antihyperglycemic benefits are related to their capacity to improve pancreatic role by enhancing insulin excretion, reducing gut glucose uptake, or facilitating

metabolites in insulin-dependent process [21]. Additionally, Kate *et al.,* 2018 revealed that the liver enzymes glucose-6-phosphatase, glycogen synthase, glycogen phosphorylase, glucose-6-phosphate dehydrogenase and phospho-fructokinase were all more active after receiving treatment with medicinal herbs. *Lepidagathis fasciculata* nees belongs to the Acanthaceae family. *Lepidagathis fasciculata* is commonly known as Striped *Lepidagathis* and this herb has historically been used to cure various skin conditions such as eczema, psoriasis, epilepsy, burns, mouth ulcers, wounds and itching [18].

The goal of the current study is fractionation of the methanol extract is to identify the most potent fraction, paving the way for chemical characterization and standardization. This discovery will show which substances are in charge of the antidiabetic activity. The results of the *Lepidagathis fasciculata* extract and its fractions on alpha glucosidase and alpha amylase inhibitors have not been extensively examined in earlier papers, despite the plant's significance in the treatment of diabetes. Therefore, the current work examined *Lepidagathis fasciculata* extract and fractions for *in-vitro* inhibition of both α-glucosidase and α-amylase.

MATERIALS AND METHODS:

Plant Materials and Sample Preparation: The fresh sample of *Lepidagathis fasciculata* was collected from Kamalapuram RF, Manuguru Mandal, Bhadradri Kothagudem district. The voucher specimen (KUW4562) was preserved in recognized Kakatiya University Herbarium. *Lepidagathis fasciculata* whole plant were powdered, dried and stored in an airtight container after passing through sieve number 60 and was kept in vacuum sealed bag at 4^{o}C prior to the investigation procedure.

Extraction Procedure: The extraction process was managed by maceration method (40^{o}C-60^{o}C) with about 200 g of specimen with solvents, i.e., methanol (ME), and water (WE) for 7 successive days. The resulting extracts were concentrated under vacuum using rotary evaporation at decreased pressure and 30°C after being filtered using Whatman paper No. 1. In a china plate, the concentrated extracts (ME, WE) were gathered and dried [13]. Prior to usage, in order to dissolve all of the extracts, normal saline was first mixed with 5% Tween 80.

Fractions Preparation: 25 g of the most active extract (ME) were fractionated using the liquid-liquid extraction method with n-hexane, chloroform, ethyl acetate, n-butanol, and water utilising anisolating vent and then evaporated under reduced pressure by rotary evaporation at 30°C–40°C. In a china plate, the concentrated fractions (nH, Cl, EA, nB, and WF) were gathered and dried[5]. Prior to usage, all fractions were dissolved in normal saline solution containing 5% Tween 80.

Experimental Animal and Induction of Diabetes: Animal experiments have been implemented in compatible with the guidelines established by the Indian governments Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Wistar albino male rats weighing 250-300 g purchased from Vyas labs in Hyderabad with earlier acceptance from our institutional animal ethical committee (IAEC/08/UCPSc / KU / 2020). The rats were housed in ordinary cages made of polypropylene: 22^{o}C± 3^{o}C and a 12-hour light-and-dark cycle. The rats were fed a processed food to eat and were given unlimited access to water.

After the rats had fasted for the previous night, diabetes was induced by intraperitoneally injecting 45 mg/kg b.w. of STZ reconstituted in 0.1 mol/L cold citrate buffer (pH 4.5) [16]. The glucose level was tested three days after streptozotocin (STZ) injection. Rats were weighed and given the treatment doses (45 mg/kg of STZ and 10 mg/kg of Glipizide) orally using a 16G oral needle. The rats were weighed daily to calculate the volume of sample required for dispensation. The extraction (100, 200, and 400 mg/kg b.w.) was newly blended in 10 mL of distilled water with 5% of Tween 80 for oral distribution.

Diabetic rats are those who's a fasting blood glucose level above 15mmol/L is considered high. Before the rats were separated into groups, measurements of blood sugar levels and body weight were taken. In order to properly label every rat group (n = 6) for the upcoming experiment, the blood sugar levels and body weight of individual

batch were taken into consideration when designing and placing them in a single cage [3]. Added label for the control group on the sign. Six rats are used in each group for each test, which includes five diabetic induction groups (30 rats) administered with raw extracts, one normal saline administration group serving as the negative control (n = 6) and one glipizide administration group serving as the positive group.

Blood Sample Collection: After cutting a small cut in the tail, the blood was collected. A small amount of blood was restrained out and utilized to measure the blood sugar using a glucometer. In this study, rats with fasting blood glucose levels more than 250 mg/dL were considered diabetic.

Analyzing the Anti-diabetic Effects of Various Concentrations of Methanol Extract from *Lepidagathis fasciculate*: The wistar albino male rats from each group of six were randomly assigned to receive the following treatment (oral administration) for 28 days:

Group 1: Normalcontrol (10 mL/kg normal saline).

Group 2: Glipizide 10 mg/kg (standard drug)

Group 3: Methanol extract 400 mg/kg.

Group 4: Methanol extract 200 mg/kg.

Analyzing the Antidiabetic Properties of Methanol Fractions of *Lepidagathis fasciculate:* The male wistar albino rats were given the following treatment (oral administration) for 28 days after being randomly divided into groups of six rats each:

Group 1: Normal control (10 mL/kg normal saline).

Group 2: Diabetic animals treated with 10 mg/kg Glipizide.

Group 3: n-hexane fraction of (MELF) 200 mg/kg.

Group 4: Chloroform fraction of (MELF) 200 mg/kg.

Group 5: Ethyl acetate fraction of (MELF) 200 mg/kg.

Group 6: n-butanol fraction of (MELF) 200 mg/kg.

During the experiment, the rats fasting blood sugar values and changes in body mass from the first day to the last 28 days were recorded.

***In-vitro* α- amylase Inhibition Assay:** With a small modification, the *in-vitro* α-amylase inhibition experiment was carried out using the Pradeep *et al.*, 2013 method. Calculating the inhibitory activity as follows:

% Inhibition = (Absorbance of control - Absorbance of compound) / Absorbance of control × 100

***In-vitro* α-glucosidase Inhibition Assay:** With some modifications, the *in-vitro* α-glucosidase inhibition experiment was carried out using the Pradeep *et al.*, 2013 method. The following formula was used to compute the inhibition percentage for –glucosidase.

% Inhibition = (Absorbance of control – absorbance of sample) / Absorbance of control × 100

Statistical Analysis: Values with the standard error of the mean (SEM) included. An ANOVA with one factor was used for the statistical analysis. P-values below 0.05 indicated significance.

RESULTS AND DISCUSSION:

Effect of Various Dosage of Methanol Extract on the Body Weight and Blood Glucose Levels of STZ-Induced Diabetic Rats: Fig. 1 exhibits a decline in blood sugar levels on days 7, 14, 21 and 28 following the daily administration of various dosages of methanol extracts.

All provided doses, however, demonstrated a considerable reduction in blood glucose levels, with doses of 400 mg/kg demonstrating the greatest deduction and indicating roughly the same strength as Glipizide.

The 28-day study of various doses reveals that all groups body weights steadily grew, although there was no statistically significant difference from the control group. **Fig. 2** However, the body weight significantly increased after 28 days of 400 mg/kg dosages.

While anorexia can occasionally result from loss of body weight in rats, regular increases in body weight in rats over the course of the 28 days show that the varied extract doses have not caused anorexia. These findings are consistent with earlier publications.

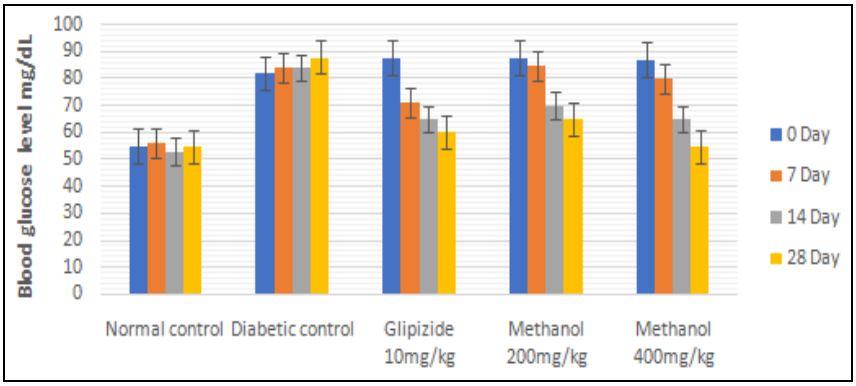

FIG. 1: EFFECT ON BLOOD GLUCOSE LEVELS IN STZ-INDUCED DIABETIC RATS AT DIFFERENT DOSAGES OF ACTIVE CRUDE EXTRACT AND GLIPIZIDE (10 mg/kg). Statistically significant *p<0.05, **p<0.01 when in contrast to the control group, values are averages of n=6± SEM.

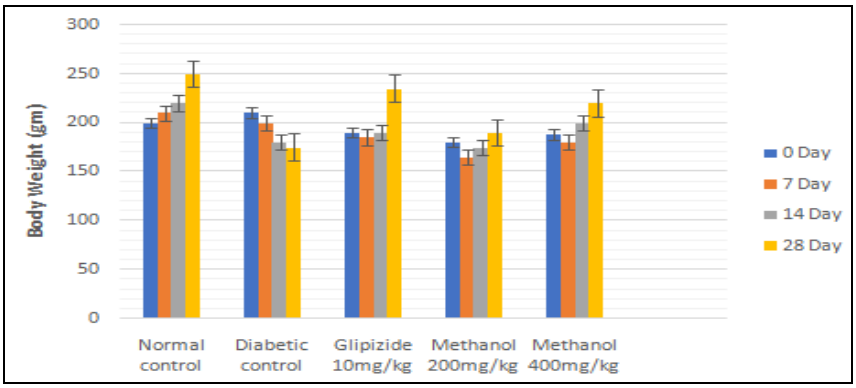

FIG. 2: EFFECTS OF TAKING ACTIVE CRUDE EXTRACT ORALLYAT VARIOUS DOSES AND GLIPIZIDE (10 MG/KG) ON THE BODY WEIGHT (GRAMS) OF DIABETIC RATS CAUSED BY STZ. Statistically significant *p<0.05, **p<0.01 when in contrast to the control group, values are averages of n=6 ±SEM.

The Impact of Various Doses of Methanolic Fractions on the Body Weight and Blood Glucose Levels of STZ-Induced Diabetic Rats: Fig. 3 demonstrates that on the 28th day after oral administration of the methanol fractions, STZ-induced rats blood glucose levels reduced by 200 mg/kg. However, the n-butanol fraction of the methanol fractions exhibited roughly the same level of normal blood glucose levels and showed a considerable drop in blood glucose levels up to 28 days differentiated to the control group. N-hexane, chloroform and ethyl acetate fractions did not differ significantly from the control group, despite lower blood sugar levels in the 14th and 28th days after medication. Oral portion delivery during the 28th period resulted in a modest increase in body weight, although it was not statistically different from the control group. **Fig. 4** On the 28th day, however, the ethyl acetate fraction significantly increased as compared to the control group.

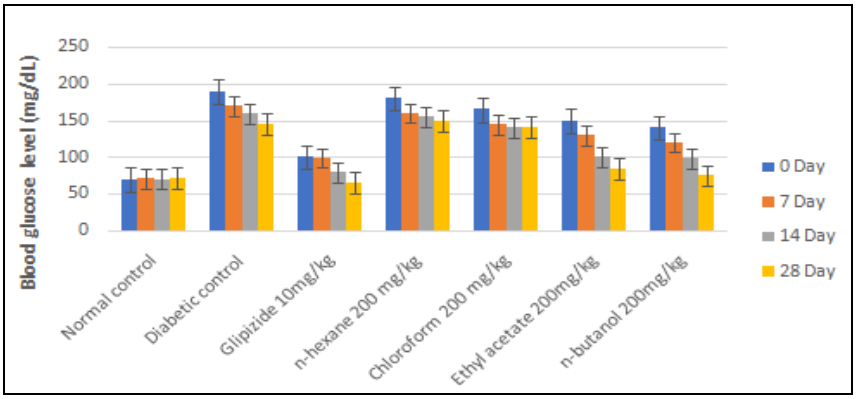

FIG. 3: EFFECT OF FRACTIONS, GLIPIZIDE (10 MG/KG) ADMINISTERED ORALLY EVERY DAY ON THE BLOOD GLUCOSE LEVELS OF STZ-INDUCED DIABETIC RATS. Statistically significant *p<0.05, **p<0.01 when compared to the control group, values are averages of n=6 ±SEM.

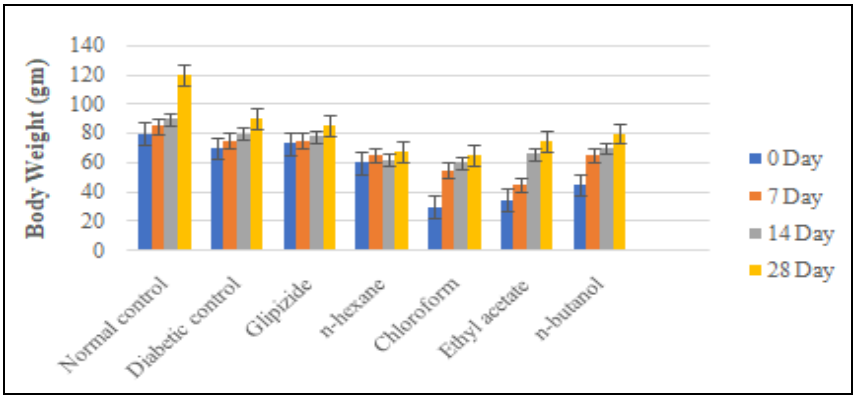

FIG. 4: EFFECTS OF FRACTIONS OF GLIPIZIDE (10 MG/KG) GIVEN ORALLY EVERY DAY TO DIABETIC RATS INDUCED BY STZ. Statistically significant *p<0.05, **p<0.01 when compared to the control group, values are averages of n=6± SEM.

***In-vitro* α-amylase Inhibition Activity:** *Lepidagathis fasciculata* whole plant methanolic extracts were separated, and the alpha amylase inhibitory activity of each fraction was assessed. In a concentration-dependent way, the amylase enzymes were all blocked from all test parts. The increased% inhibition on alpha amylase was reportedly found at 500 µg/ml, according to nBLF and MELF. The results show that additional test fractions show positive alpha amylase enzyme inhibitory effects at concentrations as low as 50 µg/ml, with a peak reaction seen at 500 µg/ml. Although the test fractions had favorable alpha amylase inhibitory effects, these effects were initially found to be less significant than those of the widely used inhibitor Acarbose.

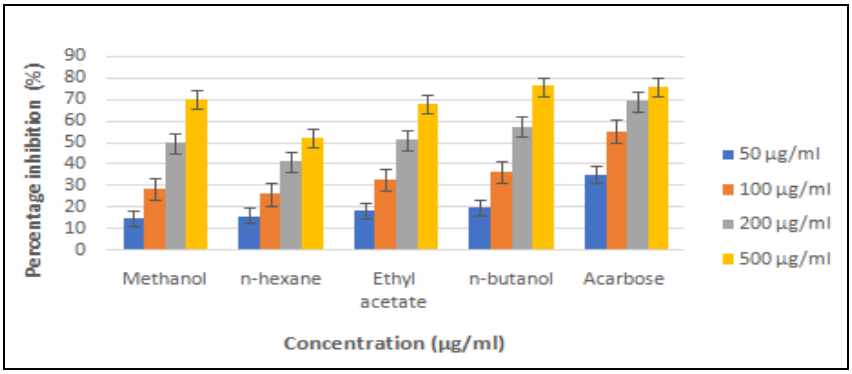

FIG. 5: PERCENTAGE OF THE WHOLE PLANT EXTRACT OF *LEPIDAGATHIS FASCICULATA* AND ITS FRACTIONS THAT BLOCK WHEAT ALPHA AMYLASE, AS WELL AS THE STANDARD ALPHA AMYLASE INHIBITOR ACARBOSE (RESULTS ARE REPORTED AS MEAN SEM, N=3).

***In-vitro* Alpha Glucosidase Inhibitory Activity:** The breakdown of carbohydrates in diet into monosaccharides, which are easily absorbed by GIT, is caused by the alpha glucosidase enzymes.

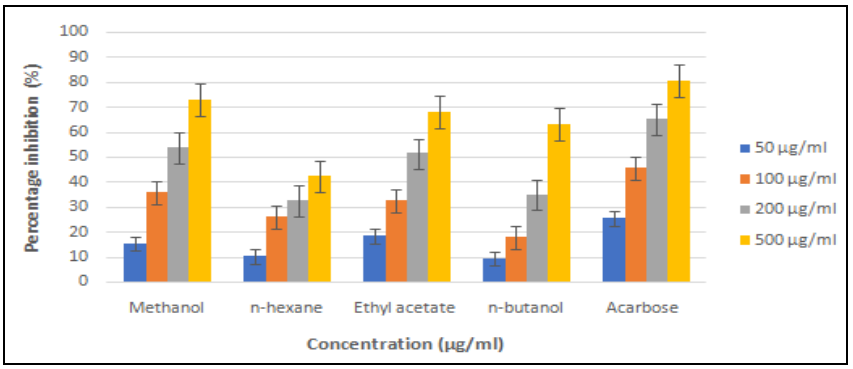

FIG. 7: PERCENTAGE OF THE *LEPIDAGATHIS FASCICULATA* ENTIRE PLANT'S METHANOL EXTRACT AND ITS FRACTIONS THAT BLOCK THE YEAST ALPHA GLUCOSIDASE ENZYME IN COMPARISON TO THE REFERENCE ALPHA GLUCOSIDASE INHIBITOR, ACARBOSE (RESULTS ARE REPORTED AS MEAN SEM, N=3).

Therefore, inhibiting this enzyme may lead to less glucose absorption from carbohydrates in the gut. The MELF caused alpha glucosidase to be inhibited in a concentration-dependent manner. The concentrations of the fractions under analysis, which ranged from 50 to 500µg/ml, resulted in a noticeable increase in alpha glucosidase activators.

CONCLUSION: These findings demonstrate that the polyphenolic active components in the vital extract of methanol crude and its active fractions (ethyl acetate and n-butanol) significantly lower glucose levels. In summary, the current finding that *Lepidagathis fasciculata's* active ingredients have been isolated may open the door to the creation of novel drugs to treat diabetes and its consequences.

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