

Original Research Article

Antidiabetic activity of methanolic leaf extract of *Trachyspermumammi* in rodents

ABSTRACT

Background: The annual and aromatic plant known as *Trachyspermumammi* L. Sprague belongs to the family Apiaceae. The *in vivo* antidiabetic efficacy of crude extract extracted from *Trachyspermumammi* leaves was assessed in the current study.

Study design: Experimental Design.

Place and duration of study: Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, India.

Methods: Maceration technique was used to extract META and preliminary phytochemical analysis was performed. Acute toxicity study was done in the From Acute toxicity studies 2000 mg/kg bd.wt., was found to be safe. The results of antidiabetic activity of META by OGTT and STZ-NIC induced diabetes model showed that the META has significant antidiabetic activity.

Conclusion: The results of this investigation suggest that *Trachyspermumammi* leaf extract has considerable antidiabetic effects. However, need further work to elucidate the mechanism of action.

Keywords: *Trachyspermumammi*, Antidiabetic activity, Oral Glucose Tolerance Test, Streptozotocin-Nicotinamide.

1. INTRODUCTION

A major concern to global public health is rising the prevalence of diabetes. The most recent survey conducted by the International Diabetes Federation estimates that there are more than 400 million diabetics globally [1]. The term "Diabetes Mellitus" (DM) refers to a group of disorders where there is a loss in carbohydrate metabolism and an upsurge in lipid and protein metabolism, both of which lead to hyperglycaemia. Insulin intolerance or lack of insulin are the two main causes of diabetes mellitus. Diabetes Type 1 (insulin dependent) accounts for just 5% of cases worldwide; Type 2 (noninsulin dependent) accounts for 95%. Traditionally, diet, exercise, and plant-based medicinal remedies were used to treat DM. Merely one-third of the thousands of plants used to treat diabetes mellitus have had their chemical composition and therapeutic qualities investigated. [2].

The annual herbaceous and aromatic plant known as Ajwain (*Trachyspermumammi* L. Sprague) belongs to the family Apiaceae. It is an ascending annual herb possessing a striate stem

that originally emerged in eastern Persia and India. Ajwain is used to treat stomach problems, asthma, acute pharyngitis, spasmodic diseases, common cold, painful and congested throats [3].

The *in vivo* antidiabetic efficacy of crude extracts extracted from *Trachyspermum ammi* leaves was assessed in the current study. The primary goal of this *in vivo* study is to establish the antidiabetic action of a META.

2. MATERIAL AND METHODS

2.1 Plant collection and drying

In the month of January, the leaves of *Trachyspermum ammi* Linn were collected in Hyderabad, Telangana, and recognized and authenticated. The leaves are coarsely grounded in a mixer grinder after drying in the shade for roughly six days. The powdered substances were stored and extracted.

2.2 Preparation of plant extract

Extraction is done by Maceration technique. Menstruum is poured on top of coarsely powdered drug material, such as leaves, stem bark, or root bark, until the drug material is thoroughly covered. The solvent then evaporates after the container is closed and kept that way for at least 3 to 7 days [4]. *Trachyspermum ammi* leaves are extracted using methanol as a solvent.

2.3 Preliminary phytochemical screening

To determine the various phytoconstituents found in *Trachyspermum ammi* leaves, a preliminary phytochemical analysis of the META was carried out.

2.4 Acute Toxicity Studies

The current study was carried out at Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India, which has an animal facility certified by CPCSEA (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

2.5 Experimental animals

Swiss Albino mice (weighing between 20 and 25 grms) were purchased from Jeeva Life Sciences in Hyderabad. The Committee for the Purpose of Control and Supervision of Experiments on Animals approved certain procedures for the upkeep and maintenance of the animals, and these procedures were followed.

2.6 *In vivo* methods for evaluation of Antidiabetic activity

2.6.1 Oral Glucose Tolerance Test:

Wistar albino rats weighing 150–200g were used in this study. Rats were fasted before the experiment. G-I was administered with the distilled water, G-II was administered with glucose, G-III and IV were administered META at a dose of 200 and 400 mg/kg bd. wt., *p.o.*, and G-V was given with glibenclamide 5 mg/kg bd. wt., *p.o.* a 40% oral glucose solution of 2gm/kg bd. wt. concentration was administered to the rats after 30 minutes. In order to measure the glucose level at 0, 0.5, 1, and 2-hours following glucose infusion, blood is then drawn from each tail vein of the rats [5].

Table 1: Experimental design for OGTT

Groups	Treatment
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Group-I	Control (Distilled water)
Group-II	Glucose (2 gm/Kg, bd. wt. <i>p.o</i> for 120 mins)
Group-III	META (200 mg/kg bd. wt. <i>p.o</i> + Glucose 2 gm/Kg, bd. wt. <i>p.o</i> for 120 min)
Group-IV	META (400 mg/kg bd. wt. <i>p.o</i> + Glucose 2 gm/Kg, bd. wt. <i>p.o</i> for 120 min)
Group-V	Standard drug-Glibenclamide(5 mg/kg bd. wt. <i>p.o</i> + Glucose 2 gm/Kg, bd. wt. <i>p.o</i> for 120 min)

2.6.2 Streptozotocin-Nicotinamide Induced Diabetes model

In this 21-days study, Wistar albino rats (weighing 150-200gms) of either sex, will be used. All groups receive STZ 50 mg/kg bd.w.t. *i.p.* and NIC 100 mg/kg bd.w.t. *i.p.*, with the exception of Groups I and III [6]. After 72 hours, STZ-NIC induced mice were deemed to be diabetic if their fasting plasma levels were more than 250 mg/dL. Reserpine 0.5 mg/Kg, bd. wt. *i.p.* from 1-14 days was given to the depression group (G-III), diabetic group (G-II), and control group (G-I) respectively. META is administered to Groups IV and V at a dose of 200 and 400 mg/kg bd. wt. *p.o.*, respectively, while G-VI receives glibenclamide 5 mg/kg bd. wt. *p.o.* for a period of 21 days starting on the day of induction. Blood is drawn from the tail tip on the first, seventh, and fourteenth days of the experiment, and blood glucose levels are calculated using a glucometer and the strip method. In order to test blood glucose levels and lipid profiles using an auto-analyzer, samples are taken on the twenty-first day using the retro-orbital bleeding technique [7].

Table 2: Experimental design for Streptozotocin-Nicotinamide induced diabetes model

Groups	Treatment
Group-I	Control (Distilled water)
Group-II	STZ (50 mg/Kg, bd. wt. <i>i.p</i> + NIC 100 mg/kg bd. wt. <i>i.p</i>)
Group-III	Reserpine (0.5 mg/Kg, bd. wt. <i>i.p</i> from 1-14 days)
Group-IV	STZ (50 mg/Kg, bd. wt. <i>i.p</i> + NIC 100 mg/kg bd. wt. <i>i.p</i> + META 200 mg/kg bd. wt. <i>p.o</i> from 1-21days)
Group-V	STZ (50 mg/Kg, bd. wt. <i>i.p</i> + NIC 100 mg/kg bd. wt. <i>i.p</i> + META 400 mg/kg bd. wt. <i>p.o</i> from 1-21days)
Group-VI	STZ (50 mg/Kg bd. wt. <i>i.p</i> + NIC 100 mg/kg bd. wt. <i>i.p</i> + Glibenclamide 5 mg/kg bd. wt. <i>p.o</i> from 1-21days)

2.6.3 Histopathology studies

In a model of reserpine-induced depression, histopathological examinations are carried out. To evaluate the antidiabetic action of *Trachyspermum ammi*, Pancreas tissue samples were fixed with neutral buffered formalin for 24 hours. Tissue sections of the pancreas were then analysed histopathologically. After being fixed with 10% buffered formalin, the tissues were processed using a tissue processor. A rotary microtome was used to slice sections of the treated tissue into 5 m thick pieces after it had been embedded in a paraffin block. Hematoxylin and eosin was used to stain these

sections using conventional methods. The slides were inspected under a microscope for morphological alterations such as shrunken pyramidal cells with empty gaps [8].

2.7 Statistical analysis:

Values are presented as the Mean \pm SEM, with (n=6). ANOVA was used for the statistical analysis, which was followed by Dunnett's test. Comparisons were made between each group with the control, disease-control, and standard groups. Significant values are expressed as standard (A= $p < 0.0001$), control group (*= $p < 0.001$, a= $p < 0.0001$ b= $p < 0.001$) and ns- nonsignificant.

3. RESULTS

Methanolic extract of *Trachyspermum ammi* leaves were explored for its antidiabetic activity. All the findings of this research are shown below.

3.1 Calculation of extract yield

$$\begin{aligned} \text{Percentage yield of extract} &= \text{Amount of extract obtained} / \text{Amount of powder used} \times 100 \\ &= 32.30 / 200 \times 100 \\ &= 16.15 \% \text{ w/w.} \end{aligned}$$

Amount of META obtained was 16.15 % w/w.

3.2 Preliminary phytochemical analysis

Terpenoids, phenolics, flavonoids, tannins, steroids, carbohydrates, and saponins were found in the methanolic extract of *Trachyspermum ammi* leaves, while glycosides, resins, and aldehydes were not identified.

3.3 Acute toxicity studies

2000 mg/kg b.d.wt., p.o. dose of a META was tested on female albino mice. Even at 2000 mg/kg b.d.wt., the extract showed no symptoms of toxicity or death. All of the animals were safe even after 14 days of observation. The pharmacological analysis was performed at doses of 200 and 400 mg/kg body weight, orally.

3.4. In vivo methods for evaluation of Antidiabetic activity

3.4.1 OGTT

The OGTT determines how well the body can utilise glucose. Statistical analysis showed no significant difference between groups at 0 mins. After 30 mins of glucose administration, the effects of META and glibenclamide 5 mg/kg revealed raised blood glucose levels. Similar to this, a significant decline in Blood glucose levels (BGLs) was seen after 60 mins compared to the BGLs in 30 minutes, and a total decline was seen after 120 minutes compared to the negative control. The extract-fed rats can consume more glucose than control rats. The decrease in glucose absorption and improvement in glucose utilisation may account for the extract's ability to lower postprandial glucose levels.

Table 3: Effect of META on OGTT

S.no	Treatment	Blood Glucose Levels (mg/dL)				
		0 min	30 min	60 min	90 min	120 min

1.	Normal control	91.33 ± 0.80	92.83 ± 0.60	94.33 ± 0.84	94.16 ± 0.47	98.66 ± 0.61
2.	Disease control	99.33 ± 0.88 [*]	157 ± 0.96 [*]	155.5 ± 0.88 [*]	132.66 ± 0.71 [*]	115.5 ± 0.99 [*]
3.	META 200 mg/kg bd wt	96.16 ± 0.94 ^{***ns}	153.33 ± 0.83 ^{####A}	127.33 ± 0.88 ^{*#A}	113.16 ± 0.79 ^{*#A}	101.17 ± 0.83 ^{ns#A}
4.	META 400 mg/kg bd wt	94.16 ± 0.79 ^{ns##}	150 ± 0.79 ^{*#B}	117.33 ± 0.88 ^{*#A}	105.33 ± 0.60 ^{*#A}	90.83 ± 0.79 ^{*#ns}
5.	Glibenclamide 5 mg/kg bd wt	92.5 ± 0.95 ^{ns#}	144.5 ± 0.99 ^{*#}	106.5 ± 0.99 ^{*#}	98.33 ± 0.55 ^{**#}	88.5 ± 0.61 ^{*#}

The results of the one-way ANOVA with Dunnett's multiple comparison test against the standard (^A = p < 0.0001, ^B = p < 0.005, ^C = p < 0.05), control (^{*} = p < 0.0001, ^{**} = p < 0.0005, ^{***} = p < 0.005) and disease control ([#] = p < 0.0001, ^{##} = p < 0.005, ^{###} = p < 0.05). Which are represented as Mean ± SEM (n=6).

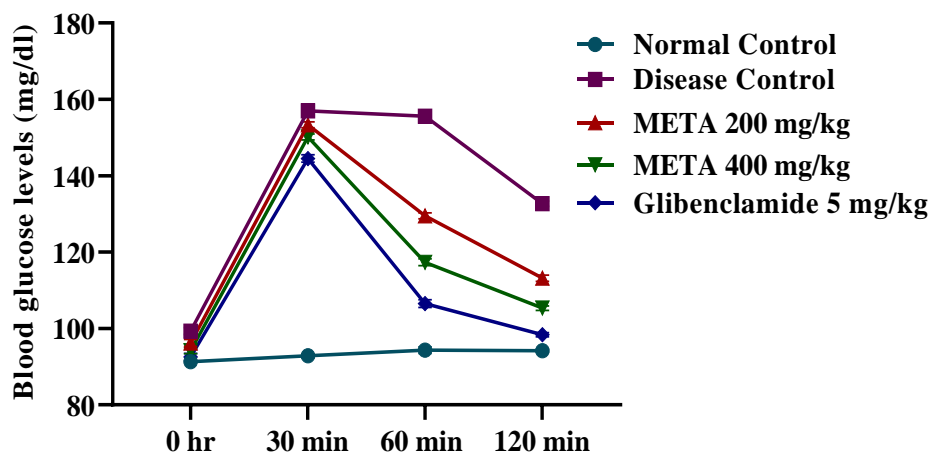


Figure 1: Influence of META on the OGTT

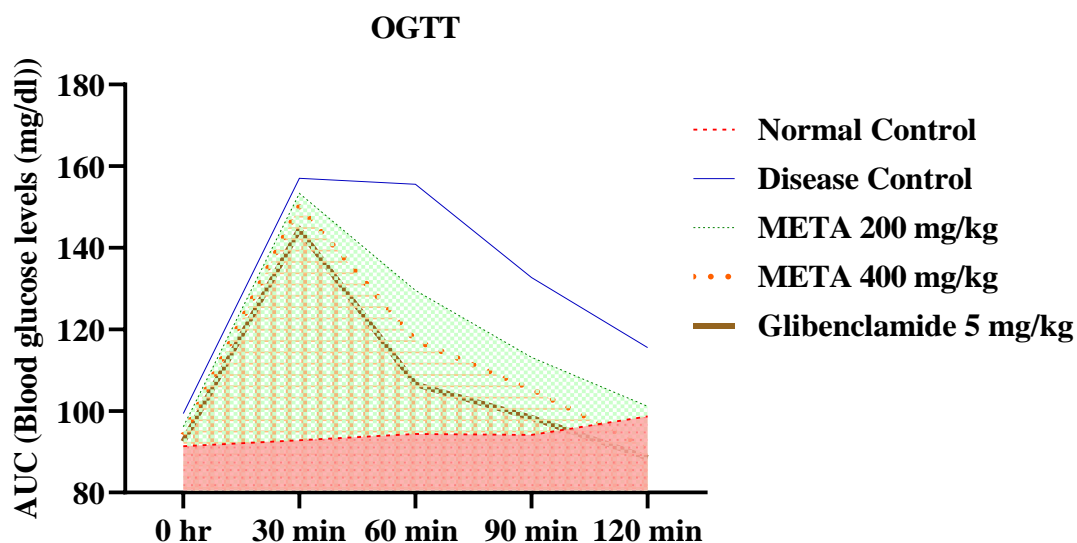


Figure 2: Impact of META on the OGTT

3.4.2 Streptozotocin-Nicotinamide induced diabetes test

In this model, BGLs were noticeably higher in the streptozotocin- Nicotinamide induced diabetes and reserpine-induced depression groups than in the control group. When compared to the STZ-NIC induced diabetic group, the META treatment groups at 200 mg/kg, 400 mg/kg, and regular glibenclamide 5 mg/kg showed a considerable drop in BGLs.

Table 4: Effect of META on blood glucose levels in a STZ-NIC induced diabetes model

S.no	Treatment	Blood glucose levels mg/dL			
		0 th Day	7 th Day	14 th Day	21 st Day
1.	Normal Control	92.66 ± 0.49	90.16 ± 0.87	96 ± 0.68	97.83 ± 0.47
2.	STZ 50 mg/kg <i>i.p</i> + Nicotinamide 100 mg/kg <i>i.p</i>	295 ± 0.89 [*]	290.83 ± 0.60 [*]	293.5 ± 0.99 [*]	294.16 ± 0.87 [*]
3.	Reserpine 0.5 mg/kg bd wt. <i>i.p</i>	223.83 ± 0.79 ^{*#}	252.33 ± 0.88 ^{*#}	260 ± 0.73 ^{*#}	271.5 ± 0.76 ^{*#}
4.	META 200 mg/kg bd wt	287.33 ± 0.66 ^{*#}	275.83 ± 0.70 ^{*#A}	192.66 ± 0.91 ^{*#A}	135.33 ± 0.91 ^{*#A}
5.	META 400 mg/kg bd wt	281 ± 0.63 ^{*#}	242.33 ± 0.98 ^{*#A}	164.83 ± 0.98 ^{*#A}	116.5 ± 0.76 ^{*#A}
6.	Glibenclamide 5 mg/kg bd wt	286 ± 0.96 ^{*#}	207.16 ± 0.83 ^{*#}	146.16 ± 0.60 ^{*#}	99 ± 0.93 ^{**#}

The data is displayed as Mean SEM (n=6). One-way ANOVA is used followed by a multiple comparison test with the standard (A = p<0.0001), control (* = p <0.0001, ** = p <0.05) and disease control (# = p<0.0001).

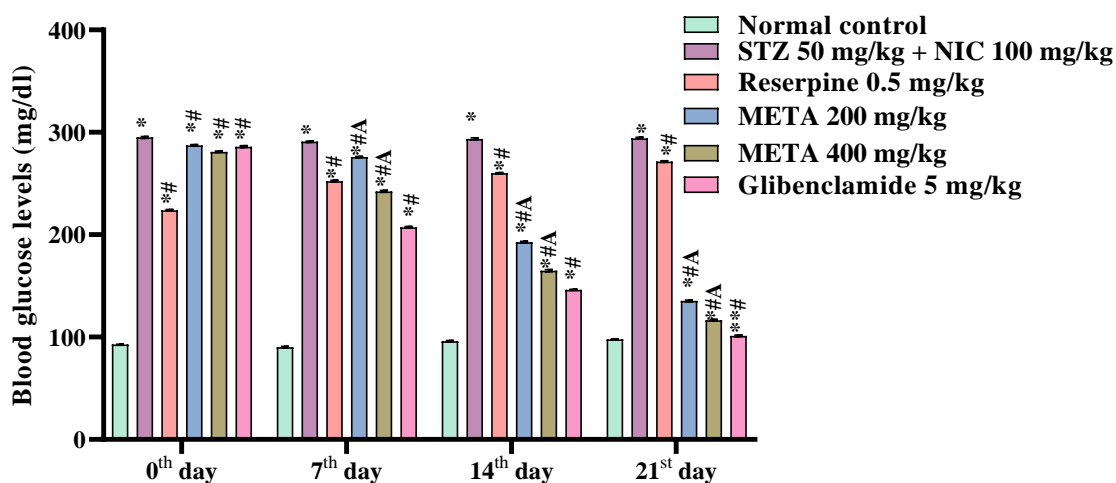


Figure 3: Effect of META on anti-diabetic activity using streptozotocin-induced diabetic model

The lipid profile levels are assessed on days 0 and 21 in streptozotocin-induced diabetes. On day 0, all of the rats had higher levels of TC, TG, LDL, and VLDL, whereas HDL levels were reduced in comparison to the control group. In comparison to the diabetic group, the META-treated groups at 200 mg/kg, 400 mg/kg, and regular glibenclamide 5 mg/kg displayed a significant decrease in TC, TG, LDL, and VLDL levels and an increase in HDL levels.

Table 5: Effect of META on lipid levels (0th day) in a streptozotocin-induced diabetes model

Treatment	Lipid profile (0 th day)				
	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Normal Control	87.16 ± 0.60	74.33 ± 0.76	48.83 ± 0.94	110.63 ± 0.87	14.9 ± 0.15
STZ 50 mg/ kg <i>i.p</i> + NIC100 mg/ kg <i>i.p</i>	141 ± 0.95*	112.66 ± 0.95*	23.83 ± 0.79*	228.46 ± 0.84*	22.73 ± 0.18*
META 200 mg/ kg <i>bd wt.</i>	156.16 ± 0.70*#	126.66 ± 0.88*#	29.5 ± 0.99*##	250.8 ± 0.89*#	24.73 ± 0.57*##
META 400 mg/ kg <i>bd wt.</i>	152.33 ± 0.95*#	102.33 ± 0.98*#	28.66 ± 0.95*###	223.8 ± 0.81*###	20.5 ± 0.19*#

Glibenclamide 5 mg/ kg bd wt.	148.66 ± 0.42 ^{*#}	128 ± 0.96 ^{*#}	28.33 ± 0.71 ^{####}	246.46 ± 0.98 ^{*#}	25.6 ± 0.19 ^{*#}
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The information was displayed as Mean SEM (n=6). One-way ANOVA was used to perform the investigation, and it was followed by Dunnett's multiple comparison tests against the control group (* = p< 0.0001, ** = p< 0.0005, *** = p< 0.05) and disease control (# = p< 0.0001, ## = p< 0.0005, ### = p< 0.005).

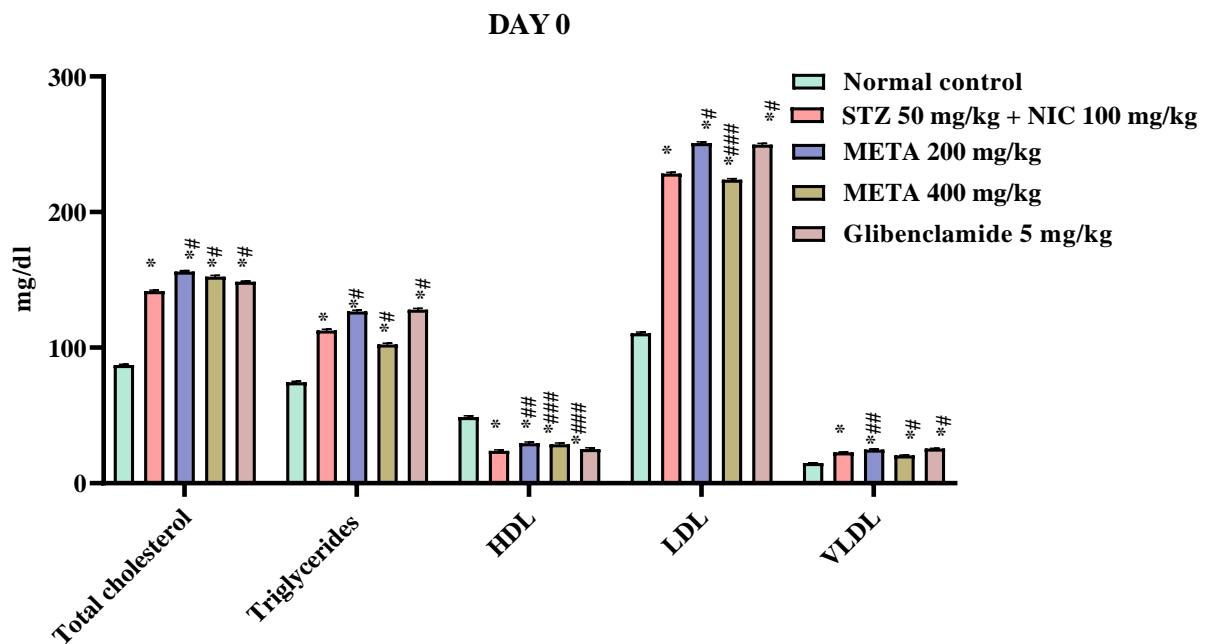


Figure 4: Effect of META on lipid levels (0th day) of STZ-induced diabetic model

Table 6: Effect of META on lipid levels (21st day) in a streptozotocin-induced diabetes model

Treatment	Lipid profile (21 st day)				
	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Normal control	82.66 ± 0.66	76.66 ± 0.76	49.33 ± 0.91	107.8 ± 0.81	15.33 ± 0.15
STZ 50 mg/ kg + NIC 100 mg/ kg	153 ± 0.68 [*]	123.83 ± 0.94 [*]	25 ± 0.73 [*]	249.8 ± 0.77 [*]	24.8 ± 0.20 [*]
META 200 mg/kg bd wt	124.83 ± 0.79 ^{*#A}	112 ± 0.93 ^{*#A}	30.66 ± 0.71 ^{*#A}	203.8 ± 0.94 ^{*#A}	22.4 ± 0.18 ^{*#A}
META 400 mg/kg bd wt	113.83 ± 0.87 ^{*#A}	96.5 ± 0.42 ^{*#A}	37.83 ± 0.60 ^{*#B}	170.3 ± 0.84 ^{*#A}	19.4 ± 0.14 ^{*#A}

Glibenclamide 5 mg/kg bd wt.	90.5 ± 0.99**#	84.16 ± 0.94*#	41.16 ± 0.47*#	131.3 ± 0.92*#	16.83 ± 0.18*#
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ANOVA was used to analyse the values, which are shown as Mean SEM (n=6). Dunnett's multiple comparison test was then performed against the standard (^A = p< 0.0001, ^B = p< 0.005), control (^{*} = p< 0.0001, ^{**} = p< 0.01) and disease control ([#] = p< 0.0001).

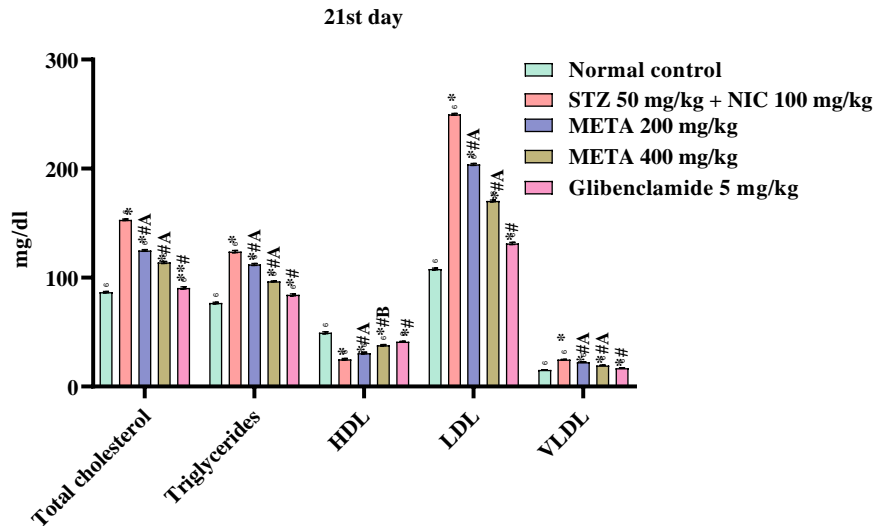
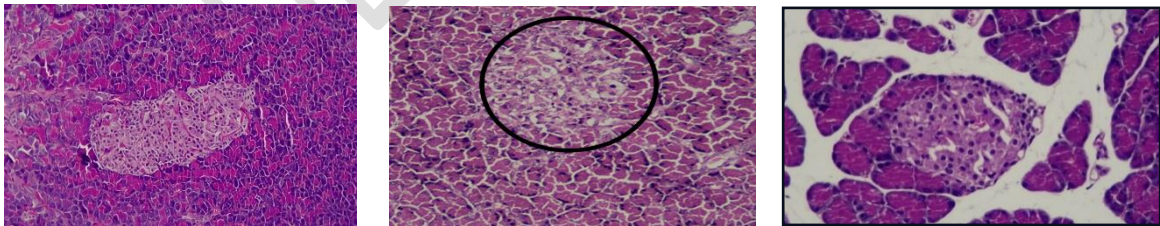


Figure 5: Effect of META on lipid profile levels (21st day) in a streptozotocin-induced diabetes

3.5 Histopathological studies of Streptozotocin-induced diabetes

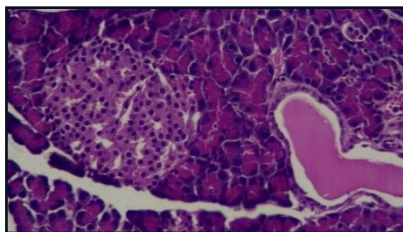
Rat pancreas was used for histopathological analysis in a diabetic model induced by STZ. The pathological changes are stained with hematoxylin and eosin and then studied under a light microscope.



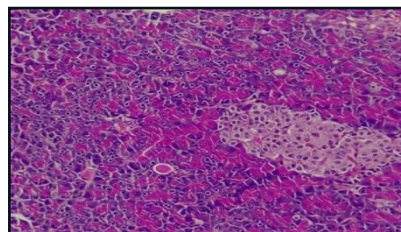
B. Normal pancreas

A. STZ-NIC Induced

C. META 200mg/kg



D. META 400 mg/ kg



E. Glibenclamide 5 mg/kg

Figure 6: Histopathology of the rat pancreas in a streptozotocin-induced diabetes model

- A. Control group: Pancreatic beta-cells and acinar cells were determined to have normal morphology.
- B. STZ-induced diabetes group: Pancreatic beta-cells were destroyed and found to be devoid of connective tissue.
- C. META 200 mg/kg group: Pancreatic -cells and acinar cell regeneration was mildly observed.
- D. META 400 mg/kg group: Acinar and pancreatic -cells regenerated to a moderate extent.
- E. Glibenclamide 5 mg/kg group: Significant improvement in the architecture of the pancreatic islets of Langerhans.

4. DISCUSSION

In the OGTT, control mice's blood glucose levels peaked at about 120–130 mg/100 ml after 30 minutes. After 30 minutes of sugar infusion, the META decreased the maximum values, indicating that the extracts had a more potent hypoglycaemic impact. The most likely scenario is that increasing sugar triggers islet-cells to release insulin [9].

Type 2 diabetes was caused by streptozotocin/nicotinamide, whereas type 1 diabetes was caused by streptozotocin alone [10,11].

Diabetes is brought on by streptozotocin's preference for destroying pancreatic insulin-secreting cells. Inadequate insulin levels also limit how well cells can use glucose to produce ROS[12]. By restricting the activity of poly (adenosine diphosphate ribose-ribose) polymerase-1 and serving as a promoter of nicotinamide adenine dinucleotide, nicotinamide, on the other hand, protects pancreatic beta-cells from STZ. Additionally, those experimental rats get diabetes in the form of heart, kidney, nerve, eyesight, and other conditions that are all brought on by ROSproduction [13].

Lipid abnormalities, which are characterised by an increase in blood TC, TG, and minimum HDL-C, are one of the effects of diabetes mellitus [14]. Insufficient insulin causes lipase to be activated, which can increase lipolysis and liver VLDL generation[15]. Chylomicron and VLDL clearance are also decreased as a result of lipoprotein lipase insufficiency, which is decreased by insulin[16]. The enzymatic activity of cholesteryl ester transfer protein is also increased by hypertriglyceridemia, increasing the triglyceride content of LDL and HDL. While LDL particles are digested by hepatic lipase or lipoprotein lipase, resulting in smaller LDL pieces, triglyceride-enriched HDL molecules are easily catabolized[4].

Glibenclamideaction prompts rapid insulin delivery to block ATP-sensitive K⁺ channels. Compared to other drugs, it causes hypoglycaemia more frequently [17].

Thymol significantly decreased the levels of aspartate aminotransferase, alanine aminotransferase, leptin, HbA1c, insulin resistance, and plasma glucose in diabetic rats induced with STZ. Additionally, it reduced plasma LDL cholesterol, free fatty acids, total cholesterol, and triglycerides. Levels of high-density lipoprotein cholesterol therefore rose[18]. Plasma hyperglycaemia can be reduced and cell function can be maintained by pinene [19]. The therapy of diabetes, a complex condition involving interplay between inherited and environmental factors[20],benefits

particularly from such effects. In rats given a high-fat diet, terpineol and its structural isomer 4-terpineol decreased pro-inflammatory cytokine levels in the blood, inhibited amylase activity, and improved insulin sensitivity[8]. A key site of entry for glucose, the small intestine, is prevented from absorbing glucose into the bloodstream by lupeol's inhibition of glucosidase, an enzyme located on the apical barrier of the small intestine that aids in carbohydrate breakdown and transfers into circulation. It thus reduces blood sugar levels [21]. Phellandrene stimulates the activity of the enzymes (glucokinase, alpha-amylase, PTP1B, alpha-glucosidase, and hexokinase-II) (HK-II) involved in glycolysis and glucose conversion [22].

By preventing pancreatic β -cell damage and reducing induced oxidative signalling, apigenin reduces hyperglycaemia [23]. Antioxidant luteolin is recognised as risk-free. The formation of free radicals, which results in oxidative damage to pancreatic islet cells, is the root cause of diabetes [24]. In addition to preserving the pancreas and improving insulin secretion, it has the capacity to scavenge reactive oxygen species (ROS), block the enzymes that create them, and protect the components of other antioxidant systems. The management of lipid peroxidation and the suppression of free radical production have been validated in diabetic animal investigations [25].

BGL and glycated haemoglobin are both reduced bytocopherol [26]. Fucosterol, a naturally occurring chemical primarily found in sea algae, is employed for the treatment of diabetes by preventing the liver breakdown of glycogen. Due to this, glucose absorption and carbohydrate digestion may be significantly delayed [27].

The rats in the control grouppancreas had normal pancreatic islets, according to the histological results. Rats given STZ-NIC and reserpine (owing to increasing levels of corticosterone being secreted) both show signs of pancreatic islet breakdown. The pancreatic islets in the rats receiving 200 mg/kg of META showed a slight restoration, where the cells seemed less shrunken and had sporadic inflammatory infiltration. The architecture of the Langerhans islets significantly improved and rejuvenated in the rats given 400 mg/kg of META. The pancreatic islets of rats receiving glibenclamide 5 mg/kg showed reformation.

4. CONCLUSION

The results of this investigation suggest that *Trachyspermumammi* leaf extract has considerable antidiabetic-like effects. A novel study method is necessary to elucidate *Trachysperumammi* mode of action.

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