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Research Article

PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF HYPOGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT OF *MUNTINGIA CALABURA L* STEM BARK AGAINST NORMAL AND STREPTOZOTOCIN-INDUCED DIABETES

MOHANAVAMSI YEMINENI[1]*, VENKATA RAMANA KANCHARLAPALLI[1], SUBRAHMANYESWARI PN[2]

[1]Department of Pharmaceutical Chemistry, A. S. N. Pharmacy College, Tenali, [2]Department of Pharmacology, Vignan Pharmacy College, Guntur, Andhra Pradesh, India. Email: vamsiyemineni@gmail.com

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ABSTRACT

Objective: This research was attributed to the investigation of preliminary phytochemical constituents and evaluation of hypoglycemic activity of the methanolic extract of *Muntingia calabura L* stem bark against normal and streptozotocin-induced diabetes.

Methods: The investigation of preliminary phytochemical study includes a methanolic extract of *M. calabura L* was prepared through using Soxhlet apparatus. The methanolic extract was qualitatively analyzed to find out the plant secondary metabolites such as alkaloids, glycosides, flavonoids, terpenoids, steroids, and sterols. The hypoglycemic activity was determined by streptozotocin-induced diabetic rats. Screening for hypoglycemic activity of the methanol extract was performed on albino Wistar rats, and glibenclamide (2.5 mg/kg, p. o.) was used as a reference standard for activity comparison. All the test samples were administered by the oral route.

Results: The preliminary phytochemical investigation of the methanolic extract of *M. calabura L* stem bark revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, steroids, and sterols as secondary metabolites. The methanolic extract of *M. calabura L* stem bark showed a significant reduction in blood glucose levels. The antihyperglycemic activity of *M. calabura L* extract may be due to the presence of several bioactive antidiabetic principles such as alkaloids, glycosides, flavonoids, terpenoids, steroids, and sterols.

Conclusion: The results indicate that the hypoglycemic activity of crude methanolic extract of *M. calabura L* stem bark has been proved; hence, there is ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic activity of *M. calabura L* stem bark in *in vivo* animal studies.

Keywords: *Muntingia calabura*, Phytochemical analysis, Hypoglycemic activity, streptozotocin-induced diabetic rats, Glucometer.

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INTRODUCTION

Plants have an almost limitless ability to synthesize secondary metabolites. These substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores [1]. The compounds that are superintended for the therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical investigation for the detection of various plant constituents [2].

The *Muntingia calabura L* (Jamaica cherry) is an indigenous plant found throughout the world. *M. calabura* belongs to the family Muntingiaceae. It is a fast-growing tree. The leaf infusion is drunk as a tea-like beverage [3].

The flowers are said to possess antiseptic properties and also taken to diminish headaches and colds. An infusion of the flowers is used as an antispasmodic. It is taken to diminish headache and the first symptoms of a cold. The fruits are also used for treatment of gout, strong antioxidant, and lipid peroxidation inhibition activities [4].

Diabetes is a shape in which the body either does not secrete enough or does not properly counter to insulin, a hormone secreted in the pancreas. Insulin does not mediate cells to absorb glucose to turn it into energy. This causes glucose to accumulate in the blood, often leading to various complications [5].

Majorly three types of diabetes were recognized, they were:
Type 1 diabetes mellitus is designated by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to a deficiency of insulin. This type of diabetes can be later classified as immune-mediated or idiopathic. The most of type 1 diabetes is of the immune-mediated nature, whereas beta cell loss is a T-cell mediated autoimmune attack [6]. There is no preventive measure which can be taken against type 1 diabetes. Type 1 diabetes can affect children or adults but was traditionally termed “juvenile diabetes” because it represents most of the diabetic cases in children. The average glucose level for the type 1 patient should be as close to normal (80–120 mg/dl and 4–6 mmol/L) as is safely possible [7].

Type 2 diabetes mellitus is significantly differentiated and is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute [8]. Type 2 diabetes is the most common type. Type 2 diabetes is primarily treated by increasing physical activity, decreasing carbohydrate intake, and losing weight [9].

Gestational diabetes mellitus similar to type 2 diabetes in several aspects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in 2%–5% of all pregnant women and may improve or disappear after delivery. Gestational diabetes is completely treatable but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women build type 2 diabetes later in life [10].

Inspite of the presence of known antidiabetic medication in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Many traditional plant medications for diabetes are used throughout the world. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important [11]. The presence of antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the action of metabolites in insulin-dependent processes. Hence, treatment with herbal medication has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as a drug of choice rather than individual [12].

MATERIALS AND METHODS

Standard drugs used for activity comparison

Glibenclamide (2.5 mg/kg, p. o.) was used as a reference standard for activity comparison in the antidiabetic study [13].

Chemicals used

1. Normal saline
2. Tween 20 and 80: The test samples were suspended in 1:1 ratio of Tween-20 and 80 in normal saline, respectively, and used for the activity study
3. Glucose
4. Streptozotocin (55 mg/kg).

Animals used

Adult Wistar albino rats (150–200 g) of either sex were used for evaluation of pharmacological studies, namely, antidiabetic activity on normoglycemic and streptozotocin-induced diabetic animals. The animals were housed for at least 1 week in the laboratory animal room before testing in standard polypropylene cages at room temperature of 34°C ± 2°C and 60%–65% relative humidity. The food and water were given *ad libitum* as per protocol. The Institutional Animal Ethics Committee approved all the experimental protocols (IAEC/GIP-1287/PHD/IP/SM-BU/04/2017-18).

Plant material collection

The plant materials were collected from the young and matured plants of *M. calabura L* stem bark and authenticated by the taxonomist P. Satyanarayana Raju, Head of the Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The collected materials were washed; shade dried and pulverized using a mechanical grinder to obtain a coarse powder.

Preparation of extracts

Dried plant powder (500 g), was defatted with petroleum ether at 40–60^{0}C and was extracted with methanol using Soxhlet extractor. The methanol extraction was done for 48 h with the air-dried marc left after petroleum ether extraction. After the completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined. Purification of solvents was done by distillation and used for further extraction.

Preliminary phytochemical studies

The methanolic extract of *M. calabura L* stem bark was subjected to preliminary phytochemical screening for the detection of various plant constituents [14-16].

Useful agents used in a chemical test

Lieberman-Burchard reagent

Acetic anhydride (5 ml) is carefully mixed under cooling with 5 ml concentrated sulfuric acid. This mixture is added carefully to 50 ml of absolute ethanol with cooling.

Mayer's reagent

Mercuric iodide (1.36 g) in 60 ml of water, mixed with a solution containing 5 g of potassium iodide in 20 ml of water.

Dragendroff's reagent

Bismuth nitrate (1.76 g) and 20 g to tartaric acid are dissolved in 80 ml of water. This solution is mixed with a solution containing 16 g of potassium iodide and 40 ml of water.

Wagner's reagent

Iodide (1.27 g) in 28 g of potassium iodide in water and made up to the volume 100 ml with distilled water.

Phosphomolybdic acid reagent

It is a 5 % w/v solution of phosphor molybdic acid in ethyl alcohol.

Dilute ammonia solution

It contains approximately 10 % w/v ammonia in distilled water.

Fehling's solution A

Copper sulfate (34.64 g) is dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

Fehling's solution B

Sodium potassium tartrate (176 g) and sodium hydroxide (77 g) are dissolved in sufficient water to produce 500 ml. equal volume of fehling's solution A and B are mixed at the of used.

Benedict's reagent

Sodium citrate (173 g), anhydrous sodium carbonate (10 g), and cupric sulfate (1.73 g) are dissolved in a water and volume made up to 100 ml with water.

Molisch reagent

Pure α-napthol (2.5 g) is dissolved in 25 ml of ethanol.

Ferric chloride solution (Alcoholic)

Ferric chloride (5% w/v) solution in 90% alcohol.

Hager's reagents

It is a saturated solution of picric acid.

General chemical test method

Tests for alkaloids

Mayer's reagent

a. Dissolve 1.36 g of mercuric chloride ($HgCl_2$) in 60 ml distilled water
b. Dissolve 5 g Potassium iodide in 60 ml distilled water.

Mix (a) and (b) and make up the volume to 100 ml with distilled water. With alkaloids, it produces white to buff colored precipitate.

Wagner's reagent

It is dissolved 1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and make up the volume 200 ml with distilled water. With alkaloids, it produces reddish-brown precipitate.

Dragendorff's reagent

Boil 14 g of sodium iodide with 5.2 g of bismuth carbonate in 50 ml glacial acetic acid for a few minutes. Allow it to stand for overnight and filter out the precipitate of sodium acetate crystals. Preserve the stock solution in an amber colored bottle. When required, add 20 ml of acetic acid to 10 ml of this stock solution and makeup to 100 ml with water. With alkaloids, it produces orange-brown precipitate.

Hager's reagent

A saturated aqueous solution of picric acid used for detection of alkaloids. It gives characteristics crystalline precipitate with many alkaloids.

Test for carbohydrates

Molisch's test

To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α-naphthol. Shake well and add a few drops of concentrated sulfuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates.

Fehling's test

Add 2 ml of Fehling's solution A and 2 ml of Fehling's solution B to 2 ml of liquid extract in a test tube and boil. If yellow or bricked precipitate appears, then reducing sugars are present.

Benedicts test

Add 5 ml of Benedict's reagent to 3 ml of test solution in a test tube and boil on a water bath. The appearance of brick red precipitate at the bottom of the test tube shows the presence of monosaccharides.

Barfoed's test

The extract was treated with Barfoed's reagent was added and heated on a boiling water bath for a few minutes. Showed a red precipitate of cuprous oxide indicated the presence of monosaccharides.

Test for glycosides

Test for cardiac glycosides

Legal's test

The extract was hydrolyzed for a few hours in a water bath; the hydroxylate was added with 2 ml of sodium nitroprusside solution and was made alkaline with sodium hydroxide solution. The change of color from yellow to orange indicates the presence of cardiac glycosides.

Liebermann-Burchard's test

Hydroxylates were treated with a few drops of acetic anhydride, boiled, and cooled. Few drops of sulfuric acid were added through the sides of the test tube. Formation of a brown ring at the junction of two liquids and green color in the upper layer indicates the presence of glycosides.

Keller–Killiani test

To an extract of the drug in glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid are added. A reddish brown color is formed at the junction of two layers and the upper layer turns bluish green. The test confirms the presence of cardiac glycosides with the presence of digitoxose as the glycone moiety.

Test for anthraquinone glycosides

Borntrager's test

Boil 0.1 g of the powdered drug with 5 ml of 10% sulfuric acid for 2 min filter while hot, cool the filtrate, and shake gently with an equal volume of benzene. Allow the benzene layer to separate completely from the lower layer. Pipette out and transfer the benzene layer to a clean test tube. Add about half of its volume of an aqueous solution of ammonia (10%). Shake gently and allow the layer to separate. The lower ammoniacal layer will acquire pink to red color due to the presence of free anthraquinones.

Modified Borntrager's test

The C-glycosides of anthraquinones requires more drastic conditions for hydrolysis, and thus a modification of the above test is used. Ferric chloride and hydrochloric acid are used to effect oxidative hydrolysis. 0.1 g of the drug is boiled with 5 ml of dilute hydrochloric acid and 5 ml of 5% solution of ferric chloride for 5 min cool the solution and filter. This filtrate is shaken with benzene. Separate the benzene layer and add an equal volume of a dilute solution of ammonia. This ammoniacal layer shows pink to red color.

Test for saponins

Foam test

The sample was diluted with 20 ml of distilled water, and it was agitated in a graduated cylinder for 15 min. A 1 cm layer of foam was formed in all the four extracts, which indicated the presence of saponins.

Observation for the formation of a stable froth

A small quantity of each of the extract was diluted separately with water, shaken well and observed for the formation of stable froth.

Observation for the formation of honeycomb-like froth

Few drops of sodium bicarbonate were added to the alcoholic solution of extract, which was shaken well and observed for the formation of honeycomb-like froth.

Test for gums and mucilages

Precipitation with 95% alcohol

Gums and mucilages precipitate with the addition of 95% alcohol, being insoluble in alcohol.

Molisch's test

To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α-naphthol. Shake well and add a few drops of concentrated sulfuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates, gums, and mucilages.

Ruthenium red test

It is dissolved 0.8 g of ruthenium red in 10 ml of 10% solution of lead acetate. It stains mucilage to red color.

Test for tannins

A pinch of the dried extract was dissolved in ethanol, mixed thoroughly and filtered. The filtrate is tested for the presence of tannins by the following test:

Ferric chloride test

To the filtrate dilute ferric chloride solution was added, the formation of deep blue color, black, violet (or) greenish blue precipitate indicates the presence of tannins.

Lead acetate test

To the filtrate lead acetate solution was added (10%). The formation of white color precipitate shows the presence of tannins.

Gelatin test

To the filtrate 1% solution of gelatin solution containing 10% sodium chloride was added, the formation of white color precipitate shows the presence of tannins.

Bromine test

To the filtrate few drops of bromine solution were added, the formation of a precipitate indicates the presence of tannins.

Potassium dichromate test

On addition of a solution of potassium dichromate in a test filtrate, developing dark color shows the presence of tannins.

Test for steroids and sterols

Lieberman–Burchard reagent

To about 2 ml of a solution extract in chloroform in a dry test tube, add 2 ml of acetic anhydride and 2–3 drops of concentrated sulfuric acid mix and stand for a few minutes. An emerald green color develops if steroids or sterols are present.

Salkowski's test

To 5 ml of a solution of extract in chloroform in a dry test tube add gently along the sides, on an equal volume of concentrated sulfuric acid.

Observe the upper chloroform layer and the lower acid layer. The acid layer develops a yellow color with green fluorescence. The chloroform layer will give a play of colors first from bluish red to gradually violet red.

Liebermann's test

To a few mg of residue in a test tube, few ml of acetic anhydride was added and gently heated. The contents of the test tube are cooled. Few drops of conc. H_2SO_4 were added from the side of the test tube. A blue color indicates the presence of sterols.

Test for proteins and amino acids

Biuret test

To 2 ml of extract, 2 ml of 10% NaOH solution and 2 to 3 drops of 1% $CuSO_4$ solution are added and mixed. The appearance of violet or purple color confirms the presence of proteins.

Ninhydrin test

To 2 ml of extract add 0.5 ml of ninhydrin solution. Boil for 2 min and cool. If blue color appears, then proteins are present.

Xanthoproteic test

To 2 ml of extract add 1 ml of concentrated nitric acid, boil, cool and add 40% sodium hydroxide drop by drop. The appearance of the colored solution indicates the presence of proteins.

Million's test

To 2 ml of extract add 2 ml of Million's reagent, boil, cool and add a few drops of $NaNO_2$ solution. The appearance of red precipitate or coloration indicates the presence of proteins.

Test for Triterpenoids

Noller's test

To the extract dissolved in chloroform, add piece of metallic tin. Add one drop of thionyl chloride. If the pink color develops, then triterpenoids are present.

Test for flavonoids

With NaOH

The extract dissolved in water, filtrate treated with sodium hydroxide, a yellow color is observed if flavonoids are present.

With H_2SO_4

A drop of concentrated sulfuric acid when added to the above, the yellow color disappears.

Shinoda test (Mg/HCl)

To the aqueous or alcoholic solution of the extract, add a piece of magnesium ribbon and few drops of concentrated hydrochloric acid. A pink color develops which indicates the presence of flavonoids.

Test for phenolic compounds

With ferric chloride

A 5% W/V solution of ferric chloride in 90% alcohol is used for detection of phenols.

With lead acetate

Tannins are precipitated with lead acetate.

With gelatin solution

To a solution of tannins (0.5%–1%) aqueous solution of gelatins (1%) and sodium chloride (10%) are added. A white to buff colored precipitate is formed.

Detection of anthocyanins

The extracts were treated with sodium hydroxide solution. Formation of blue-violet color indicates the presence of anthocyanins. The substance was treated with concentrated sulfuric acid. Formation of yellowish orange color indicates the presence of anthocyanins.

Detection of quinines

To the extract, sodium hydroxide was added. Formation of bluish-green or red color indicates the presence of quinines.

Detection of fixed oils and fats

Spot test

A small quantity of various extract was separately between two filter papers. Oil stained on the paper indicates the presence of fixed oils.

Saponification test

A few drops of 0.5% N alcoholic potassium hydroxide were added to a small quantity of the extract along with the drop of phenolphthalein. Then, the mixture was heated on a water bath for 1–2 h. The development of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Detection of resins

Distilled water (5.0 ml) was added to the extract. The formation of turbidity indicates the presence of resins.

A mixture of extracts in acetone (3 ml) and hydrochloric acid (3 ml) was heated on a water bath for 30 min. The formation of pink color shows the presence of resins.

Screening for the hypoglycemic activity of *M. calabura L* stem barks

Screening for hypoglycemic activity of the methanol extract was performed on albino Wistar rats, and glibenclamide (2.5 mg/kg, p.o.) was used as a reference standard for activity comparison. All the test samples were administered through the oral route [17].

Using normoglycemic rats

The method of Ponnachan *et al.*, 1993, was followed [18] where the normal rats were divided into four groups of six animals in each. Group-I served as negative control and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the extract at doses of 250 and 500 mg/kg, p. o., in a similar manner. Blood glucose levels were measured after 30, 60, 90, and 120 min of administration of a single dose of test samples. The animals were fasted for 18 h, were allowed free access to water before and throughout the duration of the experiment. At the end of the fasting period, taken as zero time (0 h), blood collection was done by tail vein method of each rat under mild anesthesia. The blood glucose level was measured with SensoCard blood glucometer supplied by M/s Avecon Health Care Pvt. Ltd., Himachal Pradesh, India.

Oral glucose tolerance test in rats

The method of Dash *et al.*, 2008 [19], was followed. Fasted rats were divided into four groups of six rats each. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the test extract at doses of 250 and 500 mg/kg, p. o., respectively, in a similar manner. After 30 min of treatment, all groups of rats were loaded orally with glucose (2 g/kg, p. o). Blood samples were collected before and at 60, 90, and 120 min after glucose administration as per the method described earlier.

Using hyperglycemic rats

The method of Ahmed and Urooj., 2008 [20], was followed. The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 55 mg/kg of streptozotocin in normal saline [21]. After 1 h, the animals were provided with standard laboratory diet *ad libitum*. Diabetes was induced in experimental groups

by a single intramuscular injection of streptozotocin (55 mg/kg body weight) after 24 h of fasting. Rats with fasting blood glucose level more than 250 mg/dl were selected for the study (Vidyarthi RD., 1977) and grouped into four groups consisting of six animals each. This condition was observed at the end of 48 h after administration. The first group (Group-I) served as diabetic control and received orally 1% Tween 80 solution (2 ml/kg p. o), second group (Group-II) received glibenclamide (2.5 mg/kg), third and fourth groups (Group-III and IV) received the methanolic extract at doses of 250 mg/kg and 500 mg/kg (in 1% Tween 80), respectively, in a similar manner. Blood glucose levels were measured after 1, 2, 4, and 6 h of administration of a single dose of test samples.

Table 1: Preliminary phytochemical tests to identify the presence of various phytoconstituents in *Muntingia calabura* stem bark

Serial number	Phytoconstituents	Methanolic extract
1	Alkaloids	++++
2	Glycosides	++++
3	Carbohydrates	++
4	Gums and mucilages	-
5	Proteins and amino acids	+
6	Tannins and phenolic compounds	++
7	Steroids and sterols	+++
8	Triterpenoids	++
9	Saponins	+
10	Flavonoids	++++

-: Absent, +: Less present, ++: Moderate, +++: High, ++++: Very high

Results were expressed as the mean ± standard error of mean. The statistical significance was also assessed by the usage of ANOVA followed by (Dunnett's *t*-test) comparison tests. *p<0.05, **p<0.01 was also considered statistically significant.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical studies were tabulated (Table 1).

Reports of the normoglycemic study (Table 2) reveals that the methanolic extract of *M. calabura* barks exhibited a significant reduction in blood glucose concentration in a dose-dependent manner as compared to control. It was observed that methanol extracts 250 mg/kg and 500 mg/kg showed 85.66 ± 1.92 and 77.16 ± 3.15 mg/dl blood glucose levels, respectively, whereas the standard drug glibenclamide 2.5 mg/kg showed 71.16 ± 2.5 mg/dl blood glucose levels in rats after 120 min.

The effect of test extracts on glucose tolerance test in normal rats is shown in Table 3. At 30 min after the glucose administration, the blood glucose level was increased rapidly from the fasting value and then subsequently decreased. The tested extract at 250 and 500 mg/kg, p. o., was exhibited a significant hypoglycemic effect when compared with the control group.

In an antihyperglycemic study (Table 4), the rise in the blood glucose level was observed after 24 h of streptozotocinization to the animals. A single administration of methanol extracts of bark of *M. calabura* (250 and 500 mg/kg) in diabetic rats showed a significant reduction in blood glucose level. The results of the methanol extract are comparable to that of the reference standard glibenclamide.

Table 2: Effect of methanolic extract of *Muntingia calabura* stem bark on the blood glucose level in normal rats

Treatment	Dose	Blood glucose concentration (mg/dl), normoglycemic study				
		Fasting	Time (min) after treatment			
			30	60	90	120
Control (%)	2 ml/kg	104.57±1.17	99.66±5.92 (4.69)	104.16±1.37 (0.39)	102.5±2.01 (1.97)	104.66±2.24 (−0.08)
Glibenclamide (%)	2.5 mg/kg	103.5±2.34	83.36±1.93** (19.45)	81.47±1.08** (21.28)	74.89±2.27** (27.64)	71.16±2.5** (31.24)
Methanolic extract (%)	250 mg/kg	102.83±2.46	92.83±3.18 (9.72)	87.16±2.42* (15.23)	88.66±2.3** (13.78)	85.66±1.92** (16.69)
	500 mg/kg	103.33±3.11	80.83±4.35* (21.77)	76.33±3.63** (26.12)	89.55±4.03** (13.33)	77.16±3.15** (25.32)

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's *t*-test). SEM: Standard error of means

Table 3: Effect of methanolic extract of *Muntingia calabura* stem barks on oral glucose tolerance test in normal rats

Treatment	Dose	Blood glucose concentration (mg/dl), oral glucose tolerance study			
		Fasting	Post-treatment		
			60 min	90 min	180 min
Control	2 ml/kg	107.83±2.62	125.33±3.33	123±1.61	128.83±6.33
Glibenclamide	2.5 mg/kg	104.5±1.94	110.33±2.53** (11.96)	105.16±1.75** (14.50)	95.66±1.47** (25.74)
Methanolic extract	250 mg/kg	109.83±3.17	111.00±4.03** (11.43)	112.33±3.42** (8.67)	104.00±3.30** (19.27)
	500 mg/kg	105.00±3.13	109.33±2.21** (12.76)	107.33±3.21** (12.73)	100.83±3.97** (21.73)

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01 compared with vehicle control (ANOVA followed by Dunnett's *t*-test). SEM: Standard error of means

Table 4: Effect of methanolic extract of *Muntingia calabura* stem barks on streptozotocin-induced rats

Groups	Blood glucose concentration (mg/dl)				
	0 min	30 min	90 min	120 min	240 min
Diabetic control	580±0	578.5±0.22	576.5±0.22	574.5±0.22	560.5±0.22
Glibenclamide (2.5 mg/kg, p.o.)	568.33±5.89	457.5±6.037* (19.50)	215.33±3.37** (62.11)	213.33±3.37** (62.46)	210.17±10.58** (63.01)
Methanolic extract (500 mg/kg, p.o.)	565.33±40.88	488±36.60 (13.67)	415.67±33.8* (26.47)	395.33±35.60* (30.07)	330.33±22.49** (41.56)

Values are expressed as mean ± SEM (n = 6). *p < 0.05, **p < 0.01 compared with vehicle control (ANOVA followed by Dunnet's *t*-test). Figures in parenthesis represent the percentage decrease in blood glucose levels. SEM: Standard error of means

CONCLUSION

In this study, the antihyperglycemic activity of *M. calabura L* stem bark extract was tested against normoglycemic, oral glucose tolerance test, and hyperglycemic rats. The results indicate the significant antidiabetic activity. This was may be due to the extraction of bioactive antidiabetic principles from *M. calabura L* using methanol as a solvent.

The active ingredient in the extract that reduces the blood sugar is unknown at present. There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic activity of *M. calabura L.* From the present study, it is apparent that the barks of *M. calabura L* possess hypoglycemic activity and it justifies the use of the barks of the plant for treating diabetes as suggested in the folklore remedies. Beneficial multiple activities such as manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of beta-cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in medicinal plants, offer an exciting opportunity to develop them into novel therapeutics [22].

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AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST STATEMENT

We declared that we have no conflicts of interest.

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