Bioscene

Bioscene
Volume- 21 Number- 04
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

In-Vivo and *In-Vitro* Assessment of Antidiabetic Activity of *Spathodea Campanulata* Leaves Against Experimentally Induced Diabetes

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Abstract

Problem: Diabetes mellitus is a chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycaemia. The pursuit of new therapeutic agents from medicinal plants has gained prominence due to limitations of conventional treatments. The study aims to explore the antidiabetic activity of ethanolic extracts of leaves of *Spathodeacampanulata* (EELSC) using experimentally induced diabetic models. **Approach:** Ethanolic extract of leaves of *Spathodea campanulata* (EELSC) was prepared by using soxhlet extraction process and evaluated for antidiabetic potential by using dexamethasone induced diabetes model. The dexamethasone induced diabetes model were divided into 5 groups using rats as experimental animals. The groups consist of a normal control group that receives normal saline, positive control group that receives dexamethasone (1 mg/kg, sc), a standard group that receives metformin (40 mg/kg), a low dose group that receives 200 mg/kg of EELSC, and a high dose group that receives 400 mg/kg of EESLC. Standard medications and all testing materials were administered for ten days. Body weight of the rats and biochemical indicators such as fasting blood sugar levels (FBS), oral glucose tolerance test (OGTT), lipid profile and *in vivo* antioxidant status using lipid peroxidation and catalase assay were evaluated. On the tenth day, the animals were sacrificed in order to assess the outcomes. This study also involved the assessment of *in-vitro* antidiabetic effect using α-amylase and α-glucosidase assays with acarbose serving as the standard medication. **Findings:** According to the current study findings, in the dexamethasone-induced diabetes model, the standard group OGTT improvements were highly significant (****P<0.001), but the high dosage group's results ranged from moderate (**P<0.01) to extremely significant (***P<0.001). At some time points, the low dose group had significant (*P<0.05) impacts. Changes in body weight and fasting blood sugar were found to follow similar trends. Lipid profiles were tested, low dose of EELSC shows less significant and high dose of EELSC exhibits more significant values. Antioxidant status of rats shows more significant values in high dose of EELSC when compared to low dose of EELSC. The *in-vitro* tests indicate dose dependent inhibition of α-amylase and α-glucosidase assays by EELSC, comparable to acarbose. **Conclusion:** The study concludes that the Ethanolic extract of leaves of *Spathodea* campanulata possess the antidiabetic activity

Keywords: Diabetes; Dexamethasone; Acarbose; Metformin; *Spathodea campanulata*; Antioxidant.

Introduction

Diabetes mellitus is a chronic metabolic disease characterised by high levels of blood glucose resulting from the impaired secretion of insulin, insulin insensitivity and inflammation response. After cancer and cardiovascular disease, diabetes has been ranked as the third most dangerous chronic illness to human health. Chronic hyperglycaemia damages several body tissues and organs, resulting in a number of chronic diabetes consequences, including retinopathy, neuropathy, and nephropathy.[1]

Many chronic diseases, including type 2 diabetes have been linked to oxidative stress. The production of free radicals during hyperglycaemia alters the way glucose is absorbed, results in abnormal glucose release from the liver, interferes with insulin secretion, interferes with the activity of antioxidant enzymes in plasma, and mediates metabolic pathways. One treatment approach for hyperglycaemia is to inhibit pancreatic amylase, an essential enzyme that breaks down dietary carbohydrates into simple monosaccharides, which are subsequently broken down by glucosidase to glucose, which is absorbed and enters the bloodstream. Therefore, inhibiting the glucosidase and amylase enzymes can reduce the digestion of carbohydrates, slow down the absorption of glucose, and lower blood sugar levels.[2]

Spathodea campanulata (Bignoniaceae),also known as the African tulip tree is one of the world's most exquisite flowering trees. It is grown as an ornamental tree in tropical and subtropical areas, including the United States, and is extensively distributed throughout Africa. *Spathodea campanulata* is used in traditional herbal medicine for the treatment of ulcers, filaria, gonorrhoea, diarrhoea and fever. [3]

A common synthetic glucocorticoid, dexamethasone is essential for the treatment of immune-related and inflammatory conditions. Dexamethasone administration has been connected to negative outcomes such diabetic mellitus and hypertension, so its therapeutic use is not without risks. Dexamethasone injection has been linked to disturbances in glucose homeostasis in animal models, especially rodents, which eventually result in diabetes. Dexamethasone induced insulin resistance, which is brought about by changes in the insulin signaling pathway and the encouragement of glycogen destruction, is responsible for this diabetogenic effect. Together, these consequences exacerbate hyperglycaemia and aid in the onset of diabetes. Moreover, it is well recognized that administering Dexamethasone can cause dyslipidaemia and a redox imbalance, both of which accelerate the onset and progression of problems associated with diabetes. [4]

Current allopathic treatment for diabetes is associated with side effects and adverse effects. Based on the above considerations the present study aimed to evaluate the antidiabetic activity of the ethanolic extract of leaves of *Spathodea campanulata* against experimentally induced diabetes in rats.

Materials and Methods

Collection and Authentication of Plant:

The leaves of*Spathodea campanulata* were collectedfromlocal areas of Chitradurga, Karnataka and they were washed, then the leaves were shade dried in fresh circulating air for three weeks. The selectedleaf material was identified and authenticated by botanist. The sample specimen is stored in institution museum.

Preparation of Plant Extract:[5]

The shade dried leaves of *Spathodea campanulata* were subjected to pulverisation.The powdered leaves of *Spathodea campanulata*was packedin Soxhlet apparatus and extracted with ethanol (70% v/v). The extract was filtered through Whatman No.1 filter paper. Concentrated under reduced pressure and stored in an airtight container for further use. The percentage yield of the corresponding extract was calculated.

Preliminary phytochemical screening:[6,7]

Preliminary phytochemical investigations were carried out on the ethanolic extract ofleavesof*Spathodea campanulata* forthe detection of various phytoconstituents by using standard procedures.

Experimental Animals:

Animal ethical clearance was obtainedfrom Institutional Animal Ethics Committee (IAEC) for experimental purpose **(Ref No: 03D/SJMCP/IAEC/September 2023/2022-23).** Healthy Adult Wistar Albino rats weighing about 150-200g of either sex was used for this study. The animals were obtained from Biogen Laboratory Animal Facility, Bangalore – 562107. Before the initiation of the experiment, the animals were acclimatized for 10 days and randomized under standard environmental conditions such as temperature (26 ± 2^0C), relative humidity (45-55%), and 12hrs light/dark cycle maintained as per Committee for Control and Supervision of Experiments on Animal (CCSEA) guidelines. All the animals were allowed free access to standard laboratory pellets and drinking water *ad libitum* under strict hygiene conditions.

Selection of Screening Dose:[8]

Screening of Antidiabetic activity dose was considered based on the literature of acute toxicity studies of Ethanolic Extract of leaves of *Spathodea campanulata* (EELSC) is given by oral route according to Organisation for Economic Co-operation and Development (OECD) guidelines 425. EELSC was found safe up to 2000mg/kg, so the dose is selected as follows: -**Low dose**: 200mg/kg and**High dose**400mg/kg.

***Invivo* Evaluation of Anti-Diabetic Activity:-**

Dexamethasone induced diabetes model.[9]

Procedure:

30 overnight fasted rats were divided into five groups of six rats each. Animals of each group i.e control, standard and test group was treated with a dose of normal saline, standard drug metformin(40mg/kg) and Ethanolic extracts of leaves of *Spathodea campanulata*at 200mg/kg and 400mg/kg respectively. One hour after drug treatment, the rats of groups 2 to 5 were subcutaneously administered with Dexamethasone (1mg/kg) each day all over the experiment (10 days). Body weight of the animals and Fasting Blood glucose level was estimated on the first day, just before all the treatments, on fifth and tenth day. Oral glucose tolerance test, lipid profile and antioxidant parameters such as lipid peroxidation and catalase levels was estimated in these rats on 10^{th} day.The animal was divided into five groups each group comprising of 6 rats.

Table 1: Experimental design for Dexamethasone induced diabetes.

Group 1 (Negative control)	Standard diet, water *ad libitum*
Group 2 (Positive control)	Normal saline (10ml/kg, p.o) + Dexamethasone (1mg/kg, sc) for 10days
Group 3 (Standard group)	Metformin (40mg/kg, p.o) + Dexamethasone (1mg/kg, sc) for 10days
Group 4 (Test group 1)	Low dose of EELSC (200mg/kg, p.o) + Dexamethasone (1mg/kg, sc) for 10days
Group 5 (Test group 2)	High dose of EELSC (400mg/kg, p.o) + Dexamethasone (1mg/kg, sc) for 10days

***Invitro* Evaluation of Anti-Diabetic Activity:-**

A. α- amylase inhibitory assay.[10]

Using the 3, 5-dinitrosalicylic acid (DNSA) technique, the α-amylase inhibition test was carried out. Amylase (10 mL), phosphate buffer (50 mL, 100 mM, pH=6.9), and plant extract (20 mL, 20–100 μg/mL) at varied quantities are combined. The substrate was then added, and the mixture was incubated at 37°C for 30 minutes with 20μL of 1 % soluble starch (in phosphate buffer, 100 mM, and pH 6.8). Following that, 100 μL of di-nitro salicylic acid (DNSA) reagent was added to the mixture, and it was then given 10 minutes to boil. Absorbance at 540 nm was measured using a UV-visible spectrophotometer.

Theα- amylase inhibition activity was calculated using the formula:

$$\textbf{\% of inhibition} = \frac{\textbf{Absorbance 1} - \textbf{Absorbance 2}}{\textbf{Absorbance 1}} \textbf{ x 100}$$

Where Absorbance 1 – control, Absorbance 2 – standard

B. α- glucosidase inhibitory assay.[11]

0.2ml ofα- glucosidase enzyme solution was prepared and preincubated with different concentrations of the test and standard drug solution for 5 minutes. To all the test tube 0.2ml of 37Mm sucrose are added. All the tubes were incubated for 30 minutes at 37°c to allow the enzymatic action and drug action. After 30min, the tubes are taken out from the incubator and heated at 100°c for 10 minutes. The liberated glucose is determined by glucose oxidase peroxidase method at 546nm and calculating with relative blank control.The α- glucosidase inhibition activity was calculated using the formula:

$$\textbf{\% of inhibition} = \frac{\textbf{Absorbance 1} - \textbf{Absorbance 2}}{\textbf{Absorbance 1}} \textbf{ x 100}$$

Where Absorbance 1 – blank, Absorbance 2 – standard/test

Biochemical Parameters

1. **Measurement of Body Weight and Fasting Blood Glucose.**[12]

   Weekly monitoring of body weight was done using a weighing scale until they were sacrificed and expressed as mean body weight in grams.

   Fasting Blood glucose levels was also determined using a caresens fit monitoring system by collecting blood drops on the test strips after tail pricking using a sharp surgical blade.

2. **Measurement of lipid profile.**[13]

   Plasma total concentration, Triglycerides, and HDL cholesterol was estimated by enzymatic colorimetric end point method using span diagnostic kit.

   LDL cholesterol and VLDL cholesterol was obtained by calculations using the formula provided in cholesterol diagnostic kit booklet.

3. **Oral glucose tolerance test (OGTT).**[14]

   At the end of experiment OGTT was performed on 12hr fasted rats. Glucose was administered into the stomach of the rats through a gastric catheter at the final dose of (2g/kg) body weight. The blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0 (immediately after glucose load), 30, 60, 90 and 120min after glucose administration. Blood glucose levels were estimated by the enzymatic glucose oxidase method using a commercial glucometer.

***In-vivo* Antioxidant parameters assessment:**

4. **Determination of Catalase assay.**[15]

   Catalase was measured in the brain homogenate by continuous spectrophotometric rate determination. Phosphate buffer (2.5 mL, pH 7.8) was added to the supernatant and incubated at 250° C for 30 min. After transferring

into the cuvette, the absorbance was measured at 240 nm spectrophotometrically. Hydrogen peroxide was added and change in absorbance was measured for 3 min. The values are expressed as µmol of H_2O_2 /min/mg wet tissue.

5. **Measurement of lipid peroxidation.** [16]
   Malondialdehyde (MDA) level was estimated as described by Satoh. 75mg of Thiobarbituric acid (TBA) is dissolved in 15% Trichloroacetic acid (TCA). To this 2.08ml of 0.2N HCl was added. The final volume was made up to 100ml using 15% TCA. 3.0ml of this reagent was then added to 0.75ml of brain homogenate. The test tubes were kept in a boiling water bath for 15 min.Then it was cooled and centrifuged for 10min at 10,000 rpm. Absorbance of the supernatant is read against the blank at 535nm. The results were expressed in mol/mg of protein.

Statistical analysis:

The data obtained from the above findings were subjected to statistical analysis using one way ANOVA followed by Tukey's Kramer Multiple Comparision test to assess the statistical significance of the results using Graph pad Prism software.

Results:

a. **Preliminary phytochemical screening:**
   Preliminary phytochemical screenings of EELSC shows positive results and confirm the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins,and terpenoids.
b. **Anti-diabeticactivity:**A test sample of *Spathodea campanulata* leaves was evaluated for antidiabetic activity by experimentally induced diabetes in rats by employing the Body weight of rats and biochemical markers such as Fasting Blood sugar (FBS) level, Oral glucose tolerance test (OGTT), lipid profile and Antioxidant status like catalase and lipid peroxidation, inWistarAlbinoratsofeithersexweighing 150-200g.

Dexamethasone Induced Diabetes

1. **Effect ofEthanolic extract of leaves of *Spathodea campanulata*on body weight by Dexamethasone induced diabetes.**
   The ethanolic extract of leaves of *Spathodea campanulata* wasevaluated for antidiabetic activity in dexamethasone induced diabetes model. The result indicated that, the dexamethasone treated animals produced a state of diabetes in positive control group, as evidenced by decreased weight. In contrast, metformin treated group (40 mg/kg) and EELSC (200 mg/kg & 400mg/kg) showed a significant inhibition of weight reduction when compared to positive control group. EELSC at high dose (400mg/kg) had shown a more significant(***$p<0.001$) effect when compared with low dose of EELSC (200mg/kg). The results are shown in Table 2.

Table 2: Effect of Ethanolic extract of leaves of*Spathodea campanulata*on body weight by Dexamethasone induced diabetes

Groups	Treatment	Body weight		
		Day 1	Day 5	Day 10
Group I	Negative control (Normal saline)	187.67±5.48	188.50±5.29	189.67±5.12
Group II	Positive control (dexamethasone 1 mg/kg)	179.17±12.27	170.00±11.10	167.33±10.91
Group III	Standard (dexa + Metformin) 40mg/kg	181.33±3.52	176.33±3.23***	169.50±4.20***
Group IV	Low dose (200mg/kg) of EELSC (Dexa + low dose)	183.17±2.35	177.33±2.02	168.50±1.72*
Group V	High dose (400mg/kg) of EELSC (Dexa + high dose)	187±1.78	183.83±1.35*	176.67±1.74**

Values were expressed as Mean ± SEM (n=6); Significance values are ***P< 0.001, **P < 0.01,*P < 0.05 and nsP > 0.05.

2. **Effect of Ethanolic extract of leaves of *Spathodea campanulata*on fasting blood glucose levels by Dexamethasone induced diabetes.**

The ethanolic extract of leaves of *Spathodea campanulata*was evaluated for antidiabetic activity in Dexamethasone induced diabetes model. The result indicated that, the Dexamethasone treated animals produced a state of diabetes in positive control group, as evidenced by increase in fasting blood glucose levels. In contrast, metformin treated group (40 mg/kg) and EELSC (200 mg/kg & 400mg/kg) showed a significant decrease in fasting blood glucose levels when compared to positive control group. EELSC at high dose (400mg/kg) had shown a more significant(**p<0.001) effect when compared with low dose of EELSC(200mg/kg). The effect of EELSC on fasting blood glucose levels by fructose pellet induced diabetes was depicted in table 3.

Table 3: Effect of Ethanolic extract of leaves of *Spathodea campanulata*on fasting blood glucose levels by Dexamethasone induced diabetes

Groups	Treatment	Fasting Blood glucose level of Rats		
		Day 1	Day 5	Day 10
Group I	Negative control (Normal saline)	75.50 ±5.53	76.33 ±4.84	82.66 ±3.09
Group II	Positive control (dexamethasone 1 mg/kg)	75.33 ± 3.33	139.00 ± 3.98	166.17 ±4.26

Group III	Standard (dexa + Metformin) 40mg/kg	76.16 ±1.42	120.50 ±1.83***	127.00±2.00***
Group IV	Low dose (200mg/kg) of EELSC + Dexa	75 ±1.39	132.50 ±5.07*	149.83 ±5.64*
Group V	High dose (400mg/kg) of EELSC+ Dexa	73.83 ±1.55	126.33 ±4.60**	139.83 ±5.64**

Values were expressed as Mean ± SEM (n=6); Significance values are ***P< 0.001, **P < 0.01,*P < 0.05 and nsP > 0.05.

3. Effect of Ethanolic extract of leaves of *Spathodea campanulata*on lipid profile by Dexamethasone induced diabetes.

In order to determine the possible anti-diabetic impact of *Spathodea campanulata* ethanolic leaf extract at several doses in dexamethasone-induced diabetic rats, the lipid profile was assessed as a biochemical measure on the tenth day of treatment. As a point of contrast, the Normal Control Group, which received normal saline treatment, showed normal lipid profile characteristics. After receiving dexamethasone (1 mg/kg), the Positive Control Group showed markedly increased levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and a decrease in high-density lipoprotein (HDL). This suggests that diabetes-related dyslipidemia was induced. LDL and HDL values in the Standard Group rats treated with dexamethasone and metformin (40 mg/kg) improved significantly (****p < 0.0001). Additionally, TC, TG, and VLDL levels were significantly lower (***P < 0.001) than in the positive control group, indicating the strong lipid-lowering and anti-diabetic benefits of metformin. TC, TG, LDL, and VLDL were significantly reduced (*P < 0.05) with a low dose of EELSC (200 mg/kg). Remarkably, HDL levels showed a relatively substantial improvement (**P < 0.01). This implies that the low dosage modifies lipids moderately, especially HDL. The EELSC group at a high dose (400 mg/kg) improved all lipid profile measures, including TC, TG, LDL, VLDL, and HDL, with a moderately significant effect (**, p < 0.01). The effects were not as robust as those of the regular metformin-treated group, but they were more noticeable than those of the low-dose group.The results are presented in table 4.

Table 4: Effect of Ethanolic extract of leaves of *Spathodea campanulata*on lipid profile by Dexamethasone induced diabetes.

Lipid profile						
Groups	**Treatment**	**TC**	**TG**	**LDL**	**VLDL**	**HDL**
Group I	Negative control (Normal saline)	50.83 ± 3.64	78.50 ± 7.40	74.00 ± 8.66	67.66 ± 7.77	62.83 ± 8.33
Group II	Positive control (dexamethasone 1 mg/kg)	91.83 ± 6.81	100.33 ± 13.59	98.50 ± 19.97	100.50 ± 20.82	84.83 ± 5.72
Group III	Standard (dexamethasone + Metformin) 40mg/kg	70.00 ± 3.53***	80.33 ± 7.89***	81.66 ± 6.76****	82.50 ± 7.21***	101.67 ± 5.28****
Group IV	Low dose (200mg/kg) of EELSC + dexamethasone	84.83 ± 1.81*	94 ± 3.42	93.33 ± 2.38	92 ± 3.42*	90.16 ± 3.80*
Group V	High dose (400mg/kg) of EELSC + dexamethasone	79.83 ± 2.55**	88 ± 1.61**	86.66 ± 1.60*	85 ± 1.57**	93.16 ± 2.52**

Values were expressed as Mean ± SEM (n=6); Significance values are ***P< 0.001, **P < 0.01,*P < 0.05 and nsP > 0.05.

4. **Effect of Ethanolic extract of leaves of *Spathodea campanulata*on Oral glucose tolerance test (OGTT) by Dexamethasone induced diabetes.**

The oral glucose tolerance test (OGTT), a crucial biochemical metric in this investigation, was carried out on the tenth day of the trial. To evaluate each group's ability to remove glucose, the test was run at 0 minutes (baseline) and then at 30, 60, 90, and 120 minutes. Rats were divided into five groups: the standard group received 40 mg/kg of dexamethasone and metformin, the normal control group received saline, the positive control group received 1 mg/kg of dexamethasone, the low dose received 200 mg/kg of dexamethasone and EELSC, and the high dose received 400 mg/kg of dexamethasone and EELSC. The OGTT results showed a significantly significant decrease in blood glucose levels at 30, 60, and 90 minutes in the Standard group (***P < 0.001). Furthermore, the

reduction was highly significant (***P < 0.001) at 120 minutes, suggesting that metformin is efficient in enhancing glucose tolerance in rats with diabetes induced by dexamethasone. The blood glucose reduction at 60 and 90 minutes was moderately significant for the high dose (400 mg/kg) group (**P < 0.01). A highly significant decrease was seen at 120 minutes (***P < 0.001), indicating that the larger dose of the ethanolic extract may have an antidiabetic impact. At 30 minutes, a substantial effect was also observed (*P < 0.05). Blood glucose levels in the low dose (200 mg/kg) test group decreased statistically significantly after 60 and 90 minutes (*P < 0.05), and moderately at 120 minutes (**P < 0.01).Both the regular group and the high-dose EELSC group showed considerably higher glucose clearance, especially at the 120-minute point, as compared to the positive control group, which displayed impaired glucose tolerance throughout the test. Although not as much, the low-dose EELSC group also showed improvement. The results are analysed in table 5.

Table 5: Effect of Ethanolic extract of leaves of *Spathodea campanulata*on Oral glucose tolerance test (OGTT) by Dexamethasone induced diabetes.

Groups	**Treatment**	**Oral Glucose tolerance test**				
		0 min	**30min**	**60 min**	**90 min**	**120 min**
Group I	Negative control (Normal saline)	84.16 ± 2.95	93.16 ± 2.65	91.83 ± 2.52	90.16 ± 2.83	88.83 ± 2.84
Group II	Positive control (dexamethasone 1 mg/kg)	167.17 ± 4.23	179.50 ± 3.14	177.33 ± 3.24	175.67 ± 3.26	174.17 ± 3.22
Group III	Standard (dexa + Metformin) 40mg/kg	128.67 ± 2.21	135.50 ± 2.37***	146.67 ± 9.15***	145.17 ± 9.13***	139.00 ± 8.32***
Group IV	Low dose (200mg/kg) of EELSC + Dexa	148.17 ± 1.30	155 ± 1.23	153 ± 1.23*	151.50 ± 1.11*	150.33 ± 1.20*
Group V	High dose (400mg/kg) of EELSC + Dexa	142.82 ± 1.66	150 ± 1.86*	148 ± 1.86**	146.17 ± 1.75**	144.33 ± 1.49**

Values were expressed as Mean ± SEM (n=6); Significance values are ***P< 0.001, **P < 0.01,*P < 0.05 and nsP > 0.05.

***In-vivo* Antioxidant parameters:**

5. **Effect of Ethanolic extract of leavesof*Spathodea campanulata* on Lipid Peroxidation and catalase assay by dexamethasone induced diabetes.**

In a study evaluating the effects of various treatments on lipid peroxidation and catalase activity in dexamethasone-induced diabetic rats, five groups were tested. The Positive Control Group showed elevated lipid peroxidation, indicating increased oxidative stress due to dexamethasone-induced diabetes. The Standard Group, treated with metformin, exhibited a significant reduction in lipid peroxidation (**P < 0.001), demonstrating strong antioxidant effects. The ethanolic extract of *Spathodea campanulata* also reduced lipid peroxidation in a dose-dependent manner, with a significant effect at 400 mg/kg (**P < 0.01), though less potent than metformin. Similarly, catalase activity was significantly lower in the Positive Control Group, indicating compromised antioxidant defenses. Metformin treatment significantly restored catalase activity (***P < 0.001), while the high-dose *Spathodeacampanulata* extract (400 mg/kg) showed a modest increase in catalase activity (*P < 0.01), suggesting a mild but dose-dependent antioxidant effect. Overall, *Spathodea campanulata* demonstrated antioxidant potential in diabetes but was less effective than metformin. The results were tabulated in table 6.

Table 6: Effect of Ethanolic extract of leavesof *Spathodea campanulata*on Lipid Peroxidation and catalase assay by dexamethasone induced diabetes.

Sl.No	Treatment	MDA (nmol/mgofprotein)	Catalase (µmol/min/mg protein)
Group I	Negative control	.14 ± 0.01	3.08±0.01
Group II	Positive control	.90 ± 0.02	0.89±0.05
Group III	Standard Metformin(40mg/Kg)	0.78 ± 0.14***	3.80±0.10***
Group IV	Low dose of EELSC (200mg/Kg)	1.45 ± 0.09	1.72 ± 0.5
Group V	High dose of EELSC (400mg/Kg)	1.38 ± 0.04*	2.73 ± 0.15*

Values were expressed as Mean ± SEM (n=6); Significance values are ***P< 0.001, **P < 0.01,*P < 0.05 and nsP > 0.05.

***Invitro* Antidiabetic Activity:**

The EthanolicExtractofleaves of *Spathodea campanulata* were used for evaluating their α amylase inhibitory activity using 3-5 dinitrosalicylic acid method, acarbose was used as standard comparison, α-glucosidase inhibitory activity using P-

nitrophenyl-α-glucopyranoside method acarbose was used as standard comparison.

1. **Assay for α-amylase Inhibitory Activity:**

The results of *in-vitro* antidiabetic activity using a-amylase inhibitory assay of the EthanolicExtractofleaves of *Spathodea campanulata* are portrayed in Table 7. The percentage inhibition at 20-100µg/ml concentrations of EELSC showed a dose dependent increase in percentage inhibition. The percentage inhibition of EELSC varied and have shown from 7.47% to 33.90% with an IC_{50} value of 91.03 µg/ml and Acarbose is a standard drug for a-amylase inhibitor. Acarbose at a concentration of (20-100µg/ml) showed have shown from 6.89 to 75.28% with an IC_{50} value of 48.13 µg/ml.

Table 7: *In-vitro* Anti diabetic activity of EELSC by α-amylase inhibitory assay

Sl. No	Concentration (µg/ml)	Acarbose		EELSC	
		Mean	% of inhibition	Mean	% of inhibition
1.	20	1.62	6.89 %	1.61	7.47 %
2.	40	1.31	24.71 %	1.52	13.79 %
3.	60	0.82	52.87 %	1.33	23.56 %
4.	80	0.51	71.92 %	1.28	26.43 %
5.	100	0.43	75.28 %	1.15	33.90 %
	IC_{50}	48.13 µg/ml		91.03 µg/ml	

2. **Assay for α-glucosidase inhibitory activity:**

The results of *in-vitro* antidiabetic activity using α-glucosidase inhibitory assay of the EthanolicExtractofleaves of *Spathodea campanulata*are expressed in Table 8. The percentage inhibition at 20-100µg/ml concentration of EELSC had shown a dose dependent increase in percentage inhibition. The percentage inhibitions of EELSC varied and have shown from 9.84 to 52.84% with an IC_{50} value of 92.19µg/ml and Acarbose is a standard drug for α- glucosidase inhibitor. Acarbose at a concentration of (20-100µg/ml) have shown from 24.87 to 66.83% with an IC_{50} value of 51.23 µg/ml.

Table 8: *In-vitro* Anti diabetic activity of EELSC by α-glucosidase inhibitory assay

Sl.No	Concentration (µg/ml)	Acarbose		EELSC	
		Mean	% of inhibition	Mean	% of inhibition
1.	20	1.45	24.87 %	1.74	9.84 %
2.	40	1.21	37.30 %	1.13	27.97 %
3.	60	0.91	52.84 %	1.39	41.45 %
4.	80	0.83	56.99 %	1.09	43.52 %

5.	100	0.64	66.83 %	0.91	52.84 %
	IC_{50}	51.23 µg/ml		92.19 µg/ml	

Discussion:

Diabetes mellitus (DM) is an endocrine disorder that is characterized by hyperglycemia and altered metabolism of carbohydrates, lipid and proteins. It is caused by inherited and/or acquired deficiency in production of insulin by beta cells of pancreas or by the ineffectiveness of the insulin produced, which leads to hyperglycemia, and at later stages lipid metabolism is also affected.[17]

Type 2diabetes is a complex illness that is often linked to a number of conditions, such as insulin resistance, obesity, hypertriglyceridemia, and poor glucose tolerance. A higher risk of type 2 diabetes may be linked to fructose consumption via a number of molecular pathways. Because of the positive energy balance, consuming more fructose may contribute to weight gain.[18]

Dexamethasone increases triglyceride levels, causing an imbalance in lipid metabolism leading to hyperlipidemia and an increase in glucose levels leading to hyperglycemia. Pharmacological doses of glucocorticoids induce gene expression in rat adipocyte tissue within 24 h. This is followed by complex metabolic changes resulting in decrease in food consumption, reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and triglyceride levels.[19]

In the present study, dexamethasone induction for 10 days in rats resulted in increased lipid profile, fasting glucose levels. A higher dose of EELSC (400mg/kg) prevented the rise in triglyceride and glucose caused by dexamethasone. Further, EELSC (400mg/kg) also prevented the progressive decrease in body weight caused by dexamethasone. The ethanolic extracts of leaves of *Spathodea campanulata* depictedthe hypoglycaemic effect in dexamethasone induced diabetes in rats. However, further studies are needed to establish their action mechanism.

An important target for managing diabetes is α-amylase, an enzyme involved in the conversion of starch to glucose, whose inhibition can effectively lower postprandial hyperglycemia. The last stage of the digestion of carbohydrates is catalyzed by the enzyme α-glucosidase, which transforms oligosaccharides into monosaccharides that are quickly absorbed in the small intestine. By delaying the absorption of glucose, inhibiting this enzyme aids in blood sugar regulation. The starch-iodine assay evaluates an extract's capacity to prevent starch digestion, a sign of its capacity to prevent the release of glucose from carbohydrates.

In the current study, the EELSC demonstrated significant α-amylase inhibitory activity in comparision to the standard drug acarbose, suggesting its ability to reduce the rapid breakdown of carbohydrates. The EELSC exhibited noteworthy α-glucosidase inhibitory activity, comparable to acarbose, further highlighting its potential in managing postprandial glucose levels. The dual

inhibition of both α-amylase and α-glucosidase is a promising mechanism for controlling glucose levels more effectively, making EELSC a potential natural alternative for diabetic management. In this study, the EELSC effectively inhibited starch breakdown, as evidenced by the starch-iodine assay, further confirming the extract's role in delaying carbohydrate digestion and absorption.

In light of the results this study indicates that *Spathodea campanulata* have good antidiabetic activity. The Ethanolic extract of leaves of *Spathodea campanulata* exhibited anti-hyperglycaemic activity in dexamethasone induced diabetes modeland *in-vitro* models.

Conclusion:

The study found that the EELSC exhibited significant antidiabetic potential against dexamethasone induced diabetes in Rats. Using various parameters and antioxidant models, the leaf extract demonstrated effectiveness in enhancing body weight, fasting blood glucose levels, lipid profiles, oral glucose tolerance test and increased catalase activity, reduced lipid peroxidation. The results of *in-vitro* tests suggest that the extract exhibitshypoglycemic effects. This suggests that the extract has notable antidiabetic properties, likely due to the presence of compounds like alkaloids, flavonoids,tannins,steroids. However, further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of *Spathodeacampanulata*extract So, that it can be better projected as a therapeutic agent for anti-diabetic activity.

Acknowledgement:

I would like to convey my heartfelt gratitude and respectful thanks to S.J.M College of Pharmacy, Chitradurga for providing necessary facilities to carry out this work.

Research funding:

The funding for the present study was provided by SJM College of Pharmacy, SJM Campus, Chitradurga-577502, Karnataka, India

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