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In-vivo and *In-vitro* Screening of Anti-Diabetic Activity of *Pongamia pinnata* Leaves on Experimentally Induced Diabetes

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Abstract

Diabetes mellitus, a chronic metabolic disorder marked by hyperglycemia, affects millions worldwide. This study explores the antidiabetic potential of ethanolic extract of *Pongamia pinnata* leaves (EEPPL) using dexamethasone-induced diabetic rat models. EEPPL was prepared via Soxhlet extraction and tested in five groups (n=6 each): normal control (saline), positive control (dexamethasone 1 mg/kg), standard (metformin 40 mg/kg), low dose (EEPPL 250 mg/kg), and high dose (EEPPL 500 mg/kg). Treatments were administered for 10 days, with assessments on day 10 including body weight, fasting blood glucose (FBS), oral glucose tolerance test (OGTT), lipid profile, lipid peroxidation, and catalase levels. In vitro α-amylase and α-glucosidase assays were also conducted with acarbose as a reference. Results showed significant antidiabetic and antioxidant effects in dexamethasone-induced diabetic rats. The standard group had highly significant improvements in FBS and OGTT. The high-dose EEPPL group exhibited moderate to highly significant effects on FBS, OGTT, and lipid profile, with weight improvements and reductions in cholesterol levels. Antioxidant assessments revealed reduced lipid peroxidation and increased catalase activity in the high-dose group. The in vitro assays demonstrated dose-dependent inhibition of α-amylase and α-glucosidase, comparable to acarbose. In conclusion, the study suggests that EEPPL has notable antidiabetic and antioxidant properties, supporting its potential as a natural therapeutic for managing diabetes and oxidative stress.

Keywords: Diabetes, Dexamethasone, Metformin, *Pongamia pinnata*, *In-vivo*, Anti-oxidant.

INTRODUCTION

Diabetes is a complex multisystem disorder [1] which is characterized by hyperglycaemia resulting from defects in insulin secretion, action or both. It is made up of two types: Type I and Type II. Type I diabetes often referred to as juvenile diabetes, which is insulin dependent and known to affect only 5% of the diabetic population. The Type II, which is non-insulin dependent, usually develops in adults over the age of 40. It has already been established that chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels. It has an adverse effect on carbohydrate, lipid and protein metabolism resulting in chronic hyperglycaemia and abnormality of lipid profile. These lead to series of secondary complications including polyurea, ketosis, retinopathy as well as cardiovascular disorder [2].

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials [3].

The '*Pongamia Tree'* is known as one of the richest and brightest trees of India. The tree is named as '*Pongamia pinnata'* in science. The tree is member of

the 'Leguminosae' family. It contains various phytoconstituents belonging to alkaloids, glycosides, flavonoids, fixed oils, and carbohydrates. Leaves of *Pongamia pinnata* possess activities like digestive, laxative, anthelmintic and are good for diarrhea, leprosy, dyspepsia and cough [4]. Thus, the present study was undertaken to evaluate the antidiabetic effect of *Pongamia pinnata* leaves in experimentally-induced diabetic rats. A comparison was made between the action of ethanolic extract of *Pongamia pinnata* Leaves (EEPPL) and a known antidiabetic drug metformin [5].

MATERIALS AND METHODS

Collection and Authentication of plant:

The Leaves of *Pongamia pinnata* were collected from local areas of Chitradurga, Karnataka and they were washed, then the leaves were dried in fresh circulating air under shade for fifteen days. Selected leaf material was identified and authenticated by Botanist. The sample specimen is stored in institute museum.

Preparation of Plant Extract [5]:

Pongamia pinnata leaves were collected, cleaned, shade dried at room temperature and pulverised. The coarse powder of leaves of *Pongamia pinnata* was packed in Soxhlet apparatus and extracted with ethanol (70% v/v) as a solvent at 60°C for 21 days. The extract was filtered through Whatman No.1 filter paper, concentrated under reduced pressure and extract is stored in an airtight container for further use. The percentage yield of the corresponding extract was calculated.

Preliminary phytochemical investigation [6]:

The obtained extract was subjected to the preliminary phytochemical investigation to detect the presence of phytoconstituents.

Experimental Animals:

Animal ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) for experimental purpose **(Ref No:3A/SJMCP/IAEC/September2023/2022-23).** 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 randomized into various groups and acclimatized for 10 days under standard environmental conditions such as temperature (26±2ºC), 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 [5]:

Screening of anti-diabetic activity dose was selected based upon previous literature. After detailed literature survey we found and fixed the dose of experimental studies which is given by oral route according to Organization for Economic Co-operation and Development (OECD) guidelines 423. The dose for the study is fixed as, Low dose: 250 mg/kg and High dose: 500mg/kg**.**

***IN-VIVO* ANTI-DIABETIC EXPERIMENTAL MODEL:**

Dexamethasone induced insulin resistance model [7]

30 overnight fasted rats were divided into five groups of six rats each which includes control, standard and treated will be given a dose of normal saline, standard drugs(subcutaneous) and extracts of *Pongamia pinnata* leaves at 250mg/kg and 500mg/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 and fasting blood glucose level was estimated on the first, fifth and tenth day just before all the treatments, also the oral glucose tolerance test and lipid profile in addition to that, the *in-vivo* anti-oxidant models like lipid peroxidation and catalase assays were performed in these rats on the 10^{th} day.

Experimental design:

Groups Treatment

Group I: Standard diet, water *ad libitum*
Group II: Normal saline (10ml/kg, p.o.) + Dexamethasone (1mg/kg, Sc) for 10days
Group III: Metformin (40mg/kg, p.o.) + Dexamethasone (1mg/kg, Sc) for 10days
Group IV: Low dose of EEPPL *(*250mg/kg, p.o.) + Dexamethasone (1mg/kg, Sc) for 10days
Group V: High dose of EEPPL *(*500mg/kg, p.o.) + Dexamethasone (1mg/kg, Sc) for 10days

***IN-VITRO* ANTI-DIABETIC EXPERIMENTAL MODELS:**

I. α- amylase inhibition activity [8]

The plant extract of different concentration such as 50, 100, 250, 500 and 1000µg were taken and dissolved with 0.25ml of α- amylase solution and mixed thoroughly. The sample was incubated at 37°c for 5minutes. Added 5ml of starch solution and incubate for 3 minutes at 37°c. Then 3ml of DNSA (3-dinitrosalicylic acid) reagent was added and boiled at 100°c for 5 minutes to stop the reaction. The reaction mixture was cooled to room temperature and the absorbance was read at 540nm in spectrophotometer. The α- amylase inhibition activity was calculated using the formula:

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

Where,

Absorbance 1 – control
Absorbance 2 – standard

II. α- glucosidase inhibition activity [9]

0.2ml of α- 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 was added. All the tubes were incubated for 30 minutes at 37°c to allow the enzymatic action and drug action. After 30min, the tubes were 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:

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

Where,

Absorbance 1 – blank

Absorbance 2 – standard/test

Biochemical parameters assessed:

1. Determination of body weight [10]:

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

2. Determination of Fasting Blood glucose [10]:

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

3. Determination of lipid profile [11]:

Triglycerides, and HDL cholesterol were estimated by enzymatic colorimetric end point method using span diagnostic kit. LDL cholesterol and VLDL cholesterol were obtained by calculations using the formula provided in cholesterol diagnostic kit booklet.

4. Oral glucose tolerance test (OGTT) [12]:

At the end of experiment OGTT were performed on 12hr fasted rats. Glucose was administered into the stomach of the rats through a gastric catheter at the final dose of (2mg/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 models assessed:**

5. Determination of lipid peroxidation [13]:

Malondialdehyde (MDA) level was estimated by below procedure.75mg of Thiobarbituric acid (TBA) is dissolved in 15% trichloroacetic acid (TCA). To this 2.08ml of 0.2N HCl were 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**.**

6. Determination of Catalase assay [14]:

Catalase was measured in the brain homogenate by continuous spectrophotometric rate determination by the Beers and Sizer method for antioxidant status. Phosphate buffe0r (2.5ml, 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 measure for 3 min. The value is expressed as µmol of H_2O_2/min/mg wet tissue.

Statistical Analysis

The data obtained from the above findings was subjected to statistical analysis using one way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results by using GraphPad prism.

RESULTS

a) Preliminary phytochemical screening:

Preliminary phytochemical screening of EEPPL leaves confirm the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, amino acid, and carbohydrate.

b) Anti-diabetic activity:

A test sample of *Pongamia pinnata* Leaves were evaluated for anti-diabetic activity by employing the behavioural and biochemical test in Wistar albino rats of either sex weighing 150-200g.

***IN-VIVO* ANTI-DIABETIC ACTIVITY:**

Dexamethasone induced diabetes:

1. Effect of Ethanolic extract of *Pongamia pinnata* on body weight by dexamethasone induced diabetes.

Body weight in this group showed significant recovery compared to the positive control group. Highly significant improvement (***$p < 0.001$) was observed on both day 5 and day 10, indicating the efficacy of metformin in mitigating the dexamethasone-induced weight loss and, by extension, its antidiabetic activity. In low dose of EEPPL (250mg/kg) rats exhibited a moderate improvement in body weight. On day 10, a significant improvement (*$p < 0.05$) was observed compared to the positive control group, suggesting a modest effect of the low-dose extract in reducing the catabolic effects of dexamethasone. High dose of EEPPL (500 mg/kg) group showed better results compared to the low-dose group. A significant increase in body weight was noted on day 5 (*$P < 0.05$) and a more pronounced effect on day 10 (*$P < 0.01$), indicating a dose-dependent response of the extract in counteracting the weight loss induced by dexamethasone. The results are showed in Table 1.

Table 1: Effect of Ethanolic extract of *Pongamia pinnata* leaves on body weight by dexamethasone induced diabetes

Body weight				
Groups	**Treatment**	**Average 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 1mg/kg)	179.17±12.27	170.00±11.10	167.33±10.91
Group III	Standard (dexa+metformin 40mg/kg)	181.33±7.52	180.33±7.05***	179.83±5.90***
Group IV	Low dose(250mg/kg) of EEPPL (Dexa + low dose)	180.33±3.52	176.00±3.23	169.50±4.20*
Group V	High dose(500mg/kg) of EEPPL (Dexa + high dose)	190.67±4.92	178.33±4.36*	175.00±4.50**

Values were expressed as Mean ± SEM (n=6); Significance values are: ***P < 0.001, **P < 0.01, *P < 0.05 and nsP > 0.05. positive Control group vs all groups.

2. Effect of Ethanolic extract of *Pongamia pinnata* leaves on Fasting blood glucose level by dexamethasone induced diabetes.

In this present study, with regarding of fasting blood Glucose levels there was no significant difference showed on day 1 between the groups, confirming the initial diabetic state induced by dexamethasone in all groups except the normal control. On day 5 the Standard Group showed a highly significant reduction (***p < 0.001) in fasting blood glucose levels compared to the Positive Control Group, indicating the effectiveness of metformin in reducing hyperglycaemia. High dose also demonstrated a moderately significant reduction (**p < 0.01) in blood glucose levels. Low dose showed a lower but statistically significant reduction (*p < 0.05) in fasting blood glucose levels compared to the Positive Control Group. On 10th day the Standard Group continued to show a highly significant reduction (***p < 0.001) in blood glucose levels compared to the Positive Control Group. High dose exhibited a moderately significant decrease (p < 0.01), while Test Group 1 maintained a statistically significant reduction (p < 0.05) in fasting blood glucose levels. The results are depicted in Table 2.

Table 2: Effect of Ethanolic extract of *Pongamia pinnata* leaves on Fasting blood glucose level by dexamethasone induced diabetes

Fasting blood glucose level				
Groups	**Treatment**	**Blood glucose level**		
		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 1mg/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(250mg/kg) of EEPPL (Dexa + low dose)	74.00±3.81	134.83±4.61*	154.33±3.76*
Group V	High dose(500mg/kg) of EEPPL (Dexa + high dose)	73.66±4.24	128.50±4.16**	144.17±6.88**

Values were expressed as Mean ± SEM (n=6); Significance values are: ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. positive Control group vs all groups.

3. Effect of Ethanolic extract of *Pongamia pinnata* leaves on Oral glucose tolerance test by dexamethasone induced diabetes.

The test was performed at 0 minutes (baseline), followed by intervals at 30, 60, 90, and 120 minutes to assess the glucose clearance capacity of each group. In Standard group the OGTT results in this group demonstrated a highly significant reduction in blood glucose levels at 30, 60, and 90 minutes (***P < 0.001). Moreover, at 120 minutes, the reduction was also highly significant (****P < 0.001), indicating the effectiveness of metformin in improving glucose tolerance in dexamethasone-induced diabetic rats. high dose (500 mg/kg**)** group exhibited moderate significance in blood glucose reduction at 60 and 90 minutes (**P < 0.01). At 120 minutes, a highly significant reduction was observed (****P < 0.001), confirming the potential antidiabetic effect of the higher dose of the ethanolic extract. A significant effect was also noted at 30 minutes (*P < 0.05). The low dose **(**250 mg/kg**)** test group showed a statistically significant reduction in blood glucose at 60 and 90 minutes (*P < 0.05), with moderate significance at 120 minutes (**P < 0.01). The results are tabulated in Table 3.

Table 3: Effect of Ethanolic extract of *Pongamia pinnata* leaves on oral glucose tolerance test by dexamethasone induced diabetes

Oral Glucose Tolerance Test						
Group	**Treatment**	**0min**	**30min**	**60min**	**90min**	**120min**
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 1mg/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(250mg/kg) of EEPPL (Dexa + low dose)	155.83 ± 3.67	167.33 ± 3.80	165.83 ± 3.92*	164.33 ± 3.79*	163.00 ± 3.96**
Group V	High dose(500mg/kg) of EEPPL (Dexa + high dose)	145.67 ± 6.82	155.00 ± 6.26*	153.00 ± 6.42**	151.33 ± 6.40**	150.17 ± 6.33****

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001 ***P < 0.001, **P < 0.01, *P < 0.05 and nsP > 0.05. positive Control group vs all groups.

4. Effect of Ethanolic extract of *Pongamia pinnata* leaves on Lipid profile by dexamethasone induced diabetes.

In the present study, the lipid profile was evaluated as a biochemical parameter on the 10th day of treatment to assess the potential anti-diabetic effect of *Pongamia pinnata* ethanolic leaf extract at different doses in dexamethasone-induced diabetic rats. Normal Control Group treated with normal saline, this group displayed normal lipid profile parameters, serving as a baseline for comparison. Positive Control Group treated with dexamethasone (1 mg/kg), this group exhibited significantly elevated levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and a decrease in high-density lipoprotein (HDL), indicating the induction of dyslipidaemia, which is characteristic of diabetes. Standard Group the rats treated with dexamethasone and metformin (40 mg/kg) showed highly significant improvement (****p < 0.0001) in LDL and HDL levels. There was also a highly significant reduction (***P < 0.001) in TC, TG, and VLDL levels compared to the positive control group, demonstrating the potent lipid-lowering and anti-diabetic effects of metformin. Low Dose (250 mg/kg) of EEPPL showed a significant reduction (*P < 0.05) in TC, TG, LDL, and VLDL. Interestingly, it exhibited a moderately significant improvement (**P < 0.01) in HDL levels. This suggests that the low dose has a moderate lipid-modulating effect, particularly on HDL. High Dose (500 mg/kg) of EEPPL group showed a moderate significant effect (**P < 0.01) in improving all lipid profile parameters, including TC, TG, LDL, VLDL, and HDL. The effects were more pronounced than in the low-dose group but not as strong as the standard group treated with metformin. The results are analysed in Table 4.

Table 4: Effect of Ethanolic extract of *Pongamia pinnata* leaves 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 1mg/kg)	91.83 ± 6.81	100.33 ± 13.59	98.50 ± 19.97	100.50 ± 20.82	84.83 ± 5.72
Group III	Standard (dexa+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(250mg/kg) of EEPPL (Dexa + low dose)	86.00 ± 6.50*	95.33 ± 25.27*	92.66 ± 4.52*	91.83 ± 9.14*	88.50 ± 6.44**
Group V	High dose(500mg/kg) of EEPPL (Dexa + high dose)	77.83 ± 2.45**	89.00 ± 10.41**	85.33 ± 5.05**0	85.66 ± 7.52**	91.83 ± 3.44**

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and nsP > 0.05. positive Control group vs all groups.

***In-vivo* Antioxidant Models:**

Effect of Ethanolic extract of *Pongamia pinnata* leaves on Lipid Peroxidation and catalase assay by dexamethasone induced diabetes

In this study on dexamethasone-induced diabetic rats over 10 days, lipid peroxidation and catalase activity were evaluated as key markers of oxidative stress. The positive control group, treated with dexamethasone alone, showed significantly elevated lipid peroxidation and reduced catalase activity, indicating increased oxidative stress. The standard group, receiving dexamethasone and metformin, exhibited a highly significant reduction in lipid peroxidation (***P < 0.001) and a marked increase in catalase activity (***P < 0.001) compared to the positive control, reflecting metformin's strong antioxidative and antidiabetic effects. In the test groups treated with ethanolic extract of *Pongamia pinnata* leaves, the low dose (250 mg/kg) led to a modest reduction in lipid peroxidation (*P < 0.05) and a slight, non-significant improvement in catalase activity, suggesting mild antioxidant effects. The high dose (500 mg/kg) had a more pronounced impact, with significant reductions in lipid peroxidation (**P < 0.01) and improved catalase activity (**P < 0.01), indicating a dose-dependent antioxidant effect. Overall, while *Pongamia pinnata*

extract showed promise, particularly at higher doses, its effects were lower than those of metformin, underscoring metformin's superior antioxidant and antidiabetic properties in mitigating dexamethasone-induced oxidative stress. The results are showed in Table 5.

Table 5: Effect of Ethanolic extract of *Pongamia pinnata* leaves on Lipid Peroxidation and catalase assay after dexamethasone induced diabetes

Sl. No	Treatment	MDA (nmol /mg of protein)	Catalase (µmol/min/mg protein)
Group I	Negative control	1.14 ± 0.01	3.08±0.01
Group II	Positive control	1.90 ± 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 EEPPL (250mg/Kg)	1.43 ±0.08	1.65±0.322
Group V	High dose of EEPPL (500mg/Kg)	1.32±0.03*	2.57±0.13**

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05 and ****P* < 0.001. Positive control group vs all groups.

***INVITRO* ANTIDIABETIC ACTIVITY:**

Assay for α-amylase Inhibitory Activity:

Alpha-amylase is a key enzyme involved in the breakdown of starch into simpler sugars, particularly maltose and glucose. Inhibition of alpha-amylase can slow down the release of glucose into the bloodstream after meals, thus helping to manage postprandial hyperglycaemia, a common issue in diabetic patients. The results of *in-vitro* antidiabetic activity using a-amylase inhibitory assay of the Ethanolic Extract of leaves of *Pongamia pinnata* of the percentage inhibition at 20-100µg/ml concentrations showed a dose dependent increase in percentage inhibition. The percentage inhibition of EEPPL varied and have shown from 4.02% to 46.55% with an IC50 value of 93.06 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 IC50 value of 48.13 µg/ml. The results are shown in Table 6.

Table 6: *In-vitro* Anti diabetic activity of EEPPL using α-amylase inhibitory assay

Sl. No	Concentration (µg/ml)	Acarbose		EEPPL	
		Mean	% of inhibition	Mean	% of inhibition
1.	20	1.62	6.89 %	1.67	4.02%
2.	40	1.31	24.71 %	1.43	17.81 %
3.	60	0.82	52.87 %	1.31	24.71 %
4.	80	0.51	71.92 %	1.12	35.63 %
5.	100	0.43	75.28 %	0.93	46.55 %
	IC_{50}	**48.13 µg/ml**		**93.06 µg/ml**	

α-glucosidase inhibitory activity:

Alpha-glucosidase, another enzyme that plays a significant role in carbohydrate digestion, is responsible for converting disaccharides into absorbable monosaccharides in the small intestine. By inhibiting this enzyme, the absorption of glucose into the bloodstream can be delayed, helping to prevent rapid increases in blood sugar levels following a meal. The results of *in-vitro* antidiabetic activity using α-glucosidase inhibitory assay of the Ethanolic Extract of leaves of *Pongamia pinnata* of the percentage inhibition at 20-100µg/ml concentration of EEPPL shown a dose dependent increase in percentage inhibition. The percentage inhibition of EEPPL varied and have shown from 3.62% to 62.69% with an IC50 value of 84.10µg/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 IC50 value of 51.23 µg/ml. The results are analysed in Table 7.

Table 7: *In-vitro* Anti diabetic activity of EEPPL using α-glucosidase Inhibitory assay

Sl. No	Concentration (µg/ml)	Acarbose		EEPPL	
		Mean	% of inhibition	Mean	% of inhibition
1.	20	1.45	24.87 %	1.86	3.62 %
2.	40	1.21	37.30 %	1.57	18.65 %
3.	60	0.91	52.84 %	1.33	31.08 %
4.	80	0.83	56.99 %	1.09	43.52 %
5.	100	0.64	66.83 %	0.72	62.69 %
	IC_{50}	**51.23 µg/ml**		**84.10 µg/ml**	

DISCUSSION

The origin and etiology of DM can vary greatly but always include defects in either insulin secretion or response or in both at some point in the course of disease. Mostly patients with diabetes mellitus have either type 1 diabetes (which is immune-mediated or idiopathic) Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycaemia, insulin resistance, and relative insulin deficiency [15].

The phytochemical screening of *Pongamia pinnata* revealed the presence of various flavonoids, fur flavones, triterpenoids, carbohydrates, tannins, phytosterols and other polyphenolic compounds [16]. The presence of such properties suggests a multifaceted mechanism of action, which may contribute to its observed antidiabetic. Screening of anti-diabetic activity dose was selected based upon previous literature. After detailed literature survey we found and fixed the dose of experimental studies which is given by oral route according to Organisation for Economic Co-operation and Development (OCED) guidelines 423. The dose for the study is fixed as, Low dose: 250 mg/kg and High dose 500mg/kg [5].

In our present study 30 overnight fasted rats were divided into 5 groups of 6 rats each and diabetes was induced in rats by feeding the animals with dexamethasone 1mg/kg for 10 days. Animals of each group i.e. control, standard and treated were given a dose of normal saline, standard drug like metformin and extracts of *Pongamia pinnata* leaves at the dose of 250mg/kg and 500mg/kg respectively. After administration we have screened the biochemical parameters for the assessment of diabetic status. Assessing the body weight in this study allowed us to monitor the catabolic effects of dexamethasone evaluated the protective or restorative effects of the treatments. The significant reductions in fasting blood glucose levels were observed. OGTT results suggest the effectiveness of treatment interventions in regulating blood glucose levels. The assessment of lipid profile which includes TC, TG, LDL, VLDL, and HDL are one of the significant biochemical parameters in evaluating the efficacy of anti-diabetic treatments. In this present study, the administration of the ethanolic extract of *Pongamia pinnata* showed a dose-dependent improvement in glucose regulation, comparable to the standard antidiabetic drug, metformin. The modulation of lipid levels suggests that it may offer both glycaemic control and protection against diabetes-related lipid abnormalities. The *In-vivo* anti-oxidant assessment of lipid peroxidation showed reduction in lipid peroxidation levels which can help to mitigate oxidative damage, which may contribute to better glycaemic control. Evaluating Catalase activity serves as a key indicator of the antioxidant and antidiabetic potential of the treatments which is identified by increase in the catalase levels in all treatment groups.

In the present study, the *In-vitro* assays like α-amylase inhibition assay and α-glucosidase inhibition assays evaluated the antidiabetic potential of bioactive compounds and it was assessed for its inhibitory activity against these carbohydrate-metabolizing enzymes which provides valuable insights into its potential mechanism in managing diabetes mellitus.

α-amylase is an enzyme in the breakdown of starch into glucose, and its inhibition can effectively reduce postprandial hyperglycaemia, making it a significant target for diabetes management. The α-glucosidase enzyme catalyses the final step in carbohydrate digestion, converting oligosaccharides into monosaccharides, which are rapidly absorbed in the small intestine. Inhibiting this enzyme delays glucose absorption, thus helping control blood sugar levels.

In the current study, the EEPPL demonstrated significant α-amylase inhibitory activity in comparison to the standard drug acarbose, suggesting its ability to reduce the rapid breakdown of carbohydrates. The EEPPL 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 EEPPL a potential natural alternative for diabetic management.

CONCLUSION

The present study concludes that the effect of Ethanolic extract of *Pongamia pinnata* leaves exhibited significant dose dependent anti-diabetic activity against dexamethasone induced diabetes in rats. Hence, the research justifies that EEPPL can be effectively used in the treatment of anti-diabetic Activity. However, further study is required for identification and isolation of its active constituents and to confirm its extract mechanism of protection. So, that it can be better projected as a therapeutic agent for anti-diabetic activity.

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