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Original Research Article

## Antidiabetic effect of *Pterocarpus marsupium* seed extract in gabapentin induced diabetic rats

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### ABSTRACT

**Background:** Type 2 diabetes mellitus is the most common type of diabetes. Diabetes mellitus is a leading cause of morbidity and mortality among Indian population and all over the world with more than hundreds of millions of patients worldwide. *Pterocarpus marsupium* is a medicinal plant used in Ayurvedic system of medicine to control blood sugar and strong antidiabetic. The purpose of this study was to assess the hypoglycemic effect of the ethanolic extract of *Pterocarpus marsupium* seeds in diabetic rats.

**Methods:** The present work was designed to evaluate the anti-hyperglycaemic activity of *Pterocarpus marsupium* seed extract (100 mg/kg and 200 mg/kg) on gabapentin induced hyperglycaemia in wistar albino rats. Blood glucose level, serum triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol were evaluated in gabapentin induced diabetic rats. The results of the test drug were compared with the standard drug.

**Results:** Ethanolic seed extract of *Pterocarpus marsupium* at 100 mg/kg and 200 mg/kg had significantly reduced the blood glucose level compared to disease control rats on day 1, 7, 14 and 21. *Pterocarpus marsupium* shows significant decrease in triglycerides levels, serum cholesterol levels, LDL levels and increased HDL levels, total protein levels compared to the disease control group.

**Conclusions:** In conclusion, the present study shows that the ethanolic seed extract of *Pterocarpus marsupium* has potential antidiabetic action in gabapentin induced diabetic rats and the effect was found to be more similar to the standard drug metformin.

**Keywords:** Diabetes mellitus, Gabapentin, Metformin, *Pterocarpus marsupium*

### INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Diabetes contains a

number of chronic complications like nephropathy, neuropathy, retinopathy and cardiovascular diseases. The individual with diabetes has a 20-fold increase in the risk of renal failure, a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of amputation and a 2 fold increased risk of coronary heart disease and ischemic brain damage.<sup>1</sup>

The heartwood of *Pterocarpus marsupium* is used in the treatment of inflammation and reported to have medicinal

importance in the management of diabetes since long.<sup>2</sup> The flavonoids and phenolic contents present in the tree viz., marsupin, pterosupin, and liquiritigenin are reported to have antihyperglycemic and antihyperlipidemic activities.<sup>2-4</sup> *Pterocarpus marsupium* extracts may serve as a potential source of natural antioxidant as well as for the treatment of diabetes.<sup>5-7</sup>

Serum lipid levels in rats with hyperlipidemia induced by diet as well as by triton were determined after oral administration of EtOAc extract of *Pterocarpus marsupium* heartwood and its flavonoid constituents, marsupsin, pterosupin, and liquiritigenin. Administration of EtOAc extract for 14 consecutive days produced a significant reduction of serum triglyceride, total cholesterol, and LDL and VLDL cholesterol levels without any significant effect on the level of HDL cholesterol.<sup>8</sup>

The ethanolic extract (100 mg/kg body weight) when given to streptozotocin (STZ) induced diabetic rats for 10 consecutive days declined blood glucose, improved oral glucose tolerance test and increased their serum insulin levels. The ethanolic extract also showed marked improvement on oral glucose tolerance on high fat-low dosed STZ-induced diabetic rats and neonatally STZ treated rats. The ethanolic extract of *Pterocarpus marsupium* also showed marked anti-dyslipidemic effects on high fat diet fed Syrian golden hamsters.<sup>9</sup>

*Pterocarpus marsupium* has also been documented to help in regeneration of pancreatic  $\beta$ -cells.<sup>10,11</sup> The active antidiabetic ingredients in the aqueous extract has been identified as (-) epicatechin, a benzopyran which on administration to alloxan-induced diabetic rats increased insulin secretion and number of islets in the pancreas. Insulin like activity of (-) epicatechin has been reported.<sup>12</sup> Heartwood of *Pterocarpus marsupium* has also been tested clinically and found effective in non-insulin dependent diabetes mellitus patients (type 2DM).<sup>13</sup>

The purpose of this experimental study is to investigate the antidiabetic activity of *Pterocarpus marsupium* seed extract on gabapentin induced diabetes. The anti-diabetic activity was evaluated by measuring the blood glucose levels, lipid profile and histopathological examination.

## METHODS

Male wistar albino rats weighing 200 - 250 gm were used in the study. They were housed and maintained under standard condition in the central animal house. The rats were kept in clean, clear polypropylene cages in groups of three in each cage maintained at 10 hours of light and 14 hours dark cycle. They were maintained under standard environmental conditions and fed with standard pellet diet (laboratory animal feeds) and water ad libitum.

## Identification and collection

*Pterocarpus marsupium* belongs to the family Fabaceae, class *Magnoliopsida*. Its common names are Indian kino tree, Malabar kino tree. *Pterocarpus marsupium* Roxb is a large deciduous tree commonly found in hilly region of India. The plant is distributed in India (Andhra Pradesh, Bihar, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Karnataka, Orissa, Tamilnadu and Uttar Pradesh), Nepal, SriLanka. Flowering and fruiting are observed during April-July and August-October.<sup>14</sup>

The leaves are simple, alternate on long petioles, cordate with even shallow lobes and variously serrated margins, very hairy on the abaxial surface, acute, deep green and about 7-15 cm in diameter. They are herbaceous but rigid, becoming rough on both sides, 60-200 mm long and 40-150 mm broad, sometimes unlobed, but usually deeply 3-lobed with the segments again lobed or doubly lobed, whereas the central lobe is much the largest. The leaf stalks are hairy and 150 mm long. Tendrils are simple and spiral.<sup>15</sup>

Male and female flowers grow on the same plant i.e., monoecious with the flower stalk up to 40 mm long hairy. Both have yellow petals, five in number, and sepals, also five in number and greenish in color. The fruits are globular with shallow grooves, about 14-20 cm long. Fruit is legume indehiscent, orbicular, compressed, broadly hardened winged around margin, usually single seeded, seeds subreniform, hilum small. The flesh is sweet, delicately flavoured, and juicy in nature. The corolla is green or green veined outside and inside the colour is white to pale or bright yellow and diameter is up to 30 mm.<sup>16</sup> The seeds are small, obovate to elliptical, light brown or black in colour, and smooth, between 0.5-1.5 cm  $\times$  0.5-1 cm long and 0.2-0.3 cm.<sup>17</sup>

## Extraction of *Pterocarpus marsupium*

The seeds are collected, washed, shade dried, coarsely powdered and then passed through 40 mesh sieves. The coarsely powdered materials were subjected to successive extraction with petroleum ether, ethyl acetate, and ethanol for 72 hrs. The extract solutions obtained were collected separately and the collected extract was concentrated under reduced pressure (<45 °C) using a vacuum pump for complete removal of the solvent. Pure organic part of the sample thus prepared and was stored at 4-5 °C until used.

The yield of ethanolic extract was collected and weighed. Acute toxicity study was done by oral administration of 2000 mg/kg body weight in rats. There were no clinical symptoms or mortality and no significant abnormalities were noted during post-mortem examinations. *Pterocarpus marsupium* was non-toxic to rats even at high dose.

### **Induction of diabetes in rats**

After overnight fasting, experimental diabetes was induced by single intra-peritoneal injection of gabapentin (150 mg/kg) in rats. The gabapentin was freshly prepared by dissolving 150 mg of gabapentin in normal saline solution. The animals were allowed to drink 20% glucose solution to overcome the drug induced hypoglycemia. 48 hours after injection of gabapentin, fasting plasma blood glucose was estimated. Animals with plasma glucose levels of >250 mg/dl were selected for anti-diabetic activity.

### **Experimental design**

The study was carried out after obtaining approval by the Institutional Animals Ethics Committee (IAEC). Thirty rats were used in this study. They were divided into five groups of six animals each. The vehicle/drug was administered for a period 21 days from the day of induction of diabetes. Drug/vehicle was administered orally (p.o.) under aseptic conditions. The treatment schedule was as follows:

- Group I- Normal control group, normal rats were administered with normal saline (10 ml/kg) and no drug treatment.
- Group II- Disease control group, diabetic rats were administered with normal saline (10 ml/kg) and no drug treatment. Experimental diabetes was induced by single intra-peritoneal injection of gabapentin (150 mg/kg).
- Group III- Diabetic rats were administered with standard drug metformin (150 mg/kg, p.o.).
- Group IV- Diabetic rats were administered with *Pterocarpus marsupium* (100 mg/kg, p.o.).
- Group V- Diabetic rats were administered with *Pterocarpus marsupium* (200 mg/kg, p.o.). Drug/vehicle was administered to the rats for 21 days.

### **Collection of blood**

The blood samples were collected on 1st, 7th, 14th and 21st day from tail and retro-orbital venous plexus of rats by giving anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, serum cholesterol, HDL cholesterol, LDL cholesterol, total proteins. Blood glucose levels were estimated by using glucose oxidase-peroxidase (GOD-POD) kit. On 21st day all the animals were sacrificed after collection of blood samples and the pancreas was collected. The isolated pancreas was used for study the anti-oxidant parameters.

### **Estimation of blood glucose<sup>18</sup>**

Blood glucose was estimated by using glucose kit obtained from Span Diagnostics using GOD-POD method. GOD oxidizes glucose to glucuronic acid and

H<sub>2</sub>O<sub>2</sub>. In presence of enzyme peroxidase, released H<sub>2</sub>O<sub>2</sub> is combined with phenol and 4-aminoantipyrine (4-AAP) and forms coloured quinoneimine dye. By using colorimeter absorbance of dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

### **Estimation of triglycerides (GPO-POD method)<sup>19</sup>**

Triglycerides (TGL) were estimated by using the kit obtained from Span Diagnostics. TGL were hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid (FFA). In presence of glycerol kinase (GK), adenosine triphosphate (ATP) phosphorylates glycerol to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol 3-phosphate is further oxidized by glycerol 3-phosphate oxidase (GPO) to produce dihydroxy acetone phosphate (DAP) and H<sub>2</sub>O<sub>2</sub>. In presence of peroxidase (POD), hydrogen peroxide couples with 4-amino antipyrine (4-AAP) and 4-Chloro phenol to produce red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to TGL concentration in the sample.

### **Estimation of total cholesterol (Chod-Pod/ Phosphotungstate method)<sup>19</sup>**

Pipette the samples and the reagent into 3 test tubes labelled blank (B), standard (S), and Total cholesterol (Tc). Mix well and incubate for 5 min at 37°C or 10 min at room temperature. Read the absorbance of standard (S), total cholesterol (Tc) against blank at 505 nm. Absorbance of quinoneimine was measured at 505 nm which is proportional to cholesterol concentration. Total cholesterol (in mg/dl) = (Abs. of test/Abs. of standard) × 200. Cholesterol concentration = Concentration (mg/dl) × 0.0259

### **Estimation of HDL**

Pipette out serum/plasma 0.2 ml and precipitating reagent 0.3 ml into the centrifuge tube. Mix well and allow standing at room temperature for 5 min. Centrifuge at 3000 rpm for 10 min to get a clear supernatant. If supernatant is not clear (high TGL level) dilute the sample 1:1 normal saline and multiply the result with 2. Pipette into 3 test tubes labelled blank (B), standard (S), HDL cholesterol (TH). Mix well and incubate for 5 min at 37 °C or 10 min at room temperature. Read the absorbance of standard (S), HDL cholesterol (TH) against blank at 505 nm. HDL cholesterol was calculated using the formula. HDL Cholesterol (mg/dl) = Absorbance of test/ Absorbance of standard × 50.

### **Estimation of total protein (Biuret method)<sup>20</sup>**

Proteins bind with copper ions in the alkaline medium of biuret reagent and reduce a purple colored complex, whose absorbance is proportional to the protein concentration. Pipette out the samples and reagents into

clean dry test tubes labelled blank (B), standard (S) and test (T). Mix well and incubate at 37°C for 10 min. Measure the absorbance of standard (S) and test (T) against blank (B) on a spectrophotometer at 555 nm. Total protein (gm %) = Absorbance of test / Absorbance of standard × 6.5.

### Histopathology studies

Light microscopy: the pancreas was removed, washed immediately with saline and then fixed in 10% buffered formalin. The Pancreas stored in 10% buffered formalin, were embedded in paraffin, sections cut at 5 µm and stained with hematoxylin and eosin. The hematoxylin and eosin stained sections were then examined under a light microscope for histological changes.

### Statistical analysis

The mean value±SEM will be calculated for each parameter. Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's

multiple comparison tests by using graph pad prism version 5.01.

## RESULTS

When the rats were sacrificed, the abdominal cavity was examined macroscopically before and after removing the pancreas for any abnormality. It was observed that there was no visible abnormality seen in pancreas and other viscera in the abdominal cavity in control group and the test group of rats.

Rats treated with *Pterocarpus marsupium* 100 mg/kg and 200 mg/kg has significantly reduced blood sugar compared to disease control group on 1st day, 7th day, 14th day, 21st day in gabapentin induced diabetes in epileptic rats. The fall in blood sugar level in rats treated with *Pterocarpus marsupium* 100 mg/kg (104.4±1.05) and 200 mg/kg (101.6±1.87) was comparable to the results produced by the standard drug metformin (120.7±0.54) on day 21 (Table 1).

**Table 1: Effect of extract of *Pterocarpus marsupium* seeds on plasma glucose levels in gabapentin induced diabetic rats.**

Group	Glucose levels (mg/dl) 1 <sup>st</sup> day	Glucose levels (mg/dl) 7th day	Glucose levels (mg/dl) 14th day	Glucose levels (mg/dl) 21 <sup>st</sup> day
Normal control	113.4±0.68	120.1±1.02	105.1±1.02	110.1±1.02
Disease control	289.5±1.70	293.7±0.34	274.4±0.68	288.7±0.85
Standard (metformin)	161.1±0.51*	141.1±0.51*	126.8±1.36*	120.7±0.54*
<i>P. marsupium</i> (100 mg/kg)	215.4±1.43*	161.4±0.74*	118.8±1.83*	104.4±1.05*
<i>P. marsupium</i> (200 mg/kg)	198.1±0.91*	152.8±1.4*	111.4±1.75*	101.6±1.87*

Values are expressed as mean±SEM, Oneway ANOVA followed by Dunnett's test. \*p<0.001 when compared with disease control group.

**Table 2: Effect of extract of *Pterocarpus marsupium* seeds on biochemical parameters in serum of gabapentin induced diabetic rats.**

Groups	TG	Cholesterol	HDL	LDL	Total protein
Normal control	79.47±1.95	131.1±1.02	55.83±0.78	71.04±0.88	6.97±0.03
Disease control	169.15±1.23	247.5±0.51	36.41±0.68	188.13±0.68	3.79±0.11
Standard (metformin)	105.48±0.98*	153.1±1.02**	48.32±0.71*	92.51±0.33**	5.74±0.08**
<i>P. marsupium</i> (100 mg/kg)	91.83±1.36**	136.4±1.98**	48.12±1.02*	84.53±0.42**	6.63±0.30**
<i>P. marsupium</i> (200 mg/kg)	83.35±1.41**	127.6±1.19**	52.36±1.44**	79.53±1.00**	7.84±0.11**

Values are expressed as mean ± SEM, Oneway ANOVA followed by Dunnett's test. \*p<0.05, and \*\*p<0.001 when compared with disease control group.

Rats treated with gabapentin showed a significant increase in the triglyceride's levels compared to control group. Standard drug metformin improved the cholesterol levels comparable to the control group. Rats treated with *Pterocarpus marsupium* 100 mg/kg and 200 mg/kg significantly decreased triglycerides levels, serum cholesterol levels, LDL levels and increased HDL levels, total protein levels compared to the disease control group. The improvement in lipid profile was comparable and slightly higher than the standard drug metformin (Table 2).

### Histopathological reports of gabapentin induced diabetic rats

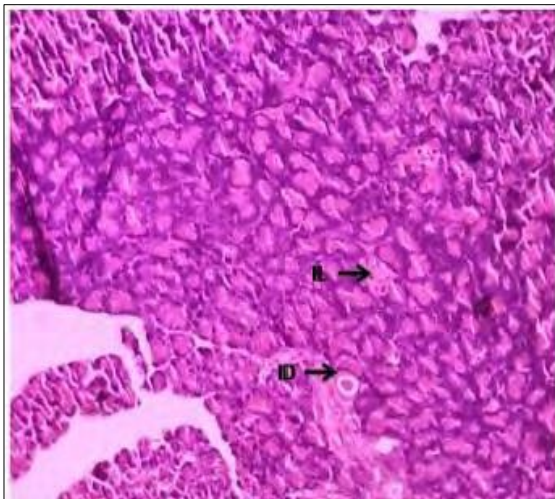
In the histopathological studies, the normal control groups of rats (Figure 1) have normal islets of Langerhans, acinar cells and β cells. In disease control group (Figure 2) shows degeneration and necrosis of pancreatic cells; extensive damage to the islets of Langerhans and damage of intralobular ducts were observed. Rats treated with standard drug (Figure 3) shows normal pancreatic β cells, normal islets of Langerhans. Rats treated with seed extract



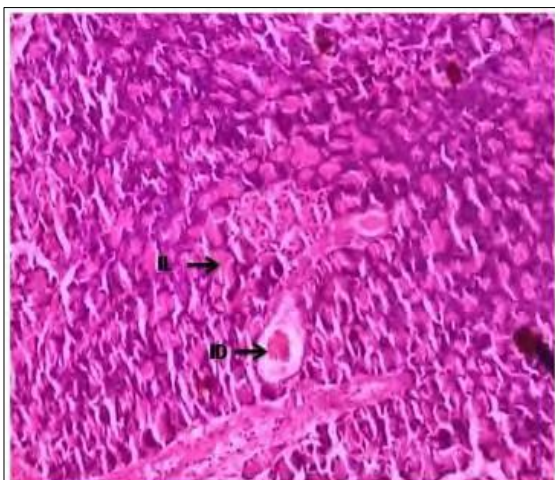
of *Pterocarpus marsupium* (Figure 4 and 5) shows the regeneration of pancreatic  $\beta$  cells with normal islets cells, normal acinar cells and normal intra lobular ducts.



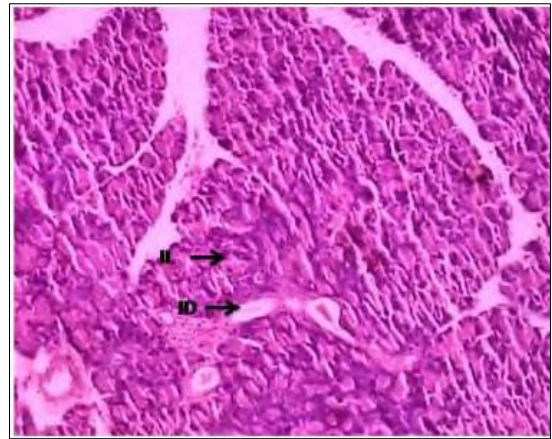
**Figure 1: Pancreas of normal control rat.**



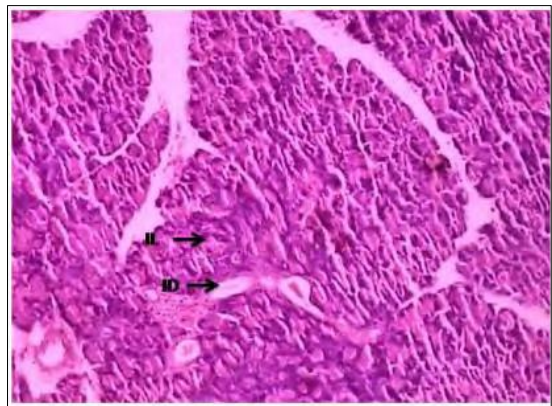
**Figure 2: Pancreas of disease control rat.**



**Figure 3: Pancreas of standard rat.**



**Figure 4: Pancreas of treatment rat (100 mg/kg).**



**Figure 5: Pancreas of treatment rat (200 mg/kg).**

## DISCUSSION

Diabetes mellitus is the one of the major metabolic disorder which affecting the three fourth of the world's population. It is fourth leading causes of death in the world. Insulin dependent diabetes is characterized by juvenile onset and insulin deficiency. Non-insulin dependent diabetes is characterized by mature onset and varying basal insulin levels. There are several reasons for the diabetes, major reason is inheritance. This diabetes can cause micro and macro vascular complications like renal failure, coronary artery disorders cerebrovascular disease, neurological complications, blindness, obesity, and hyperglycaemia.

There are different chemicals agents capable of producing the alterations related to the diabetic condition. In this present work gabapentin was used as inducing drugs. Gabapentin is used in the treatment of epileptic seizures. The induction of diabetes using gabapentin is referred as drug induced diabetes. Administration of gabapentin significantly increased the level of glucose when compared to control. The present study was designed to evaluate the hypoglycemic and hypolipidemic activity of seed extract of *Pterocarpus marsupium* in gabapentin drug induced diabetes. This ethanolic seeds extract

contains the compounds like carbohydrates, flavonoids, and alkaloids.

Administration of gabapentin significantly increases the glucose levels when compared to normal control group, which account for cytotoxic action of gabapentin. Metformin was taken as standard. The seed extract of *Pterocarpus marsupium* in lower dose (100 mg/kg), higher dose (200 mg/kg) were taken. The induced hyperglycaemia was present till end of the experiment which was compared with the normal group by observing the blood glucose levels. Induced diabetes was decreased by the protective mechanism of standard drug. The test drug at 100 mg/kg and 200 mg/kg had decreased levels of glucose  $104.4 \pm 1.05$  and  $101.6 \pm 1.87$  respectively when compare to disease control group. Similar hypoglycaemic activity was observed in studies which used *Pterocarpus marsupium* heartwood, roots and bark.<sup>21-24</sup> But the test drug does not show more protective mechanism when compare with the standard drug in gabapentin induced diabetic models.

The test drug shows reduced levels of total cholesterol, triglycerides, LDL to a significant level when compared to the disease control group. HDL and Total protein levels were increased with the test drug when compare to disease control group. Test drug at high dose (200 mg/kg) showed significant reduction of TG, total cholesterol, LDL levels in gabapentin induced diabetes models.

In the histopathological studies the pancreas of normal control group of rats showed normal islets of Langerhans, normal acinar cells and normal  $\beta$  cells. In the disease control group degeneration and necrosis of pancreatic cells, extensive damage to the islets of Langerhans and damage of intralobular ducts were observed. Seed extract of *Pterocarpus marsupium* showed the regeneration of pancreatic  $\beta$  cells with normal islets cells, normal acinar cells and normal intra lobular ducts. Metformin treated diabetic rats also shows normal pancreatic  $\beta$  cells, normal islets of Langerhans. So, the seed extract of *Pterocarpus marsupium* shows anti diabetic activity.

## CONCLUSION

In the present study, *Pterocarpus marsupium* at 100 mg/kg and 200 mg/kg has significantly reduced the blood glucose level on all the days of blood sampling. *Pterocarpus marsupium* 100 mg/kg and 200 mg/kg has significantly reduced the total cholesterol, triglycerides and LDL levels and significantly increased the HDL level compared to disease control group of rats. In conclusion, the present study shows that the ethanolic seed extract of *Pterocarpus marsupium* has potential antidiabetic action in gabapentin induced diabetic rats and the effect was found to be more similar to the reference drug metformin.

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