Evaluation of antidiabetic activity of ethanolic extract of roots of pterocarpus marsupium in streptozotocin induced diabetic rats

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Abstract

The present study evaluates the effect of ethanolic bark extract of P.marsupium in streptozotocin induced diabetic rats. Diabetes was induced in rats by administering streptozotocin (50mg/ kg) intraperitonially (ip). Animals were divided into five groups (n=6) receiving different treatments: Group I: vehicle (normal Control), Group II: (diabetic control), Group III: standard antidiabetic drug glibenclamide (1 mg/ kg bw, orally), Groups IV and V: ethanolic root extract treated (200 mg and 400 mg/ kg bw, orally respectively). Blood samples were collected and analyzed for blood glucose, SGPT and SGOT after 28 days of the treatment. The ethanolic root extract of P.marsupium at the dose level of 200 and 400 mg/ kg bw, produced significant reduction in blood glucose and serum enzymes (SGPT and SGOT) level and body weight improves by the extracts at dose of 200mg/kg and 400mg/kg during treatment period. The present investigation thereby reveals the anti-hyperglycemic potential of ethanolic root extract of P.marsupium.

Keywords: Diabetes mellitus, Streptozotocin, Glibenclamide, Pterocarpus marsupium, Blood glucose level, Body weight, Anti-diabetic activity.

Introduction

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency of insulin that leads to hyperglycemia, which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy and cardiac problems.(1) Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.

Prevalence of diabetes mellitus is increasing rapidly in both developing and developed countries. It was estimated to be 2.8% (171 million) in 2000 and it would be 4.4% (366 million) in 2030.(2) Despite insulin and other different types of anti-hyperglycemic agents available in the pharmaceutical market, diabetes and its related complications continue to be a major health problem. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. So the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus.(3) In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels.

Several species of herbal drugs have been described in the scientific and popular literature as having antidiabetic activity. Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed. Ethanopharmacological surveys indicate that more than 1200 plants are used world wide in traditional medicine for their hypoglycemic property. The investigation of antidiabetic agents of plant origin is thus of great significance because of their effectiveness, minimal side effects and relatively low cost.(4)

Pterocarpus marsupium (Roxb.) is large deciduous tree, commonly called as Indian Kino or Malabar Kino, belonging to the family fabaceae (Leguminoceae). The tree is scared with novel antidiabetic properties. Along with as an antidiabetic drug, it is also used as astringent, anti-inflammatory, haemostatic, anthelmintic, in chest pain, body pain and in indigestion, in diabetic anaemia, elephantiasis, erysipelas, urethrorrhea and opthalmopathy. The aim of the present study is to investigate the anti-hyperglycemic property of P.marsupium in streptozotocin induced diabetic rats.

Materials and Method

Collection of plant material: Pterocarpus marsupium plant material was collected from local areas of Mangalore, Karnataka, India. The taxonomic were authenticated by Ms Aparna Upadhyaya, Botanist, Madikeri, Karnataka. Pterocarpus marsupium root was

washed under tap water and were efficiently dried under shade for about one week and protected from deterioration.

Preparation of extract: The chemical compounds were extracted from the roots using successive solvent extraction process (soxhlation apparatus). The root powder (100 g) was extracted with water for 12 cycles. After completion of soxhlation process the liquid extract was collected and concentrated under reduced pressure below 50°C, until a soft mass obtained it was dried and kept in a desiccator.

Preliminary phytochemical screening:[5] About 50 mg of the solvent-free extract was stirred with little quantity of dilute HCl and then filtered. The filtrate was tested for presence of various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides, Gums, Saponins and terpenes.

Experimental Animals: Experiments were performed with male wistar rats, weighing about 180-220 g. The animals were housed in individual polypropy-lene cages under standard laboratory conditions of light, temperature (22 ± 1°C) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water ad libitum. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (Approval no SCP/IAEC/F150/P94/2016).

Acute toxicity studies: Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (ACAE) were administered orally at the dose of 2000 mg/kg. The animals were observed for toxic symptoms and behavioral changes continuously for the first 4 hrs after dosing. Finally, the number of survivors was noted after 24 hrs. From the next day onwards, each day 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for the next 14 days.

Induction of diabetes with Streptozotocin:[6] All the animals except group I were made diabetic by a single intra peritoneal injection of Streptozotocin (50mg/kg body weight) in normal saline. After two days of streptozotocin injection the blood glucose level was assessed and the animals having blood sugar level >200 was selected for the study. All the treatment was given orally once daily for entire 30 days.

Experimental Design:[7] The Wistar albino rats (150-200g) of either sex will be randomly divided into five groups of six each. The different groups will be assigned as follows.

Group I: Normal control (Vehicle)

Group II: Diabetic control (STZ 50 mg/kg)

Group III: Reference Standard (STZ 50 mg/kg + Glibenclamide 5mg/Kg)

Group IV: Diabetic animals (STZ 50 mg/kg +PMEE low dose)

Group V: Diabetic animals (STZ 50 mg/kg + PMEE high dose)

Evaluation: Starting from the first day of treatment, blood was collected every week from retro orbital puncture and glucose level was estimated by using Accu-Chek Active glucose monitoring kit. On 30th day, post treatment blood was collected; serum was separated and used for estimation of various biochemical parameters like body weight, fasting glucose, SGPT and SGOT.

Statistical Analysis: All data were expressed as Mean±SEM. The statistical significance between groups were compared using one way ANOVA, followed by Dunnett's (multiple comparison test). P value less than 0.05 was considered as statistically significant.

Methods for estimation of Biomarkers: The animals were sacrificed at the end of experimental period of 28 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 rpm for 10 minutes. serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels in the normal, diabetic control and drug treated rats was measured spectrophotometrically as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser.

Results

Table 1: Preliminary phytochemical screening of ethanolic extract of Pterocarpus marsupium root

Sl. No.	Test	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Flavonoids	+ve
4.	Glycosides	-ve
5.	Saponins	+ve
6.	Steroids	-ve
7.	Tannins	+ve
8.	Proteins	+ve
9.	Volatile oil	-ve

Table 2: Effect of Pterocarpu marsupium root extract on blood glucose level in STZ induced diabetic rats

Groups	Blood glucose level(mg/dl)				
	Initial	**7th day**	**14th day**	**21st day**	**30th day**
Normal	84.50± 1.204	82.50± 2.045	85.17± 1.493	85.00± 1.461	83.83± 1.579
Diabetic control	323.3± 3.528	342.2± 6.337	346.8± 7.002	338.8± 8.758	312.2± 12.98
Glibenclamide (5 mg/kg)	336.5± 3.871	234.8± 8.592***	180.2± 13.51***	119.0± 6.367***	100.5± 2.377**
PMEE (200 mg/kg)	331.8± 4.672	283.0± 6.763**	223.5± 4.787*	164.3± 11.43*	129.2± 4.963*
PMEE (400 mg/kg)	338.3± 3.964	283.2± 3.535**	183.7± 9.486**	131.3± 3.602**	108.0± 2.352**

Values are expressed as mean ± S.EM, n=6 in all except in diabetic control, one way ANOVA followed by Dunette's test. *p<0.05, **p<0.01 and ***p<0.001 when compared to diabetic control.

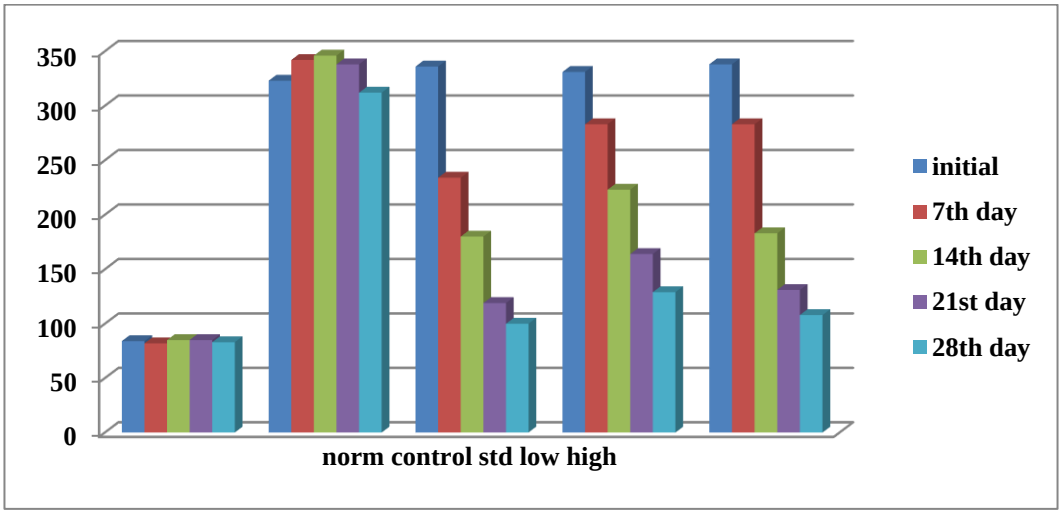

Fig. 1: Effect of PMEE on blood glucose level in STZ induced diabetic rats

Table 3: Body weight in STZ induced diabetic rats

Groups	Body Weight (Grams)			
	Day 0	**Day 7**	**Day 14**	**Day 21**
Normal control	176.8± 0.8333	192.3± 0.7601	196.0± 3.173	208.8± 0.4773
Diabetic control	181.8± 0.8724	162.5± 0.7638	143.0± 1.506	138.3± 0.6667
Glibenclamide (5mg/kg)	177.0± 0.5164***	161.0± 0.3651**	184.8± 1.579**	200.2± 1.352***
PMEE (200mg/kg)	171.3± 30.28*	180.0± 0.5774*	183.5± 0.9574*	185.3± 1.022*
PMEE (400mg/kg)	202.8± 1.493**	192.8± 1.400**	201.0± 0.3651**	207.8± 0.7923**

Values are mean ±SEM (n=6) one way ANOVA followed by Dunette's test. Where, # represents the comparision, * represents significant at p<0.05, ** represents highly significant at p<0.01 and *** represents very significant at p<0.001.

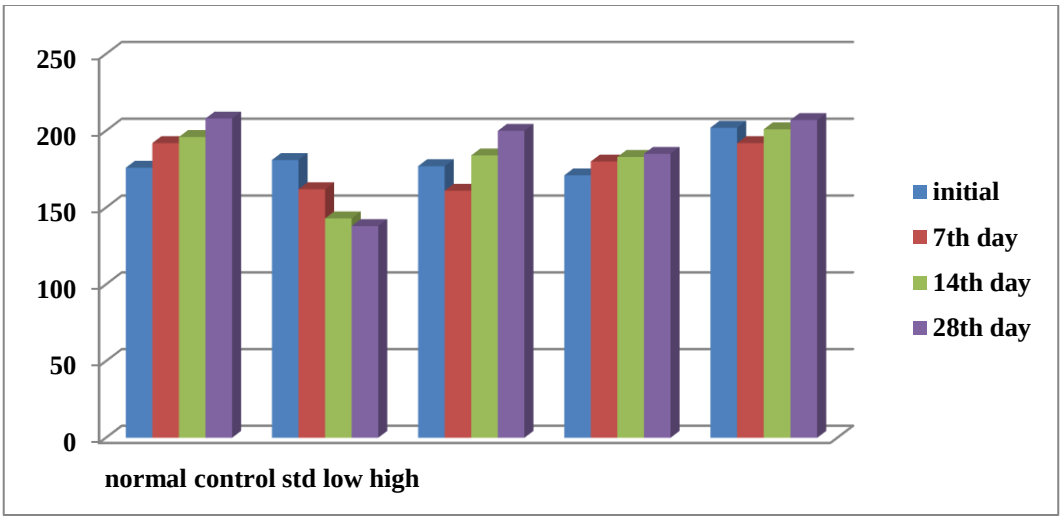

Fig. 2: Effect of PMEE on body weight in STZ induced diabetic rats

Table 4: SGPT and SGOT levels in diabetic rats

Group	STZ	
	SGPT	SGOT
Normal control	58.00± 0.3651	59.17± 0.3073
Diabetic control	103.3± 0.5578	115.7± 0.4216
Standard Gibenclamide	73.00± 0.3651***	69.67± 0.5578***
PMEE (200mg/kg)	98.50± 0.2236*	94.83± 0.3073*
PMEE (400 mg/kg)	86.33± 0.5578**	78.83± 0.4014**

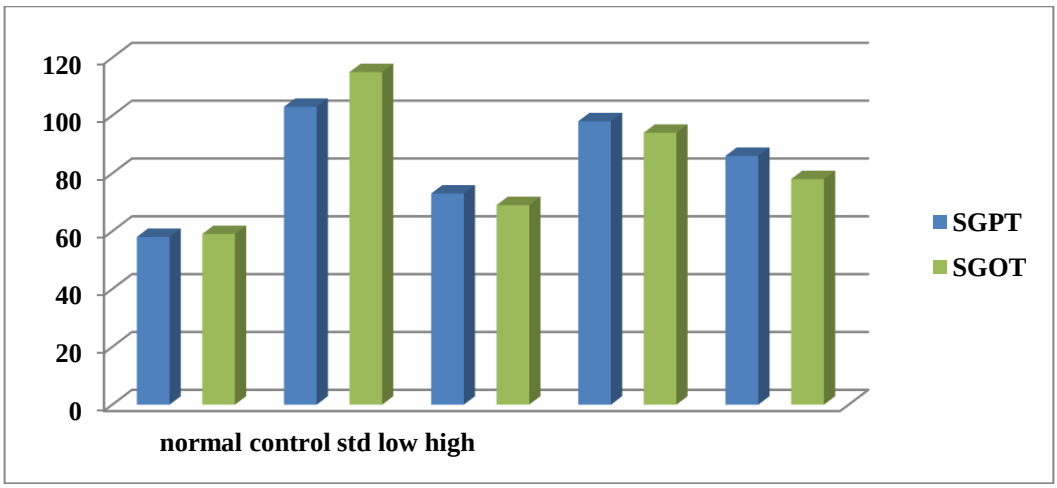

Fig. 3: Effect of PMEE on SGPT and SGOT level in STZ induced diabetic rats

The hypoglycemic effect of ethanolic root extract of Pterocarpus marsupium is shown in (Table 2). The rats of diabetic control (Group II) showed a marked increase in blood glucose (323.3±3.52 mg/dl to 342.2±12.98) when compared to normal control group (Group I). However, following the treatment with ethanolic extract of Pterocarpus marsupium(for 28 days), Group IV (200 mg/kg bw) and Group V (400 mg/ kg bw) animals showed significant ($p < 0.05$) reduction in the blood glucose (331.8 ± 4.672 to 129.2 ± 4.963 and 338.3 ± 3.96 to 108.0± 2.352 mg/dl) respectively. The maximum effect of Pterocarpus marsupium was seen at a dose level of 400 mg/ kg bw and also the Group V rats showed results comparable

with the glibenclamide treated rats (Group IV). This effect may be due to increased insulin secretion from beta cells of pancreas i.e., pancreotrophic action in P.marsupium treated animals.

Body weight of animals in all groups was recorded. Decrease in body weight during study period was found to be in diabetic control group. Glibenclamide and Pterocarpus marsupium root ethanolic extract treated groups showed increase in body weight as compared to diabetic control group (Table 3, Fig. 2). Decrease in body weight in Streptozotocin induced diabetic rats and weight gain in PMEE treated group is due to loss of tissue protein and muscle wasting in the former and having beneficial effect in preventing loss of body weight and in catabolic process in PMEE treated groups. Significant increase in weight in PMEE treated group of albino rats in comparison to vehicle treated diabetic rats indicating that ethanolic extract had beneficial effect in preventing loss of body weight of diabetic rats.

Summarized the effect of STZ on the activity of the hepatic marker enzymes in serum. In the present study, the levels of SGPT and SGOT in STZ induced diabetic rats were elevated. In this study, the ethanol root extract of P.marsupium regulated the activity of SGPT and SGOT in liver of rats intoxicated with STZ. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and ethanol extracts of P. marsupium further strengthen the anti-diabegenic effect of these extract. More over SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver.

The major chemical constituents found in preliminary phytochemical tests are carbohydrates, glycosides, saponins, tannins and proteins (Table 1). The number of investigators have reported that these phyto constituents are known to possess anti diabetic activity in animals. It is therefore to speculate that the phytoconstituents present in the PMEE may attribute to observed potential anti-diabetic activity.

Conclusion

Ethanolic extract of Pterocarpus marsupium root is found to be more effective in the treatment of diabetes mellitus as determined by its statistically significant p-value < 0.001 in Strptozotocin induced diabetic rats. The mechanism of anti-diabetic activity of ACAE may be due to enhancing the effect of insulin and by stimulating the insulin secretion from beta cells of pancreas. Hence this study suggests that P.marsupium ethanolic extract has a potent anti diabetic effect which could be used for the management of diabetes effectively.

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