Research Article

Effect of Ethanolic Extract of *Pterocarpus marsupium* Root on Alloxan-Induced Diabetic Rats

Abhishek N*, Karunakar Hegde, Chaithra Amin B and Ibrahim Sayeed VK

Department of Pharmacology, Srinivas College of Pharmacy, Valachil,

Post- Farangipete, Mangalore- 574 143, Karnataka, India.

ABSTRACT

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 etc. The ethanol extracts of *Pterocarpus marsupium* root was investigated for its antidiabetic effect in Wistar albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (100mg/kg,i.p). The ethanol extracts of *Pterocarpus marsupium* root at a dose of 200mg/kg and 400 mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 28 days. The effect of ethanol extracts of *Pterocarpus marsupium* on blood glucose, body weight, serum enzymes [Serum glutamate pyruvate transaminases (SGPT), serum glutamate and oxaloacetate transaminases (SGOT) were measured in the diabetic rats. The ethanol extracts of *Pterocarpus marsupium* resulted significant reductions of blood glucose ($p<0.01$), and serum enzymes and significantly increased body weight. From the above results it is concluded that ethanol extracts of *Pterocarpus marsupium* root possesses significant antidiabetic effects in alloxan induced diabetic rats.

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

INTRODUCTION

Diabetes mellitus is a universal problem affecting human societies at all stages of development. It is a condition where sufficient amount of insulin is either not produced or the body is unable to use the insulin that is produced, leading to excess glucose in the blood[1]. Insulin is the hormone that enables glucose uptake and utilization by the body cells for energy supply. The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes and an estimated 1.1 million people died of this disease condition in 2005 alone[2]. Hence despite the presence of various antidiabetic medicine on the market, diabetes and related complications continues to be a major medical problem. The search for a cure for diabetes mellitus continues along with traditional and alternative medicine. Many herbal supplements have been used for the treatment of diabetes, but not all of them have scientific evidence to support their effectiveness[3].

The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus and unexpected weight loss, etc. Over a period of time diabetes develops complications such as nephropathy, retinopathy and neuropathy[4]. Impaired glucose tolerance and the metabolic disorder often lead to development of type II diabetes. A number of important epidemiological studies revealed the relationship between hyperglycemia and an increased risk of cardiovascular disease[5]. It is the most widespread disease in the world, disturbing 25% of population and badly affect 150 million people and is set to increase up to 300 million by 2025[6].

India is the largest producer of medicinal herbs and is called as botanical garden of the world[7]. The current research focuses on herbal drug preparations and plant used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

Modern medicines like biguanides, sulphonylureas and thiozolidinediones are available for the treatment of diabetes. But they

also have undesired effects associated with their uses. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity. Medicinal plants are a rich source of natural products. Medicinal plants and their products have been widely used for treatment of diabetic population all around the world with less known scientific basis of their functioning. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity.

In folk medicine, the various parts of *Pterocarpus marsupium* is extensively used in the treatment of diabetes and it is also have insulin sensitizing activity[8]. Moreover it is reported that, the roots of the plant is known to contain bioactive flavonoids, glycoside, terpenoids etc. However no scientific data regarding the anti-diabetic activity of *Pterocarpus marsupium* root is available in scientific literature. Hence, the present study is designed to evaluate the efficacy of the ethanolic extract of root of *Pterocarpus marsupium* in experimental models of diabetes using rats.

MATERIAL AND METHODS

The fresh roots of *Pterocarpus marsupium* were collected in the month of july-august from the forest of Mangalore district, Karnataka state, India. The taxonomic were authenticated by Ms Aparna Upadhyaya, Botanist, Madikeri, Karnataka. The roots will be shade dried, The dried roots were pulverized into coarse powder at plant mill and sieved by using a mesh no. 10/44. and 100g of the powered samples were extracted using aqueous solvent in a soxhlet apparatus. The extracts obtained were dried and used for anti-diabetes studies.

EXPERIMENTAL ANIMALS

Normal healthy male Wistar albino rats (180-240g) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12 h). Rats were feed standard pellet diet and water *ad libitum*. All the experimental protocols were reviewed and approved by the institutional animal ethical committee (Approval no SCP/IAEC/F150/P94/2016) prior to the initiation of the experiment and the care of the laboratory animals were taken as per the CPCSEA regulations. The animals were acclimatized for atleast one week before use.

ACUTE TOXICITY EVALUATION [9]

Acute toxicity study of the extract of roots of the plant *Pterocarpus marsupium* will be performed as per the OECD guidelines 425 at a limit dose of 2000 mg/kg or 5000 mg/kg. The doses will be administered by oral route in albino mice (20-25g). Animals will be observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for total 14 days for sign of toxicity and/or mortality if any.

INDUCTION OF EXPERIMENTAL DIABETES

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (100 mg/kg) . Two days after alloxan injection, rats screened for diabetes having

glycosuria and hypoglycaemia with blood glucose level of 200 – 260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages. All the treatment was given orally once daily for entire 30 days.

EXPERIMENTAL DESIGN [10]

The Wistar albino rats (150-200g) of either sex were randomly divided into five groups of six each. The different groups were assigned as follows.

Group I : Normal control (Vehicle)
Group II : Diabetic control (Alloxan100 mg/kg)
Group III : Diabetic animal(Alloxan100 mg/kg + Glibenclamide 5mg/Kg)
Group IV: Diabetic animals (Alloxan100 mg/kg + PMEE 200mg/kg)
Group V : Diabetic animals (Alloxan100 mg/kg + PMEE 400mg/kg)

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.

BIOCHEMICAL ANALYSIS

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 ransaminase(SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels in the normal, diabetic induced and drug treated rats was measured spectrophotometrically by utilizing the method of Reitman and Frankel.

STATISTICAL ANALYSIS

The results were represented as Mean ± SD. The statistical significance was computed using One Way ANOVA followed by Tukeys multiple comparison test and compared with diabetic control group with Standard drug, FD39 where the n=6 animals in each group were used. P<0.001 was considered statistically significant.

RESULTS

Table 1: Effect of *Pterocarpu marsupium root* extract on blood glucose level in Alloxan induced diabetic rats

Groups	Blood glucose level(mg/dl)				
	Initial	Day 7	Day 14	Day 21	Day 30
Normal	82.83± 1.990	84.17± 2.428	82.33± 2.060	79.67± 1.542	80.17± 1.22
Diabetic control	324.8± 4.362	342.7± 6.566	348.3± 6.951	350.2± 9.854	348.7± 4.624
Glibenclamide (5mg/kg)	326.7± 4.208	238.8± 5.885***	150.7± 7.121**	120.8± 5.997***	102.8± 3.701***
PMEE (200mg/kg)	322.8± 3.458	259.0± 14.50*	191.2± 14.26	157.8± 11.31*	135.3± 10.38**
PMEE (400mg/kg)	328.5± 5.334	256.5± 14.04**	211.8± 13.33*	161.8± 8.631**	119.5± 4.372**

Values are expressed as mean ± S.E.M, n=6 in al group except in diabetic control One way ANOVA followed by Dunette's t- test. *p<0.05, **p<0.01, ***p<0.001, when compared with diabetic control rats.

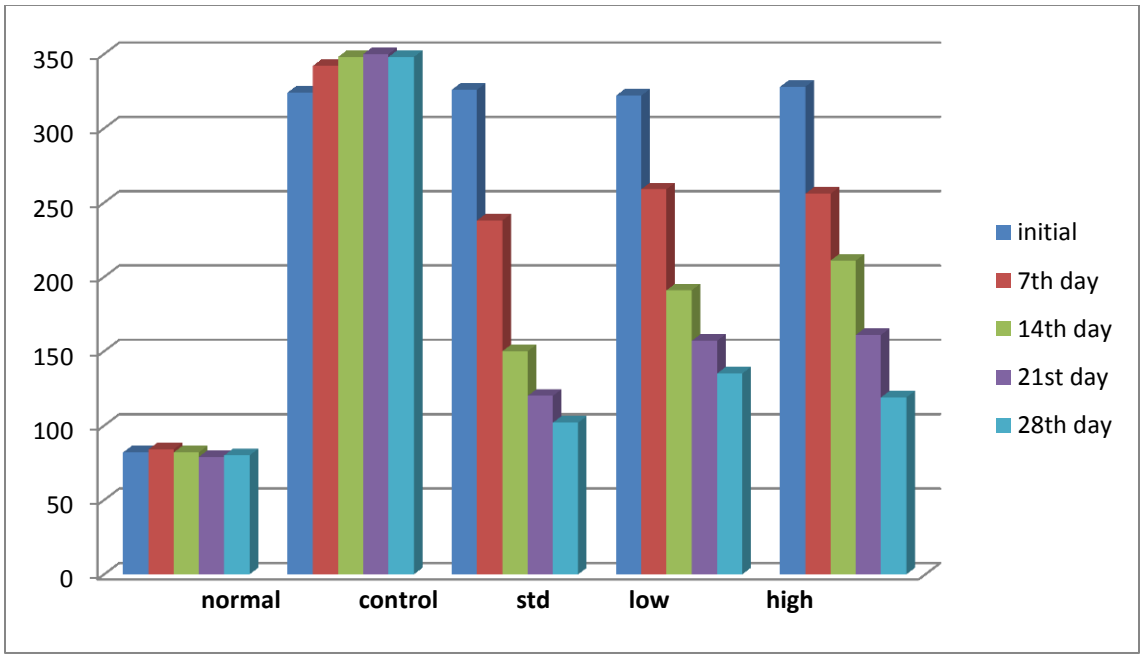

Fig. 1: Effect of PMEE on blood glucose level in Alloxan induced diabetic rats

Table 2: Body weight in Alloxan induced diabetic rats

GROUPS	BODY WEIGHT (Grams)			
	Day 0	Day 7	Day 14	Day 21
Normal control	176.8± 0.833	192.3± 0.7601	196.0± 3.173	208.8± 0.4773
Diabetic control	182.5± 0.8062	153.0± 1.549	153.0± 1.549	139.3± 1.085
Glibenclamide (5mg/kg)	186.2± 0.6540	189.8± 0.5426**	189.8± 0.5426**	196.0± 0.9309**
PMEE (200mg/kg)	202.5± 1.360	197.7± 0.7601**	197.7± 0.7601*	201.2± 0.9804*
PMEE (400mg/kg)	204.2± 1.470	197.3± 0.6164**	200.8± 0.4014*	208.0± 0.4472*

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.**

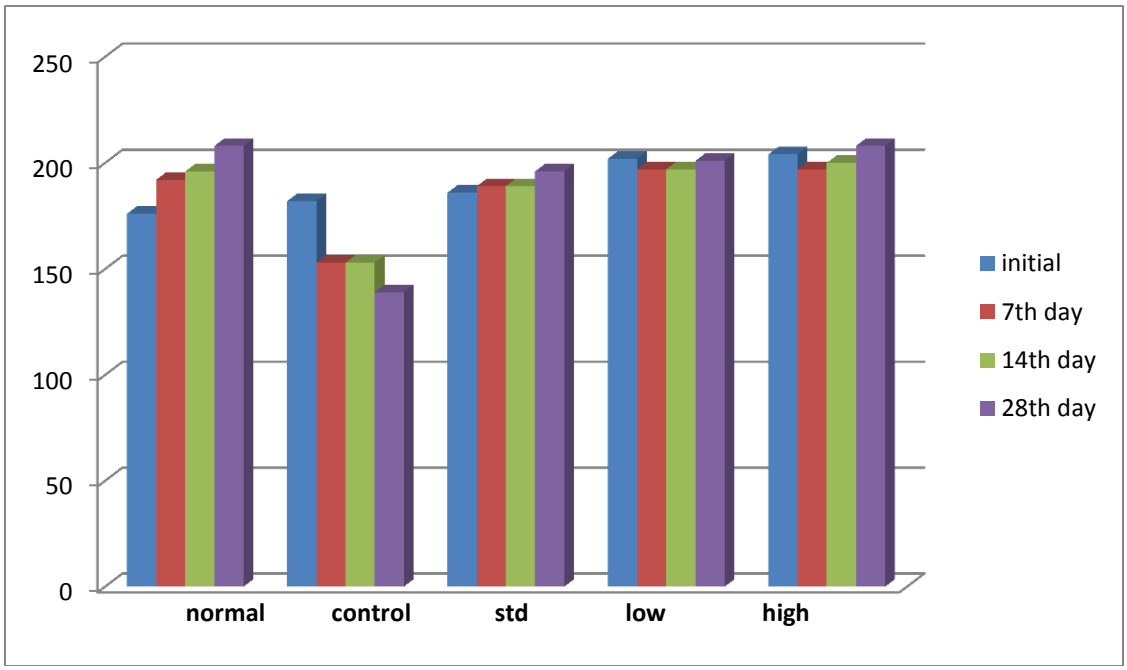

Fig. 2: Effect of PMEE on body weight in alloxan induced diabetic rats

Table 3: SGPT and SGOT levels in diabetic rats

Group	Alloxan	
	SGPT	SGOT
Normal control	59.17± 0.4010	61.00± 0.3651
Diabetic control	110.3± 0.3333	113.0± 0.3651
Standard Gibenclamide	67.67± 0.4944***	69.83± 0.3073***
PMEE (200mg/kg)	92.67± 0.3333*	87.67± 0.6146*
PMEE (400 mg/kg)	84.00± 0.5774**	79.67± 0.3333**

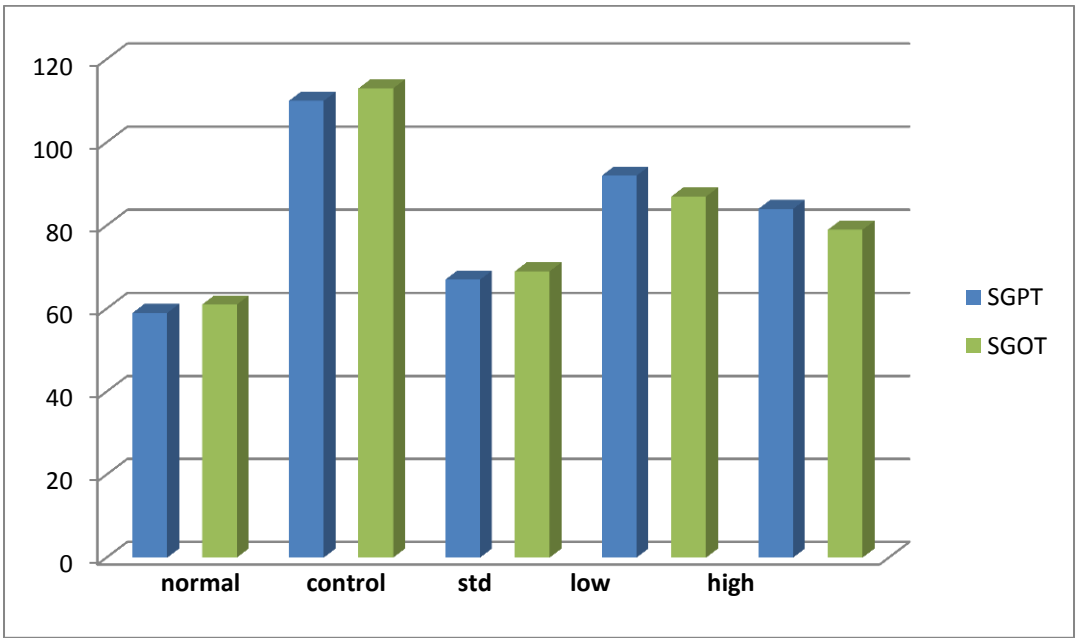

Fig. 3: Effect of PMEE on SGPT and SGOT level in Alloxan induced diabetic rats

RESULTS AND DISCUSSION

Acute toxicity study of *P.marsupium* revealed the non-toxic nature of the ethanol extract of *P. marsupium* root. The alloxan induced diabetic rats elicited significant rise in blood glucose. On the contrary, diabetic rats treated with ethanol extracts of *P. marsupium* root exhibited decrease in blood glucose level at a dose of 200 mg/kg and 400 mg/kg body weight **(Table.1).** The capacity of PMEE to decrease the elevated blood glucose to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which PMEE exerts its hypoglycemic action in diabetic rats may be due to potentiating the insulin release from pancreatic cells of diabetic rats.

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* aqueous extract treated groups showed increase in body weight as compared to diabetic control group **(Table 3, Fig 2)**. **(Table 3)** summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan. In this study, the ethanol root extract of *P.marsupium* regulated the activity of SGPT and SGOT in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study. 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 antidiabegenic 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. Since the alloxan can also affect the liver by free radical mechanism.

CONCLUSION

The roots of PMEE is a good candidate in the management of DM. The results of the present study indicate that the root extract of *P.marsupium* is capable of exhibiting significant anti-hyperglycemic activity in Alloxan induced diabetic rats and hypoglycemic activity in healthy, glucose loaded rats. The possible mechanism by which PMEE exerts its hypoglycemic action in diabetic rats may be due to potentiating the insulin release, further investigations are needed to identify the lead molecule and to elucidate exact mechanism of action for antidiabetic effect.

ACKNOWLEDGEMENT

I am thankful to my guide Dr.Karunakar Hegde. Srinivas college of pharmacy, Faculty of pharmacy, Mangalore, India, supported me to conduct work and providing a laboratory facility and infrastructure.

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