ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ANTIDIABETIC ACTIVITY OF HEART WOOD OF *PTEROCARPUS MARSUPIUM ROXB.* IN ALLOXAN INDUCED DIABETIC RATS

Nidhi Bais*, Gajendra Pratap Choudhary, Nidhi Dubey

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Ring Road Indore, Madhya Pradesh, India

**Corresponding author: baisnidhi21@gmail.com*

ABSTRACT

Diabetes mellitus is most common disease which is leading cause of morbidity and mortality among Indian population. *Pterocarpus marsupium* is a medicinal plant used in ayurvedic medicine system to control blood sugar. The aim of this study was to determine antidiabetic effect of mixture of ethanolic extract of heartwood of *Pterocarpus marsupium*. To evaluate antidiabetic activity, extract was administered in experimental mice and significant decrease in blood glucose level was shown on 7th day. Histopathology was also studied. Thus from present study it can be concluded that *pterocarpus marsupium* extract has antidiabetic activity in alloxan induced diabetic rat.

Keywords: *Pterocarpus marsupium*, Heart wood, Insulin, Antidiabetic.

1. INTRODUCTION

Diabetes mellitus is a major disease in many countries [1]. Region with greatest potential are Asia and Africa [2]. Reason for the rise in diabetes includes increase in sedentary lifestyle, consumption of energy rich diet, obesity and higher life span [3]. Therapeutic option such as dietary modification, oral hypoglycemic and insulin have limitation of their own hence many herbal medicines have been recommended for treatment of diabetes and show hypoglycemic activity in experimental animals [4]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. Increasing worldwide incidences of diabetes mellitus in adults constitute a global public health burden. Out of the two types of diabetes, the incidence of noninsulin dependent diabetes mellitus is much higher than the insulin dependent diabetes mellitus. Even insulin therapy does not reinstate a permanent normal pattern of glucose homeostasis and carries an increased risk of atherogenesis and hypoglycemia. Plants are used as traditional remedies in one or other form for the treatment of diabetes. There has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and lesser side effects. Drugs derived from natural products have played a major role in the development of pharmaceutical treatments for diabetes.

Pterocarpus marsupium Roxb., is a deciduous tree commonly grows in Sri Lanka and India [1]. It is popularly known as Vijaysar in Hindi, Indian Kino in English and is a valuable medicinal plant, used mainly in Ayurveda, for the treatment of diabetes [2-4]. It is well known to Ayurvedic medicine because of its curative and lenitive properties. Its flowers are employed against fever, its heartwood as depurative, hemostatic, and rejuvenating, its wood is used for chest and body pain as well as indigestion, etc. The gum (kino) obtained from the tree is used in diarrhea, pyrosis and toothache. Bruised leaves are used externally for boils, sores, and various skin diseases. The water kept in tumblers made out of the wood of this plant is said to be beneficial for chest pain and diabetes. The bark of *P. marsupium* is very effective in preventing cataract formation and reducing hyperglycemia in alloxanized diabetic rats [1] and the heartwood is useful as hypolglycemic agents [5]. Water extract of *pterocarpus marsupium* wood has been used in India for treatment of diabetes mellitus since time immemorial [5]. Flavonoid (-) epicatechin isolated from plant is effective in beta cell regenration in alloxan induced diabetic rat and reduced blood sugar level on further study, it was found that benzofuronone marsupin [6], dihydrochaclone, pteroscipin [7] and stilbene, pterostilbene [8] three major phenolic compound present in heartwood extract show antidiabetic activity.

2. MATERIAL AND METHODS

2.1. Plant material collection

The heart wood of *Pterocarpus marsupium* was collected from local area of Indore (M.P.) in the month of Nov 2020 to Jan, 2021. Drying of plant material was carried out under the shade. Dried *Pterocarpus marsupium* heart wood were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.2. Defatting and extraction of plant material

Plant materials were extracted in ethanol solvent by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

2.3. Acute Toxicity Studies of Extracts

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization (IEAC approval no.- RKDFCP/IAEC/2019/15).

2.4. Experimental animals

The Wister strains of male albino rats weighing between 100 and 150 g were obtained from the animal house of RKDF University Bhopal. The animals were housed in larger spacious cages and were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd., Bangalore, India, under the trade name Gold Mohur Rat Feed and had free access to water *ad labium*. The animals were well acclimatized to standard environmental conditions of temperature and 12 h light dark cycles throughout the experimental period. The animals used in the present study were approved by the Institutional Animal Ethical Committee.

2.5. Primary Screening for Diabetes

2.5.1. Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance tests (OGTT) were carried out as per the procedure previously described. Briefly, fasted mice were grouped into 4 groups of six mice each. The various groups received different treatments like

Group 1 received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control,

Group 2 received standard drug (glibenclamide, 10 mg/ kg body weight).

Groups 3-4 received, respectively, extract at doses of 500 mg per kg body weight.

All substances were orally administered by gavaging. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier, all mice were orally administered 2g glucose per kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previously published procedures. Blood glucose levels were measured with a glucometer. The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = (1 - We/Wc) X 100,

Where We and Wc represents the blood glucose concentration in glibenclamide or extract administered mice, and control mice, respectively [10].

2.6. Secondary Screening for Anti-Diabetic Activity

2.6.1. Preparation of Alloxan Monohydrates

Alloxan was prepared by weighing 1 gm of alloxan and dissolving in 20 ml of water for injection. Alloxan at this calculated dose is said to have a concentration of 50 mg/ml.

2.6.2. Hypoglycemic Activity [11-13]

Different groups of six rats each were used in the present investigation. The basal concentration of blood glucose level of all the animals was recorded and 6 animals were separated to serve as normal control. The remaining animals received a single injection of Alloxan monohydrate in water for injection at a dose of 150-mg/kg bodyweight given by intra-peritoneal route. After 4 days of Alloxan administration, the blood glucose was estimated and animals with blood glucose levels in the range 280 mg/dl and 380 mg/dl were selected and divided into groups.

Group 1:- Untreated control (Normal saline water)

Group 2:- Diabetic control (Alloxan 150 mg/kg)

Group 3:- Diabetic+ Glibenclaminde (10mg/kg)

Group 4:- Diabetic + Plant extract (500 mg)

2.7. Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) in latest computer software programme. All the obtained results are expressed as X (Mean) ± SEM, n=6. (One way ANOVA followed by Bonferroni multiple comparison test).

3. RESULTS

3.1. Acute Toxicity Studies of Extracts

The extract of *Pterocarpus marsupium* (PME) was screened for acute toxicity study by OECD guideline no. 423 for determination of $LD_{50.}$ The results showed that PME was belonging to category-5 (unclassified). Hence, LD_{50} was 5000 mg/kg, therefore, ED_{50} was 500 mg/kg. Therefore doses of 500 mg were selected for present investigation. The results were presented in Table 1.

Table 1: Determination of LD_{50} and ED_{50} of Extract

S. No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals
			pterocarpus marsupium extract
1.	3	5	0
2.	3	50	0
3.	3	300	0
4.	3	2000	0
5.	3	5000	0

3.2. Primary Screening for Diabetes

3.2.1. Oral Glucose Tolerance Test

In oral glucose tolerance tests, PME was found to be significant and dose-dependently reduced blood glucose levels in glucose-loaded mice by more than 30 % at the dose of 500 mg per kg body weight in mice. When the comparison was made with standard anti-hyperglycemic drug (glibenclamide), reduced blood glucose levels by 42.39 % at a dose of 10 mg per kg bw was observed. Another important aspect of this study is that to our knowledge this is the first description of the ability of PME to reduce blood glucose. If this holds up for the active component(s) along with their mechanism of action is identified and elucidated, this can be a highly promising means towards alleviation of high blood glucose levels in diabetic patients or people with impaired glucose metabolism. From the results obtained of OGTT for next step *i.e.,* Secondary Screening for anti-diabetic activity PME was chosen.

3.2.2. Secondary Screening for Anti-Diabetic Activity

From the results obtained of OGTT for secondary screening for anti-diabetic activity of pterocarpus extract was selected. In the present investigation, blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administration, the blood glucose levels of rats were observed. Table 2 indicates effect of administration of feeding the PME on body weight and fluid intake in normal and diabetic rats. Table 3 shows effect of administration of feeding the PME on total hemoglobin and urine sugar in normal and diabetic rats. It was observed in that significant lowering of sugar in Pterocarpus marsupium extract The administration of PME at a dose of 500 mg/kg body weight showed significant anti-hyperglycaemic effect at 21st day which was evident from the 1st day on wards as compared to standard. The anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in table 4. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Gliben-clamide. The blood glucose levels of the anti-diabetic activity of alloxan induced diabetic rats were shown in table 4. It represents the decrease in blood glucose levels. The Glibenclamide (10 mg/kg body weight) shows significant effect compared to the initial and more significant effect on the 7th day compared to the initial.

3.3. Histopathology

The results of histopathology of pancreas of aqueous extract awere as mentioned below:

Control: Normal architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. No fibrosis or any inflammation was observed.

Diabetic control: Normal architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. Focal lymphocytic infiltrate present in stroma.

Standard: The normal architecture was restored to the same as that of the standard drug treated pancreas.

***Pterocarpus marsupium Extract*:** Normal architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. No fibrosis or any inflammation seen.

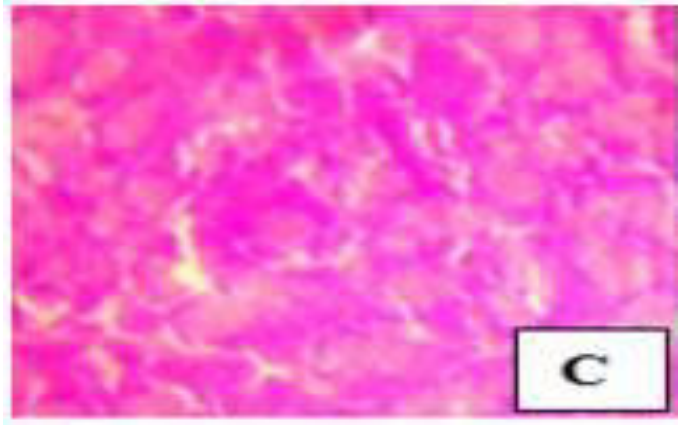

Fig. 1: Histopathology of Control group

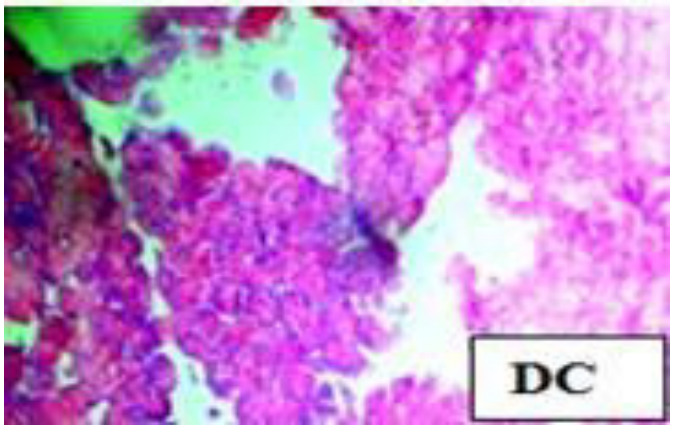

Fig. 2: Histopathology of diabetic control group

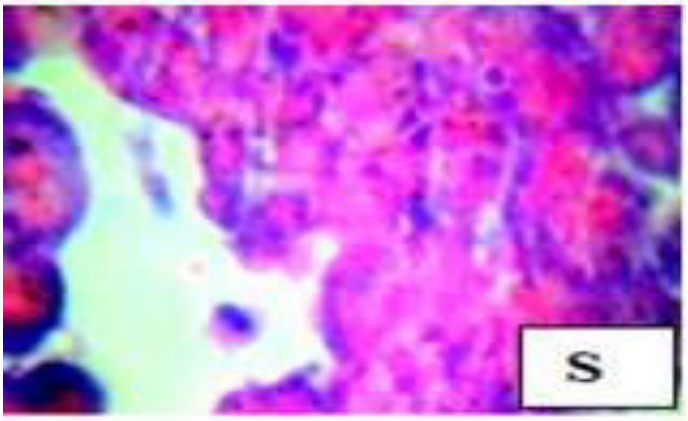

Fig. 4: Histopathology of standard group

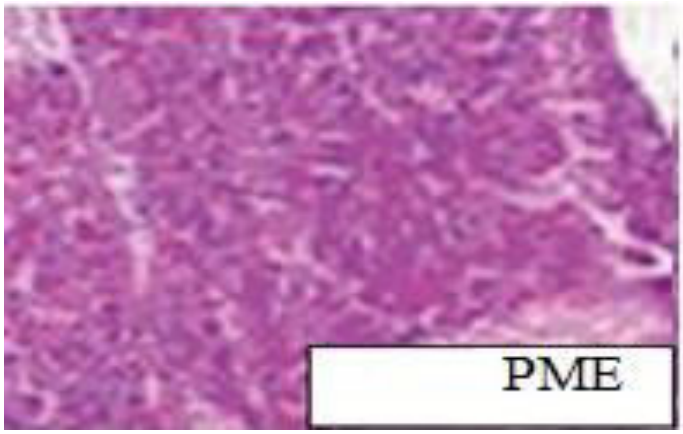

Fig. 4: Histopathology of *P. Marsupium* extract group

Table 2: Effect of Extracts on Blood Glucose Level in Hyperglycemic Mice following 120 minutes of Glucose Loading

Treatment	Dose (mg/kg bw)	Blood Glucose Level (mmol/l)	% Lowering of Blood Glucose Level
Control	10 ml	5.93±0.18	-
Glibenclamide	10 mg	3.48±0.08	42.39
Plant Extract	500 mg	4.21±0.03	30.33

All administrations were made orally only. Values are expressed as Mean±SEM, (n=6); p<0.005

Table 3: Effect of Administration of Feeding the *Pterocarpus marsupium* Extract on Body Weight and Fluid Intake in Normal and Diabetic Rats

Group	Body Weight (g)		Fluid intake g/animal/day
	Before treatment	After treatment	
Control	192±1.01	221.5±1.32	21.41±0.44
Diabetic control	201.14±1.05	167.2±2.53$^{###}$	773.0±0.11$^{###}$
Standard (10 mg/kg)	206.16±1.02	221.02±1.06***	54.12±0.05***
Plant Extract(500 mg)	196.09±2.02	218.1±1.14***	57.10±0.11***

*All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared diabetic control (normal saline), **P<0.01 as compared diabetic control (normal saline), $^{###}$P<0.001 as compared to Control. One-way ANOVA followed by Bonferroni multiple comparison test*

Table 4: Effect of Administration of Feeding the *Pterocarpus marsupium* Extract on Total Haemoglobin and Urine Sugar in Normal and Diabetic Rats

Group	Total hemoglobin (%)		Urine sugar	
	Before treatment	After treatment	Before treatment	After treatment
Control	11.53±0.12	11.7±0.481	Nil	Nil
Diabetic control	12.62±0.12	7.59±0.724###	+4	+4
Standard (10 mg/kg)	13.12±0.62	16.21±0.39***	+4	+2
Plant Extract (500 mg)	12.89±0.11	16.03±0.11***	+4	+2

*All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared diabetic control (normal saline), **P<0.01 as compared diabetic control (normal saline), ###P<0.001 as compared to Control. One-way ANOVA followed by Bonferroni multiple comparison test*

Table 5: Effect of Administration of Feeding the *Pterocarpus marsupium* Extract on Serum Glucose Estimation in Normal and Diabetic Rats

Group	Serum glucose (mg/dL)			
	0 day	7th day	14th day	21th day
Control	84.06±0.34	84.02±0.18	84.19±0.27	85.47±0.86
Diabetic control	294.45±0.28	365.92±0.08##	411.02±0.28###	409.82±0.92###
Standard (10 mg/kg)	285.18±0.93	204.02±1.81**	162.92±1.09***	112.72±1.04***
Plant extract (500 mg)	275.08±0.96	221.41±1.03**	166.61±1.12***	117.07±1.25***

*All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared diabetic control (normal saline), **P<0.01 as compared diabetic control (normal saline), ###P<0.001 as compared to Control. One-way ANOVA followed by Bonferroni multiple comparison test.*

4. CONCLUSION

Ptrerocarpus marsupium may provide leads for the discovery of new drugs for the management of many disorders with minimal side effects. The present study shows that the ethanolic extract of heartwood of *Pterocarpus marsupium* has potential antidiabetic action in alloxan induced diabetic rats and the effect was found to be more similar to the reference drug.

Conflict of interest

Authors declare no conflict of interest.

5. REFERENCES

1. Foster DW. Diabetes mellitus In: Isselbacher KJ, Brawnwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL. (Eds.), Harrison's Principles of Internal Medicine. McGraw Hill, United States, 1994, pp. 1979-1981.
2. American Diabetes Association. *Diabetes Care* 1997; **20:**S1-S70.
3. Yajnik CS. *Nutr. Rev.*, 2001; **43:**1-9.
4. Joglekar GV, Chaudhary NY, Aiman R. *Indian Journal of Physiology and Pharmacology,* 1959;**76:** 34-50.
5. Chakarvarthy BK, Gupta S, Gambhir SS, Gode KD. *Acta Diabetologia,* 1989; **23:**291-300.
6. Maurya R, Ray AB, Duah F, Slatkin DJ. *JR Heterocycles*. 1982; **2:**2103-2107.
7. Adinarayana D, Syamasudan KV, Seligman O. *Natuforsch*, 1982; **3:**145-147.
8. Mathew J, Subba Rao AV. *Current Science*, 1977; **12:**337-338.
9. OECD. Guidelines for the testing of chemicals revised draft guideline 423: Acute oral toxicity. France: Organization for Economic Cooperation and Development; 2000
10. Manik S, Gautam S, Kalia AN. *Indian J Exp Biol.* 2013; **51(9)**:702-707.
11. D'souza JJ, JJD'souza PP, Fazal F, Kumar A, Bhat HP, Baliga MS. *Food Funct.,* 2014; **5(4):**635-644.
12. Burnett A, McKoy ML, Singh P. *West Indian Med J.* 2015; **64(4):**315-319.
13. Girija K, Lakshman K. *Asian Pacific Journal of Tropical Biomedicine*, 2011; **32:**S62-S65.