

## EFFECT OF *PTEROCARPUS MARSUPIUM* IN STREPTOZOTOCIN-INDUCED HYPERGLYCEMIC STATE IN RATS: COMPARISON WITH GLIBENCLAMIDE

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marsupium, streptozotocin

### SUMMARY

*Pterocarpus marsupium* (Family: Leguminosae) is widely used in 'Ayurveda' as 'Rasayana' for the management of various metabolic disorders including hyperglycemia. Treatment of diabetic rats with Pterocarpus marsupium methanol extract (PMMtE, 300 mg/kg b.w./day) for 7 and 14 days showed normalization of streptozotocin-distressed serum glucose. At longer duration, PMMtE exerted a protective effect by correcting glycosylated hemoglobin (HbA<sub>1c</sub>), serum protein, insulin, alkaline and acid phosphatase (ALP and ACP), and albumin levels. Furthermore, tissue biochemical parameters viz. protein and glycogen altered towards normal in comparison to diabetic group. These results suggest oral administration of Pterocarpus marsupium may have the ability to improve streptozotocin-induced chronic diabetic stress.

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### INTRODUCTION

In recent years, substantial efforts have been made to identify efficient antidiabetic agents, as synthetic hypoglycemic agents are associated with many disorders and their effectiveness is limited and prone to a variety of side effects (1). Plants used in folk medicine to treat diabetes mellitus represent a viable alternative for the control of this disease (2). So for the present study, we selected a widely used plant *Pterocarpus* (*P.*) *marsupium*, as it is cited in ayurveda for curing diabetes and has been reviewed as possessing hepatoprotective (3) and antihyperlipidemic activities (4). *P. marsupium* is for its pharmaceutical properties like an astringent used in the treatment of dysentery, diarrhea, fever and toothache (5). It is also reported to be rich in polyphenolic compounds like marsupsin, pterosupin and pterostilbene (6,7) and flavonoids pteroside, pteroisoside, marsuposide, vijayoside and vijayosine (8,9).

Anti-cataract activity of *P. marsupium* in diabetes was observed by Vats *et al.* (10) but proper scientific research to evaluate its antidiabetic activity in severe hyperglycemia is yet to be proved. Therefore, the aim of the present study was to evaluate the hepatoprotective efficacy of *P. marsupium* methanolic extract (PMMtE) in streptozotocin induced diabetic rats.

## MATERIALS AND METHODS

### Experimental animals

Colony bred, adult, male albino rats of Wistar strain (180±20 g) were housed in plastic cages and maintained under standard conditions of temperature (25±3 °C), 12 h light/12 h dark cycle and 35%-60% relative humidity. Animals had free access to food (commercial pellet diet procured from Aashirwad Industries, India) and drinking water till before 30 min of sampling.

The study was approved by The Ethics Committee of the Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India. The Indian National Science Academy, New Delhi Guidelines (11) were followed on maintenance and use of the experimental animals.

### Authentication of plants

*Pterocarpus marsupium* wood (Leguminosae) was purchased from Jaipur local market and authenticated by Dr. Mohan Shankar Dashora, Department of Dravya Guna, National Institute of Ayurveda, Jaipur, India.

### Preparation of methanolic extract of *Pterocarpus marsupium*

*P. marsupium* wood (1 kg) was powdered and subjected to Soxhlet with methanol (100%) for 72 hours and separated under reduced pressure to obtain a chocolate-brown viscous mass. This material was vacuum evaporated to get a yield of 11.87% w/w. This final extract was redissolved in double-distilled water (DDW) prior to experimentation for the evaluation of antidiabetic activity.

### Preparation of diabetic animals

Diabetes was i.p. induced in overnight fasted rats by freshly prepared 0.2 mL solution of Streptozotocin, Himedia Laboratory Limited, Mumbai, India (50 mg/kg b.w./day dissolved in 0.1 mM sodium citrate buffer). Streptozotocin treated animals were considered as diabetic when the fasting glucose levels were above 250 mg/dL. Control rats were introduced 0.1 mM sodium citrate buffer alone. Diabetic animals were allowed to drink 2% glucose solution overnight to overcome the drug-induced hypoglycemic shock.

### Experimental design

Rats were randomized into five groups of 7 rats and duration of the experiment was set at 7 and 14 days. The experiments were performed as described below:

- Group I: (control group): rats orally received 0.5 mL distilled water
- Group II: diabetic group
- Group III: diabetic rats orally administered PMMtE (300 mg/kg b.w./day for 7 days) dissolved in 0.5 mL distilled water
- Group IV: diabetic rats orally administered PMMtE (300 mg/kg b.w./day for 14 days) dissolved in 0.5 mL distilled water
- Group V: diabetic rats orally administered glibenclamide (0.3 mg/kg b.w./day) dissolved in 0.5 mL distilled water

### Chemicals

Folin and Ciocalteu's reagent (Qualigens Fine Chemicals, Mumbai), glycogen standard (S.D Fine Chemicals, Mumbai), potassium hydroxide pellets (Sarabhai M Chemicals, Baroda), sulfuric acid (BDH Chemicals Ltd., Mumbai), absolute alcohol and sodium chloride (Merck Specialities, Mumbai), phenol, trichloroacetic acid (BDH, Chemicals Ltd., Mumbai), sodium carbonate (BDH Chemicals Ltd., Mumbai), serum glucose (Ecopak<sup>®</sup>, Accurex Biomedical Ltd, Mumbai).

### Estimation of serum glucose

Fasting serum glucose was measured by using a commercially available kit based on glucose oxidase method (12).

### Biochemical analysis

After autopsy, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats. Little volume of this blood was stored in EDTA-vials for glycosylated hemoglobin (HbA<sub>1c</sub>) estimation (13), and from the rest serum was separated by centrifugation and analyzed for serum biochemical parameters, i.e. total protein (14), insulin (15), ALP and ACP (16) and albumin (17). Liver, pancreas and heart were dissected immediately from sacrificed rats

and kept at 4 °C in ice cold normal saline (0.8%) for biochemical estimation of protein (14) and glycogen (18).

### Statistical analysis

Values are given as mean ± SEM (standard error of the mean) and were compared using one way ANOVA with Tukey-Kramer multiple comparison test, to judge the difference among various groups. Values of  $P < 0.05$  were considered statistically significant.

## RESULTS

### Effect of PMMtE on serum glucose and glycosylated hemoglobin

Intraperitoneal administration of streptozotocin to overnight fasted normal animals caused marked elevations ( $P < 0.001$ ) in serum glucose (Fig. 1) and glycosylated hemoglobin (Fig. 2) levels after 72 hours; these levels were observed to continuously increase until day 7 of animal sacrifice. Regular PMMtE administration (300 mg/kg b.w. for 7 and 14 days) to diabetic rats antagonized the remarkable alterations in

serum glucose and HbA<sub>1c</sub> levels (7.81% and 18.51% decrease in serum glucose; and 14.41% and 22.58% decrease in HbA<sub>1c</sub>, respectively).

### Effect of PMMtE on biochemical parameters

As evident, the glycogen and protein contents were decreased (glycogen level 47.57% in liver and 31.16% in heart; protein level 16.58% in liver, 31.08% in kidney and 69.93% in pancreas) after streptozotocin-diabetes induction (Table 1). As compared to diabetic rats, 300 mg/kg b.w./day PMMtE treated diabetic rats showed significant to highly significant elevations on day 7 in hepatocardiac glycogen content (30.03% in liver and 18.86% in heart) and non-significant increase in protein contents of the liver, kidney and pancreas (6.31% in liver, 7.75% in kidney and 8.17% in pancreas). When 300 mg/kg b.w./day PMMtE were given for 14 days, significant to highly significant improvement was observed in hepatocardiac glycogen content (55.98% in liver and 51.47% in heart) as well as in protein content (14.23% in liver, 25.64% in kidney and 53.10% in pancreas). Orally administered glibenclamide brought back glycogen and protein contents near to the normal level.K

Figure 1. Final serum glucose levels after 7- and 14-day treatment with PMMtE (300 mg/kg b.w./day) and glibenclamide in hyperglycemic rats. Values are presented as mean ± SEM (n=7); \*\* $P < 0.001$  and \* $P < 0.01$  as compared with control rats; <sup>a++</sup> $P < 0.001$  and <sup>a</sup> $P < 0.01$  as compared with hyperglycemic rats; and <sup>ns</sup> $P > 0.05$ .

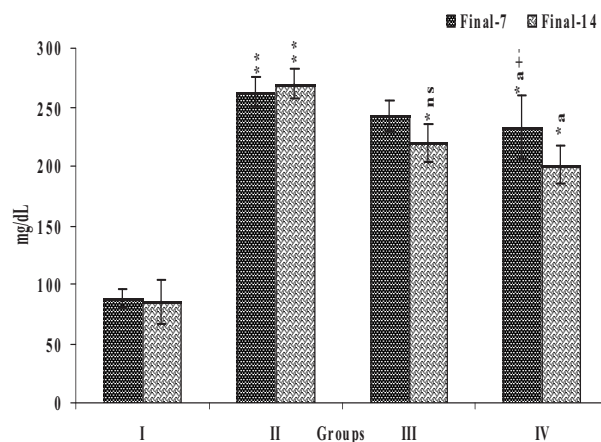
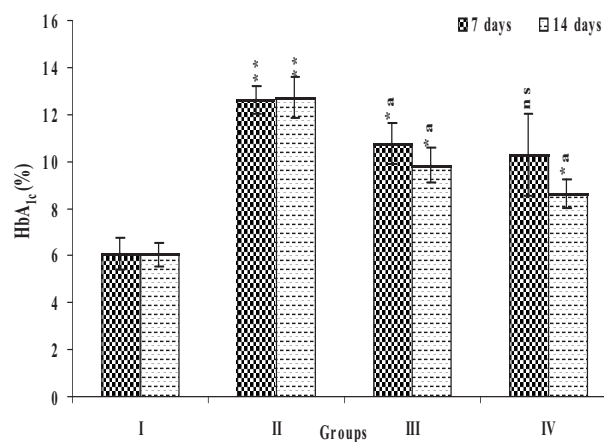


Figure 2. Changes in glycosylated hemoglobin (HbA<sub>1c</sub>) level after 7- and 14-day treatment with PMMtE (300 mg/kg b.w./day) and glibenclamide in hyperglycemic rats. Values are presented as mean ± SEM (n=7); \*\* $P < 0.001$  and \* $P < 0.01$  as compared with control rats; <sup>a</sup> $P < 0.01$  as compared with hyperglycemic rats; and <sup>ns</sup> $P > 0.05$ .



A significant reduction in serum insulin, protein and albumin was noticed after streptozotocin-diabetes induction. The 7-day PMMtE extract administration to hyperglycemic rats caused a non-significant increase in insulin level but significant increase in protein and insulin levels. Oral administration of PMMtE (300

mg/kg b.w./day) for 14 days brought significant (serum insulin and albumin) to highly significant (protein) elevations in comparison to diabetic rats (Table 2). Glibenclamide administration caused a slightly greater improvement than plant oral extract treatment.

**Table 1. Changes in glycogen and protein contents after 7- and 14-day treatment with PMMtE (300 mg/kg b.w./day) and glibenclamide in hyperglycemic rats. Values are presented as mean  $\pm$  SEM (n=7); \*\*P<0.001 and \*P<0.01 as compared with control rats; <sup>a++</sup>P<0.001 and <sup>aP</sup>P<0.01 as compared with hyperglycemic rats; and <sup>ns</sup>P>0.05**

Treatment	Glycogen (mg/g)					
	7 days			14 days		
	Liver	Heart		Liver	Heart	
Group I: Control (vehicle treated)	5.97 $\pm$ 0.37	2.31 $\pm$ 0.10		6.21 $\pm$ 0.39	2.47 $\pm$ 0.13	
Group II: diabetic group	3.13** $\pm$ 0.27	1.59** $\pm$ 0.05		3.09** $\pm$ 0.25	1.36** $\pm$ 0.11	
Group III: diabetic+PMMtE (300 mg/kg b.w./day)	4.07* <sup>a</sup> $\pm$ 0.06	1.89* <sup>a++</sup> $\pm$ 0.004		4.82* <sup>a</sup> $\pm$ 0.15	2.06* <sup>a</sup> $\pm$ 0.05	
Group IV: diabetic+glibenclamide (0.3 mg/kg b.w./day)	4.39* <sup>a</sup> $\pm$ 0.08	1.98* <sup>a++</sup> $\pm$ 0.041		5.25 <sup>nsa++</sup> $\pm$ 0.20	2.24 <sup>nsa++</sup> $\pm$ 0.04	

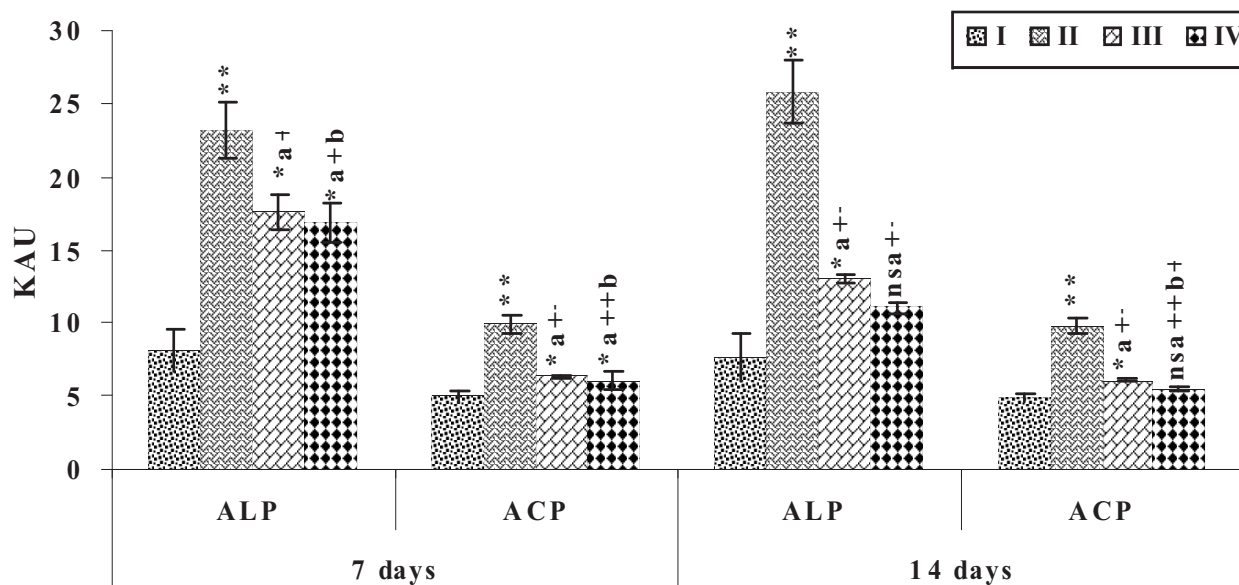
  

Treatment	Protein (mg/g)						
	Liver	7 days			14 days		
		Kidney	Pancreas		Liver	Kidney	Pancreas
Group I: Control (vehicle treated)	445.65 $\pm$ 11.13	194.12 $\pm$ 7.23	65.89 $\pm$ 4.39	428.23 $\pm$ 7.49	217.69 $\pm$ 13.21	49.18 $\pm$ 3.86	
Group II: diabetic group	371.73** $\pm$ 05.12	133.78** $\pm$ 5.57	19.81** $\pm$ 5.21	358.15** $\pm$ 5.29	127.23** $\pm$ 8.86	19.32** $\pm$ 1.16	
Group III: diabetic+PMMtE (300 mg/kg b.w./day)	395.22* <sup>a</sup> $\pm$ 07.12	144.16* $\pm$ 05.93	21.43* $\pm$ 8.18	409.13* <sup>a++</sup> $\pm$ 1.63	159.86* $\pm$ 17.19	29.58* <sup>a++</sup> $\pm$ 1.16	
Group IV: diabetic+glibenclamide (0.3 mg/kg b.w./day)	413.26* <sup>a++</sup> $\pm$ 01.12	156.26* $\pm$ 2.89	33.17* $\pm$ 5.23	426.35 <sup>nsa++</sup> $\pm$ 2.18	178.64 <sup>nsa</sup> $\pm$ 11.09	33.17* <sup>a++</sup> $\pm$ 1.87	

**Table 2. Changes in serum insulin, total protein and albumin levels after 7- and 14-day treatment with PMMtE treatment (300 mg/kg b.w./day) and glibenclamide in hyperglycemic rats. Values are presented as mean  $\pm$  SEM (n=7); \*\*P<0.001 and \*P<0.01) as compared with control rats; <sup>a++</sup>P<0.001 and <sup>aP</sup>P<0.01 as compared with hyperglycemic rats; and <sup>ns</sup>P>0.05**

Treatment	7 days			14 days		
	Insulin ( $\mu$ U/mL)	Total protein (mg/dL)	Albumin (mg/100 mL)	Insulin ( $\mu$ U/mL)	Total protein (mg/dL)	Albumin (mg/100 mL)
Group I: control (vehicle treated)	18.98 $\pm$ 0.72	6.76 $\pm$ 0.46	3.65 $\pm$ 0.16	19.10 $\pm$ 0.91	6.65 $\pm$ 0.23	3.39 $\pm$ 0.17
Group II: diabetic group	9.74** $\pm$ 1.05	3.58** $\pm$ 0.19	2.13** $\pm$ 0.12	9.61** $\pm$ 0.83	3.29** $\pm$ 0.15	2.05** $\pm$ 0.12
Group III: diabetic+PMMtE (300 mg/kg b.w./day)	12.53* $\pm$ 1.32	4.39* <sup>a</sup> $\pm$ 0.08	2.79* <sup>a</sup> $\pm$ 0.14	13.77* <sup>a</sup> $\pm$ 0.92	5.22* <sup>a++</sup> $\pm$ 0.15	2.64 <sup>nsa</sup> $\pm$ 0.10
Group IV: diabetic+glibenclamide (0.3 mg/kg b.w./day)	14.92* <sup>a</sup> $\pm$ 0.46	4.98* <sup>a++</sup> $\pm$ 0.11	3.21* <sup>a++</sup> $\pm$ 0.01	15.81* <sup>a</sup> $\pm$ 0.75	5.39* <sup>a++</sup> $\pm$ 0.21	3.07 <sup>nsa</sup> $\pm$ 0.13

Figure 3. Changes in serum ACP and ALP levels after 7- and 14-day treatment with PMMtE (300 mg/kg b.w./day) and glibenclamide in hyperglycemic rats. Values are presented as mean  $\pm$ SEM (n=7); \*\*P<0.001 and \*P<0.01) as compared with control rats; <sup>a</sup>++P<0.001 and <sup>a</sup>P<0.01 as compared with hyperglycemic rats; and <sup>ns</sup>P>0.05



A highly significant increase ( $P \leq 0.001$ ) in serum ALP and ACP was noticed after 7 and 14 days of diabetes induction. The 14-day PMMtE treatment of diabetic rats caused a highly significant decline ( $P \leq 0.001$ ) in ALP and ACP levels in treated diabetic animals, whereas oral glibenclamide (0.3 mg/kg b.w./day) treatment of hyperglycemic rats produced a highly significant decrease in these serum parameters when compared with diabetic rats (Fig. 3).

## DISCUSSION

Streptozotocin-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents (19). In our study, PMMtE treatment proved highly effective in managing hyperglycemia because both serum glucose and glycosylated hemoglobin levels were found to have normalized. As these effects were only seen in PMMtE treated diabetic rats but not in hyperglycemic rats, it was concluded that the dose-dependent glucose and glycosylated hemoglobin lowering effect might be due to *P. marsupium* extract. In addition to this, serum insulin increased following PMMtE treatment, maybe because the partially purified PMMtE caused an

increase in insulin release through stimulation in  $\beta$ -cell regeneration since histopathologic observations depicted manifold streptozotocin-destroyed  $\beta$ -cell rejuvenation after treatment with *P. marsupium* methanol extract; this may strengthen the insulinotropic role of *P. marsupium* by regenerating insulin producing cells. In this context, *P. marsupium* extract possesses a hypoglycemic activity in experimental diabetic rats (20).

Hepatocardiac glycogen was significantly reduced in streptozotocin diabetic rats (21). A significant rise in liver and heart glycogen is indicative of the role of insulin in increasing glycogen synthetase and glucokinase activity and decreasing glycolytic and glyconeolytic enzyme activity. An increase was recorded in liver and myocardial glycogen of diabetic rats after PMMtE, which might be due to a decreased output of hepatic glucose and fatty acid uptake as well as increased glycogen synthetase activity (22).

The metabolism of proteins is abnormal in diabetes due to insulin secretion defect, leading to various metabolic disorders (23). Serum total protein and albumin levels were reduced in diabetic rats as reported by Prakasam *et al.* (24) from their herbal drug antidiabetic study. Total protein and albumin level

reduction may be due to increased protein catabolism caused by streptozotocin (25). PMMtE administration caused normalization of serum protein and albumin levels, possibly through the increase in insulin-mediated amino acid uptake, enhancement in protein synthesis and inhibition in protein degradation (26).

An increased activity of serum ALP and ACP was observed in diabetic rats (27). Oral treatment with PMMtE and glibenclamide normalized these enzyme activities, suggesting that it is able to condition the hepatocytes so as to protect the membrane integrity against streptozotocin-induced leakage of marker enzymes into blood circulation. This also suggests that

it is able to nurture the streptozotocin-damaged cell membrane for getting ACP and ALP leakage into the bloodstream. Further studies are necessary to find out the mechanism of PMMtE action against streptozotocin diabetes.

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