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Studies on the ameliorative potential of aqueous extract of bark of *Pterocarpus marsupium* Roxb in streptozotocin - induced diabetic rats

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Abstract

A study was planned to evaluate the ameliorative potential of aqueous extract of bark of *Pterocarpus marsupium* Roxb (PM) in streptozotocin (STZ) induced diabetic rats. The effect of oral administration of plant extract on serum electrolytes, serum glycolytic enzymes, liver microsomal protein, hepatic cytochrome P-450 dependent monooxygenase enzymes and lipid peroxidation of the liver and kidney of streptozoticin - induced diabetic rats was studied. Administration of the bark extract to diabetic rats restored the levels of serum electrolytes, glycolytic enzymes and hepatic cytochrome P-450 dependent enzyme systems by preventing the formation of liver and kidney lipid peroxides at the end of study period of 12 weeks. Further, the bark extract at the dosage of 500mg/kg/day exhibits a significant ameliorative activity as evidenced by the histological studies in normal and PM treated STZ - induced diabetic rats. On the basis of our findings, it could be used as an effective ameliorative agent for the management of diabetes and associated metabolic alterations.

Key words: *Pterocarpus marsupium* Roxb, ameliorative potential, cytochrome P-450 dependent enzymes, lipid peroxides

1. Introduction

Diabetes mellitus (DM) associated metabolic alterations are often produces numerous long term complications. These include insulin resistance, inactivity of glycolytic enzymes, electrolyte imbalance and altered drug metabolism, accumulation of free fatty acids and formation of lipid peroxides in the affected individuals [1-3]. This disease is found in all parts of the world and is rapidly increasing in most parts of the world, and alarming increase in India with more than 40 million diabetic cases in India alone. In general, the control of blood glucose concentrations to near normal range in patients is mainly based on the use of oral hypoglycemic agents including insulin [4]. However, all these treatments have limited

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efficacy and mostly associated with undesirable side effects [5, 6]. Therefore, the search for more effective, safer and alternative medicines has continued to be an important area of investigation for the management of diabetes mellitus. Traditional medicine and medicinal plants, in general, continued to be a powerful source of new drugs and now contributing about 90% of the newly discovered pharmaceuticals [7]. Traditional medicine based approach has provided health coverage for over 80% of the world population, especially in the developing countries [8].

Pterocarpus marsupium Roxb (Leguminosae) has been traditionally used in India as folklore medicine [9] and its antidiabetic activity was tested [10-11]. The water soluble active constituent isolated from the bark of P. marsupium Roxb was (-)-epicatecin (benzopyran) and it was shown to be very effective in stimulating islets cells of pancreas for insulin secretion [12] and regulation of blood glucose. Number of other phenolic constituents including marsupsin, pterosupin and pterostilbene were isolated from *P. marsupium* [13]. Marsupsin and pterostilbene has been shown to be very effective in reducing the blood glucose level in hyperglycemic rats [13]. *P. marsupium* extract has been shown to be very effective in preventing cataract development in alloxan induced diabetic rats [14]. Further, it was reported that *P. marsupium* extract prevented the fructose induced hypertrigly-ceridaemia and hyperinsulinaemia in rats [15]. The phenolic constituent of *P. marsupium*, pterostilbene has been shown to possess significant anti-inflammatory effect and it was also reported to be safe for human use [16].

The aim of the present study was to investigate the ameliorative potential of *P. marsupium* on DM associated metabolic alterations.

2. Materials and methods

Animals: Male albino rats (Wistar strain, weighing 150-200g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Madhawaram, Chennai and housed under standard husbandry conditions (30° C ± 2°, 60 –70 % relative humidity and 12h : 12h day-night cycle) and allowed standard pellet rat feed and water ad libitum. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC), VIT University.

Plant material: The fresh bark of *P. marsupium* was collected from the Morappur forest area, Dharmapuri District, Tamil Nadu during the month of April 2005. The tree was authenticated with the help of botanist and the voucher specimen was prepared and submitted to the Forest Department (FDSC/202). The bark was washed with distilled water, shade dried, powdered using electrical grinder and stored in an air-tight container separately for further use.

Preparation of extract: Powdered *P. marsupium* (100g) was soaked in 500 ml of sterile distilled water and stirred intermittently and then left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (the yield of aqueous extract was found to be 3.9 % (w/v)) served as aqueous extract of *P. marsupium* for experimentation. The extract was concentrated under vacuum to get solid yield and freeze dried (Thermo freeze dryer, USA) and the yield was calculated.

Induction of diabetes mellitus in rats: Diabetes was induced experimentally in rats by single intraperitoneal injection of freshly prepared solution of Streptozotocin nicotinamide (STZ) (Sigma, USA) at a dose of 35mg/kg bodyweight in 0.1M cold citrate buffer, pH 4.5.

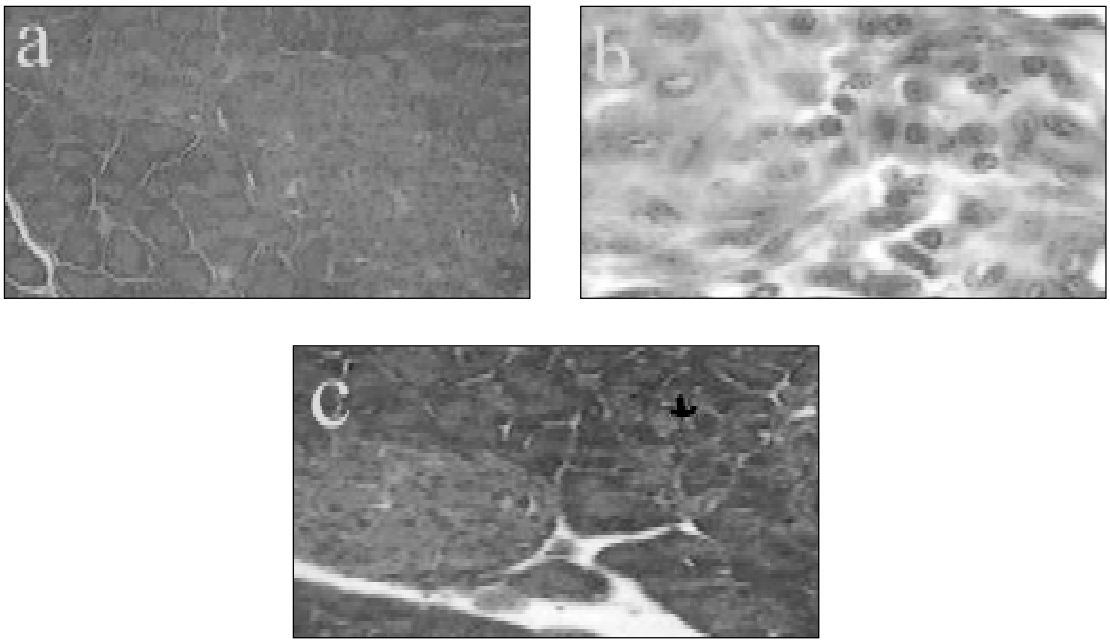

Fig 1. Histological examination of pancreas in experimental animals. A cross section of tissue was stained with haematoxlin and viewed under microscope with 100 X magnification. (a) Normal healthy control rat pancreas showing globules of acini with normal islet cells. (b) STZ-induced diabetic rat showing atrophy of islet cells with severe inflammatory infiltrate with edema. (c) *P. marsupium* bark aqueous extract (500 mg/kg/body weight) treated rat showing normal architecture and morphology of islet cells.

After 72 hours, blood was collected from the tail vein under ether anesthesia with aseptic precautions and blood glucose levels were estimated by using Autoanalyser- Microlab 2000 (Hamilton, UK). The animals were considered to be diabetic, if the blood glucose values were above 250 mg/dl and those animals alone were used for the study. Diabetes was developed and stabilized in STZ- treated rats over a period of 7 days [17]. The control rats were treated with citrate buffer (pH 4.5). The ameliorative activity of aqueous extract (100, 300 & 500 mg/kg body weight / day) was assessed in control and STZ-induced diabetic rats at the end of 12 weeks.

Experimental design: Animals were divided in to six groups of six animals each. Group I served as a control, group II was STZ- treated surviving diabetic rats, group III served as a positive control and received a hypoglycemic drug tolbutamide (Siviling Pharma, India) (100 mg/kg body weight / day), group IV, V and VI were diabetic rats treated with aqueous extract 100, 300 and 500 mg/kg body weight /day respectively by oral intubations method. At the end of 12 weeks rats were sacrificed blood samples, pancreas, liver and kidney tissue samples were collected to carry out biochemical and histological studies.

Biochemical investigations: Serum was separated and assayed for electrolytes including sodium, potassium and calcium by using flame photometry (Systronics, India). Serum marker enzymes and liver microsomal monooxygenase enzyme systems were estimated by using commercial kits purchased from Bayer Diagnistics India Ltd. using spectrophotometer (Systronics, India). Liver and kidney tissue homogenates were used to estimate thiobarbituric acid reactive substances [18] and hydroperoxides [19].

Table 1. Effect of *P. marsupium* bark aqueous extract on serum marker enzymes GS, GK, LDH, SD and MD in normal and streptozotocin-induced diabetic rats

Groups	Dose (mg/kg bw/day)	GS(U/L)	GK(U/L)	LDH(U/L)	SD(U/L)	MD(U/L)
Normal	-	8.07 ± 1.2	9.07 ± 2.52	92.74 ± 2.13	4.84 ± 125	5.85 ± 1.42
Diabetic control [a]	-	5.55 ± 1.36*	4.12 ± 2.31*	60.84 ± 1.54*	3.14 ± 1.45*	1.87 ± 1.32*
Diabetic + Tolbutamide	100	6.17 ± 1.58¶	8.65 ± 1.95¶	89.40 ± 1.45¶	6.74 ± 1.28¶	4.32 ± 1.62¶
Diabetic + *P. marsupium* bark extract	100	6.35 ± 1.95	7.44 ± 1.24	87.45 ± 1.65	6.15 ± 1.32	3.58 ± 2.45
	300	7.15 ± 1.47	7.75 ± 1.65	89.42 ± 1.95	6.95 ± 1.45	3.98 ± 1.48
	500	7.50 ± 1.01#	8.32 ± 1.42#	91.23 ± 1.45#	7.15 ± 1.85#	4.02 ± 1.24#

Each value is mean ± SD for six rats in each group, GS - Glycogen synthase, GK - Glucokinase, LDH - Lactate dehydrogenase, SD - Succinate dehydrogenase, MD - Malate dehydrogenase, [a] STZ - 35mg/kg body weight in 0.1M cold citrate buffer, pH 4.5, *F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to control, ¶, #F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to diabetic control.

Table 2. Effect of *P. marsupium* bark aqueous extract on liver microsomal protein, monooxygenase activity in normal and streptozotocin-induced diabetic rats

Groups	Dose (mg/kg bw/day)	Microsomal protein (mg/g organ wt)	Monooxygenase activity (nmol product/min/mg protein)		
			EROD	PROD	PNPH
Normal	-	12.17 ± 0.98	0.22 ± 0.01	0.25 ± 0.02	0.95 ± 0.11
Diabetic control [a]	-	13.9 ± 0.75*	0.42 ± 0.02*	0.35 ± 0.03*	1.00 ± 0.12*
Diabetic +Tolbutamide	100	12.9 ± 0.98¶	0.22 ± 0.03¶	0.25 ± 0.01¶	0.96 ± 0.13¶
Diabetic + *P. marsupium* bark extract	100	13.1 ± 2.1	0.21 ± 0.02	0.23 ± 0.01	0.90 ± 0.15
	300	13.2 ± 2.3	0.22 ± 0.01	0.24 ± 0.02	0.92 ± 0.14
	500	13.4 ± 2.4#	0.23 ± 0.02#	0.25 ± 0.02#	0.93 ± 0.18#

Each value is mean ± SD for six rats in each group, EROD: Ethoxyresorufin-O-demethylase, PROD: Pentoresorufin-O-demethylase, PNPH: p-nitrophenol hydroxylase, [a] STZ- Streptozotocin (35mg/kg body weight in 0.1M cold citrate buffer, pH 4.5), *F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to control, ¶, #F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to diabetic control.

Statistical analysis: Statistical analysis was performed using SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan' multiple range test (DMRT). All the results were expressed as mean ± SD for six rats in each group P<0.05 was considered as statistically significant.

3. Results

P. marsupium bark aqueous extract at lower doses (>100 mg) failed to show any significant effect on diabetes associated metabolic alternations and at higher doses (1000 mg) the effect was more or less similar to that of 500 mg dose. Table 1 presents the levels of glycolytic enzymes in normal and in diabetic rats. The metabolic enzymes of glucose was significantly decreased (F>0.05; P<0.001) when compared to normal control rats. Oral administration of *P. marsupium* bark aqueous extract brought back the levels of glycolytic enzymes significantly (F>0.05; P<0.001) to near normal level as that of untreated normal rats.

Table 3. Effect of *P. marsupium* bark aqueous extract on liver and kidney TBARS, hydroperoxides and malondialdehyde in normal and streptozotocin-induced diabetic rats

Groups	Dose (mg/kg bw/day)	TBARS (mM/mg)		Hydroperoxides (nM/ 100g tissue)		Tissue MDA (n mol/g wet weight)	
		Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	-	0.68 ± 0.21	1.53.08	70.64 ± 2.10	55.03 ± 2.09	295.4 ± 1.69	291.23 ± 1.02
Diabetic control [a]	-	1.45 ± 0.01*	2.64 ± 0.36*	115.57 ± 1.45*	79.00 ± 1.01*	365.3 ± 1.20*	365.23±1.65*
Diabetic + Tolbutamide	100	1.12 ± 1.01¶	1.91 ± 0.27¶	84.13 ± 1.32	65.13 ± 1.11¶	345.65 ± 1.02¶	300.95 ± 1.24¶
Diabetic + *P. marsupium* bark extract	100	1.25 ± 0.12	1.71 ± 1.24	88.19 ± 1.19	63.23 ± 1.01	324.12 ± 1.58	282.12 ± 1.65
	300	1.11 ± 0.65	1.65 ± 0.65	85.19 ± 0.85	59.30 ± 2.01	312.89 ± 1.69	276.02 ± 1.24
	500	1.00 ± 0.58#	1.61 ± 0.01#	83.10 ± 1.01#	55.65 ± 1.03#	302.03 ± 1.58#	273.21 ± 1.32#

Each value is mean ± SD for six rats in each group, [a] STZ- Streptozotocin (35mg/kg body weight in 0.1M cold citrate buffer, pH 4.5), *F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to control, ¶, #F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to diabetic control.

Table 4. Effect of *P. marsupium* bark aqueous extract on serum electrolytes in normal and streptozotocin-induced diabetic rats

Groups	Dose (mg/kg bw/day)	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mEq/L)
Normal	-	145.00 ± 5.90	6.80 ± 1.25	7.38 ± 0.15
Diabetic control [a]	-	162.00 ± 2.36*	7.90 ± 0.23*	9.10 ± 0.23*
Diabetic + Tolbutamide	100	143.12 ± 2.12¶	5.13 ± 0.62¶	7.91 ± 0.56¶
Diabetic + *P. marsupium* bark extract	100	139.00 ± 1.65	4.56 ± 0.62	6.45 ± 0.12
	300	135.00 ± 1.32	4.68 ± 0.13	7.25 ± 0.52
	500	141.54 ± 1.52#	4.79 ± 0.45#	7.86 ± 0.41#

Each value is mean ± SD for six rats in each group, [a]STZ -35 mg/kg body weight in 0.1M cold citrate buffer, pH 4.5, *F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to control, ¶, #F > 0.05 (ANOVA) and P< 0.001 (DMRT) as compared to diabetic control.

The significantly (F>0.05; P<0.001) elevated levels of microsomal protein and monooxygenase enzymes- Ethoxyresorufin-O-demethylase (EROD), Pentoresorufin-O-demethylase (PROD) and p-nitrophenol hydroxylase (PNPH) in STZ- induced diabetic rats were restored to near normal level up on treatment with *P. marsupium* bark aqueous extract (Table 2).

The significantly (F>0.05; P<0.001) elevated levels of thiobarbituric acid reactive substances (TBARS), tissue hydroperoxides and tissue malondialdehyde in diabetic rats were reduced significantly (F>0.05; P<0.001) to near normal level when compared to diabetic control up on treatment with *P. marsupium* bark aqueous extract (Table 3).

In diabetic rats there was a significant (F>0.05; P<0.001) increase in serum electrolytes including sodium, potassium and calcium. Oral administration of *P. marsupium* bark aqueous extract significantly (F>0.05; P<0.001) reduces

serum electrolytes level to near normal level (Table 4). Figure 1 shows the histological examination of pancreas of control, STZ - induced diabetic rats and drug treated rats. Changes in the morphology of pancreatic cells including mild swelling, inflammation and necrosis were observed in diabetic rats. Oral administration of the PM bark aqueous extract (500 mg/kg body weight /day) for 12 weeks decreases swelling and inflammation and restored the normal architecture and morphology of pancreas.

4. Discussion

Currently available drugs used for the management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic and ameliorative drugs. In the present study the oral treatment of *P. marsupium* bark extract for 12 weeks restored the activities of glucose metabolizing enzymes. It has been reported that chemically (STZ) induced diabetes produces partial or total deficiency of insulin that results in decreased levels of glycolytic enzymes [20]. Insulin has been shown to be a potent stimulator of hexokinse/ glucokinase activity [21]. The decreased levels of glycogen synthase, glucokinase, lactate dehydrogenase, succinate gehydrogenase and malate dehydrogenase may be due to decreased insulin level in diabetic rats. Restoration of the levels of glycolytic enzymes after oral administration of *P. marsupium* aqueous extract might be due to stimulatory and ameliorative role of the extract on insulin secretion.

Chemically induced diabetes has been shown to induce polymorphic alterations on the metabolic activities of cytochrome P-450 dependent monooxygenase enzyme systems [22]. In our study, the elevated concentrations of cytochrome P-450 dependent monooxygenase enzyme systems, such as EROD, Pentoresorufin-O-demethylase PROD and PNPH may be due to hepatocellular damage caused by the oxygen free radicals. Oral administration of aqueous extract restored the concentration of hepatic phase -I drug-metabolizing enzymes in STZ- induced diabetic rats.

The involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes has been reported by several workers [23, 24]. The oxidative stress in diabetes also include shifts in redox balance which results in altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species (ROS) by glycation and lipid oxidation with decreased antioxidant defenses. In diabetes mellitus, oxygen free radicals are generated by stimulating H_2O_2 *in-vitro*, as well as *in-vivo* and in pancreatic β-cells [25]. In our study, the observed increase in TBARS, tissue malondialdehyde and hydroperoxides in liver and kidney of STZ-induced diabetic rats served as an index of lipid peroxidation in diabetic condition. The increased lipid peroxidation indicates that more pronounced free radicals generation and oxidative stress. Administration of *P. marsupium* bark extract 500 mg/kg body weight / day for the period of 12 weeks decreased the lipid peroxidation index. Significant reduction in lipid peroxidation can be attributed to the antioxidant activity of various phytochemicals present in the aqueous extract of the plant. PM has been shown to contain 5, 7, 2, 4 - tetrahydroxy isoflavone 6-6 glucoside, reported to be a potent antioxidant and are believed to be responsible for preventing cardiovascular diseases [26]. Further, these results indicate the possibility that the major function of the extract is to protect vital tissues such as liver, kidney, pancreas and brain thereby reducing the causation of diabetes. It was already been reported that elevation of oxidative

stress causes depletion of cellular antioxidant scavenger systems and thereby it produces free radical - mediated tissue damage by a series of chemical reactions [24]. Further, increased glucose oxidation in the presence of transition metals causes membrane damage by peroxidation of membrane lipid and protein glycation [27]. This could be the reason for the altered flux in electrolyte balance results in elevated extra cellular concentration of sodium, potassium and calcium in STZ induced diabetic rats. Administration aqueous extract of PM reduces LPO and restored the antioxidant status and this could also be the possible reason for the restoration of extra cellular electrolytes concentration. Elevated levels of calcium [28] sodium and potassium have already been reported in type 2 diabetic patients [29]. The ameliorative role of *P. marsupium* bark aqueous extract was evidenced by the observed restoration of histological changes, normalization of hepatic phase I drug metabolizing CYP enzymes and reduction in lipid peroxidation index in drug treated rats. *P. marsupium* bark aqueous extract (500 mg/kg body weight per day) in addition to hypoglycemic potential, it also possesses ameliorative and free radical scavenging activity. However, further pharmacological and biochemical investigations are needed to find out the active constituent and its mechanism of action to understand the bioactive and ameliorative potential of the plant.

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