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ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF HEARTWOOD OF BIJASAR (*PTEROCARPUS MARSUPIUM* ROXB.) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT: The antidiabetic potential of ethanolic extract of bijasar (*Pterocarpus marsupium* Roxb.) heartwood was evaluated in the streptozotocin-nicotinamide induced type 2 diabetic model. Graded doses of the ethanolic heartwood extract were administered to normal and experimental diabetic rats for 12 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animals. In addition, changes in body weight, serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and liver glycogen levels assessed in the extract treated diabetic rats were compared with diabetic control and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the bijasar heartwood by Indian herbal practitioners for treatment of diabetics in different part of the Indian subcontinent.

INTRODUCTION: Worldwide nearly 150 million people are suffering diabetes and this is likely to increase the diabetics number up to 300 million or more by the year 2025 [1]. Reasons for this rise includes increase in sedentary lifestyle, consumption of energy rich diet, obesity, and higher life span, etc. [2]. Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to 2–3-folds than the present rates [3]. During past decade many herbal medicines have been investigated for the potential treatment of diabetes.

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Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones [4]. Many Indian medicinal plants have been reported for their antidiabetic activity [5, 6].

Pterocarpus marsupium Roxb. (Leguminosae) is a large deciduous tree and has a high reputation in the traditional system of Indian medicine (including folklore) and therefore is one of the drugs used in the treatment of diabetes mellitus by Ayurvedic physicians in different parts of India. The butanol subfraction of the alcohol extract of bark of bijasar bark exhibits significant antidiabetic activity and corrects the metabolic alterations in diabetic rats and this activity may resemble insulin-like properties [7]. The aqueous infusion of the heartwood has been used by herbal practitioners since time immemorial for diabetics in different part of India. Considering the potential use of infusion of heart wood of the plant, the purpose of this research was

to experimentally assess the anti-diabetic effect of bijasar heart wood extract in normal and streptozotocin-nicotinamide induced type 2 diabetic rats and to compare it with glibenclamide as a reference standard. A clinically used glibenclamide (a sulphonylurea drug) is known to lower the serum glucose by stimulating β-cells to release insulin.

MATERIALS AND METHODS:

Animals: Male Wistar rats weighing 200–230 g were housed in clean cages with temperature (22–24 ºC), 12-h light/12-h dark cycle and relative air humidity 35–60%. Rats had continuous access to food and to tap water. Permission for the study was obtained from the Institutional Animal Ethics Committee (CPSEA approved).

Plant material: The heartwood of Bijasar (*Pterocarpus marsupium* Roxb.) was purchased from a retail market of Bhopal (India) in April 2012. The sample was identified and a voucher specimen (Voucher number: PM-012), deposited in the Herbarium, section, People's Institute of Pharmacy and Research Centre, Bhopal Madhya Pradesh, India.

Preparation of heartwood extract: Dried and ground heartwood (about 200 g) were subjected to extraction with 500 ml ethanol (80%) in a Soxhlet apparatus for 72 h. The solvent was recovered by distillation in vacuo and the residue (yield 17.75 g) was stored in dessicator and used for subsequent experiments.

Acute toxicity studies: Healthy adult Wistar albino rats of either sex, starved overnight were divided into four groups (n = 6) and were orally fed with the alcoholic extract in increasing dose levels of 100, 500, 1000 and 3000 mg/kg body weight [8]. The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after 24 and 72 h for any lethality [9].

Oral glucose tolerance test: The oral glucose tolerance test (Bonner-weir, 1988) [34] was performed in overnight fasted (18 h) normal animals. Rats divided into four groups (n = 6) were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500 mg/kg) and glibenclamide (0.25 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation (to minimize the distress) at 0, 30, 60, 90 and 120 min of extract administration. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips.

Normoglycaemic study: For normoglycaemic study, rats were divided into four groups (n = 6) and were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500 mg/kg) and glibenclamide (0.25 mg/kg), respectively. The blood glucose levels were estimated on days 0, 5 and 12.

Induction of non-insulin dependent diabetes mellitus (NIDDM): NIDDM was induced (Masiello *et al.*, 1998) [10] in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ (Sigma Aldrich, Germany), 15 min after the i.p. administration of 120 mg/kg nicotinamide (Qualigens Fine Chemicals, Division of Glaxo, Mumbai, India). Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72 h and then on day 7 after injection. The rats found with permanent NIDDM were used for antidiabetic study.

Experimental design: The diabetic animals, divided into four groups (n=6) were administered 2% gum acacia solution, alcoholic extract (250 mg/kg), alcoholic extract (500 mg/kg) and glibenclamide (0.25 mg/kg), respectively, for 12 days. The fasting blood glucose levels were estimated on days 0, 5 and 12. The effects of administration of the ethanolic extract on diabetic rats were estimated on the 12th day after the animals were sacrificed by decapitation.

Serum insulin levels, serum lipid profiles, liver glycogen levels (Nicholas, 1956) [11], Glycosylated hemoglobin levels, thio barbituric acid reactive substance levels (Ohkawa *et al.*, 1979) [12] and changes in body weight were assessed in the diabetic animals treated with extracts and compared with diabetic control and normal animals. Glycosylated hemoglobin levels (turbidimetric inhibition immuno assay) were estimated by using an auto-analyzer.

Statistical analysis: Data were statistically evaluated by using one way ANOVA, followed by

post hoc Scheffe's test. The values were considered significant when $p<0.05$.

RESULTS: Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of *Pterocarpus marsupium* heartwood. There was no lethality or toxic reaction found at any doses selected until the end of study. The ethanolic extract showed a significant reduction in blood glucose levels from 30 min onwards (**Table 1**) in oral glucose tolerance test. In normal animals, significant reduction in the blood glucose level was observed as compared to the control (**Table 2**). The effect of the ethanolic heartwood extract on fasting blood glucose levels in diabetic animals is presented in (**Table 3**). Significant ($p < 0.05$) difference was observed in glycosylated hemoglobin levels (**Table 4**), serum lipid profiles (**Table 5**), liver glycogen levels, thio barbituric acid reactive substance (TBARS) levels (**Table 7**), changes in body weight (**Table 6**) of extract treated diabetic animals when compared with the diabetic control and normal animals.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON ORAL GLUCOSE TOLERANCE TEST

Group	Treatment	Blood glucose concentration (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Control (Vehicle)	89.2±7.5	111.2±4.2	105.5±8.5	102.4±10.2	98.7±6.3
II	Ethanolic extract (100 mg/kg)	85.6±7.3	98.6±7.5*	90.4±6.4*	86.6±4.5*	83.8±7.6*
III	Ethanolic extract (200 mg/kg)	86.6±6.8	90.6±6.2*	86.2±8.3*	83.1±5.3*	80.6±6.3*
IV	Ethanolic extract (300 mg/kg)	84.6±7.3	92.5±5.3*	84.4±4.3*	81.6±6.2*	78.5±4.3*
V	Glibenclamide (0.25 mg/kg)	82.1±5.1	85.8±6.1*	78.8±6.8*	76.5±5.4*	68.5±3.1*

Each value represents mean±S.E., n=6. *statistical significance *vs*. control (p<0.05).

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON NORMAL ANIMALS

Group	Treatment	Blood glucose concentration (mg/dl)		
		Day 0	Day 5	Day 12
I	Control (Vehicle)	73.3 ± 1.6	74.5 ± 3.2	75.5 ± 4.6
II	Ethanolic extract (100 mg/kg)	70.9 ± 5.1	66.5 ± 1.9*	63.7 ± 4.9*
III	Ethanolic extract (200 mg/kg)	74.8 ± 4.6	68.5 ± 1.9*	61.2 ± 3.5*
IV	Ethanolic extract (300 mg/kg)	75.5 ± 5.5	67.8 ± 8.4*	60.4 ± 5.6*
V	Glibenclamide (0.25 mg/kg)	74.7 ± 5.7	67.2 ± 5.9*	59.8 ± 4.0*

Each value represents mean ± S.E., n = 6. *Statistical significance vs. control ($p < 0.05$).

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON DIABETIC ANIMALS

Group	Treatment	Blood glucose concentration (mg/dl)		
		Day 0	Day 5	Day 12
I	Diabetic control	223.7 ± 18.6	230 ± 11.6	231.4 ± 14.3
II	Ethanolic extract (100 mg/kg)	195.8 ± 16.1	125.1 ± 12.5*	105.7 ± 17.8*
III	Ethanolic extract (200 mg/kg)	196.5 ± 12.5	129.2 ± 11.8*	100.6 ± 10.2*
IV	Ethanolic extract (300 mg/kg)	199.5 ± 16.9	132.8 ± 21.4*	88.2 ± 11.4*
V	Glibenclamide (0.25 mg/kg)	187.7 ± 15.7	126.2 ± 13.9*	103.8 ± 14.0*

Each value represents mean ± S.E., n = 6. *Statistical significance vs. control ($p < 0.05$).

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON GLYCOSYLATED HEMOGLOBIN (WHOLE BLOOD) IN DIABETIC RATS

Group	Treatment	Glycosylated hemoglobin (%)
I	Normal	3.3 ± 0.4
II	Diabetic control	6.7 ± 0.2
III	Ethanolic extract (100 mg/kg)	$4.9 \pm 0.2^{a,b}$
IV	Ethanolic extract (200 mg/kg)	$4.5 \pm 0.3^{a,b}$
V	Ethanolic extract (300 mg/kg)	$4.1 \pm 0.1^{a,b}$

Each value represents mean ± S.E., n = 6. [a]Statistical significance vs. control ($p < 0.05$). [b]Statistical significance vs. normal ($p < 0.05$).

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON SERUM LIPID PROFILE IN DIABETIC RATS

Group	Treatment	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)
I	Normal	91.4 ± 1.7	58.3 ± 2.6	52.0 ± 3.2
II	Diabetic control	183.0 ± 9.2	123.9 ± 10.2	35.2 ± 2.7
III	Ethanolic extract (100 mg/kg)	105.4 ± 10.1[a,b]	69.3 ± 1.2[a,b]	53.5 ± 4.1[a,b]
IV	Ethanolic extract (200 mg/kg)	103.4 ± 8.1[a,b]	68.3 ± 1.5[a,b]	54.8 ± 3.1[a,b]
V	Ethanolic extract (300 mg/kg)	81.5 ± 6.5[a,b]	61.1 ± 4.4[a,b]	57.9 ± 2.1[a,b]

Each value represents mean ± S.E., $n = 6$. [a]Statistical significance vs. control ($p < 0.05$). [b]Statistical significance vs. normal ($p < 0.05$).

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON CHANGES IN BODY WEIGHT IN DIABETIC RATS

Group	Treatment	Initial (g)	Final (g)
I	Diabetic control	273.3 ± 14.2	201.9 ± 13.2
II	Ethanolic extract (100 mg/kg)	256.1 ± 11.1	248.5 ± 10.1
III	Ethanolic extract (200 mg/kg)	261.5 ± 9.5	256.5 ± 12.1*
IV	Ethanolic extract (300 mg/kg)	218.2 ± 10.6	197.3 ± 10.5*

Each value represents mean ± S.E., $n = 6$. *Statistical significance vs. control ($p < 0.05$).

TABLE 7: EFFECT OF ETHANOLIC EXTRACT OF *PTEROCARPUS MARSUPIUM* HEARTWOOD ON LIVER GLYCOGEN AND TBARS LEVEL IN DIABETIC RATS

Group	Treatment	Liver glycogen (mg/kg)	TBARS (mM/mg)	Group
I	Normal	3.9 ± 0.6	0.14 ± 0.01	0.08 ± 0.05
II	Diabetic control	1.2 ± 0.1	0.30 ± 0.03	0.18 ± 0.00
III	Ethanolic extract (100 mg/kg)	1.6 ± 0.2[a]	0.30 ± 0.01[a,b]	0.14 ± 0.04[a,b]
IV	Ethanolic extract (200 mg/kg)	1.9 ± 0.2[a]	0.20 ± 0.02[a,b]	0.12 ± 0.02[a,b]
V	Ethanolic extract (300 mg/kg)	2.6 ± 0.3[a,b]	0.17 ± 0.01[a,b]	0.10 ± 0.03[a,b]

Each value represents mean ± S.E., $n = 6$. [a]Statistical significance vs. control ($p < 0.05$). [b]Statistical significance vs. normal ($p < 0.05$).

DISCUSSION: The fundamental mechanism of hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [13]. Persistent hyperglycemia, the common characteristic of diabetes can cause most diabetic complications. In all patients, treatment should aim to lower blood glucose to near-normal levels [14].

The oral glucose tolerance test and normoglycaemic studies revealed that the ethanolic extract of the heartwood of *Pterocarpus marsupium* has the capacity to lower blood glucose levels. The diabetic syndrome in rats administered STZ and partially protected with suitable dosages of nicotinamide is characterized by stable moderate hyperglycemia, glucose intolerance and altered but significant glucose stimulated insulin secretion [10]. STZ-nicotinamide induced diabetic rats administered with extract (100 mg/kg) showed 30 and 42.2% decline in the blood glucose levels whilst a decline of 31.5 and 51.3% were observed in animals treated with 200 mg/kg of the alcoholic extract on days 5 and 12, respectively. In this test the 300 mg/kg dose of extract showed a decline of 34.4 on day 5 and 56.4% on day 12. The hypoglycaemic effect may be probably brought about by an extra pancreatic mechanism. There are medicinal plants, which are known to exert antidiabetic activity without the stimulation of insulin secretion [15, 16, 17]. *In vitro* studies on Hep G2 cell lines have shown that berberine is able to exert an insulin independent glucose lowering effect glucose lowering effect in hepatocytes similar to that of metformin [18].

The marked increase in serum triglycerides and cholesterol observed in diabetic rats [19]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [20, 21]. Hypertriglyceridemia is also associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and insulin intolerance [22]. In our study, administration of the heartwood extract to the STZ induced diabetic rats significantly ($p < 0.05$) improved these parameters. The observed hypolipidaemic effect may be because of decreased cholesterogenesis and fatty acid synthesis.

Significant lowering of total cholesterol and raise in HDL cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [23, 24].

Various studies on medicinal plants have reported a similar lipid lowering activity [25, 26, 27, 28]. The characteristic loss of body weight associated with STZ induced diabetes is due to increased muscle wasting in diabetes [29]. The *Pterocarpus marsupium* heartwood extract treated animals showed a weight loss during the studies, which may be directly due to the lipid lowering activity of the extract or indirectly to the influence on various lipid regulation systems.

The glycogen content of the skeletal muscle and liver, which markedly decrease in diabetes [30, 31] increased significantly in the treated animals as compared to the diabetic control. This may be because of the activation of glycogen synthase system by the extract. Significant fall in glycosylated hemoglobin indicated the efficiency of the extract in glycemic control. A marked increase in the concentration of TBARS and hydroperoxides has been observed in STZ diabetic rats [32]. The increased susceptibility of the tissues of diabetic animals may be due to the activation of lipid peroxidation system [33]. Treatment with the extract caused a significant decrease in the TBARS level in pancreas and liver which may be due to the inactivation of the lipid peroxidation system. The significant antidiabetic activity of the ethanolic extract of *Pterocarpus marsupium* in the present study justifies its use by herbal practitioners for diabetics. Our studies are in further progress to elucidate the molecular and cellular mechanism of the extract and its principle constituent.

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