Comparative evaluation of the anti-diabetic activity of *Pterocarpus marsupium* Roxb. heartwood in alloxan induced diabetic rats using extracts obtained by optimized conventional and non conventional extraction methods

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Abstract: The aim of the present study was to assess the anti-diabetic activity of *Pterocarpus marsupium* Roxb. heartwood in alloxan induced diabetic rats using extracts obtained by optimized conventional and non conventional extraction methods. Aqueous and ethanol extracts of *Pterocarpus marsupium* heartwood were prepared by conventional methods (infusion, decoction, maceration and percolation) and non conventional methods, such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). The crude aqueous extracts were administered orally to both normal and alloxan induced male albino rats (Sprague-Dawley strain). The experimental set up consisted of 48 male albino rats divided into 6 groups: Normal control, diabetic control (sterile normal saline, 1 ml/100 g body weight), standard (gliclazide, 25 mg/1000g of body weight), groups 4-6 (crude aqueous percolation, optimized UAE and MAE extract, 250 mg/1000g of body weight). In acute treatment, the reduction of blood glucose level was statistically significant with the oral administration of UAE and percolation aqueous extracts to the hyperglycemic rats. In sub-acute treatment, the UAE aqueous extract led to consistent and statistically significant ($p<0.001$) reduction in the blood glucose levels. There was no abnormal change in body weight of the hyperglycemic animals after 10 days of administration of plant extracts and gliclazide. This study justifies the traditional claim and provides a rationale for the use of *Pterocarpus marsupium* to treat diabetes mellitus. The antidiabetic activity of *Pterocarpus marsupium* can be enhanced by extracting the heartwood by non-conventional method of UAE.

Keywords: Extraction, *Pterocarpus marsupium* Roxb., Diabetes.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by glycosuria, hyperglycemia, hyperlipemia, negative nitrogen balance and sometimes ketonemia (Tripathi, 2006). There are more than 125 millions persons with diabetes in the world today, and both Type 1 and 2 are increasing in frequency (Davis and Granner, 2001).

Pterocarpus marsupium Roxb., belonging to family Fabaceae, is grown in deciduous and evergreen forests of central, western and southern regions of India. The heartwood of *Pterocarpus marsupium* revealed the presence of following constituents: pterostilbene, isoliquiritigenin, liquiritigenin, marsupsin, pterosupin, naringenin, lupeol, pterocarpol, sitosterol and stigmasterol. *Pterocarpus marsupium* has been reported as an anti-diabetic, an anti-hypertriglyceridaemic, a cardio tonic, an anti-cataract, a COX-2 inhibitor and a hepato-protective. Several conventional methods have been used for the extraction of the heartwood, like infusion, decoction, maceration, percolation and hot water extraction (Devgun *et al*., 2009a).

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The ultrasound-assisted extraction (UAE) method involves the usage of ultrasound which refers to mechanical vibrations which are essentially the same as sound waves but of a higher frequency. Ultrasound causes rapid extraction due to:

i) increase in the permeability of the cell wall,
ii) spontaneous formation of bubbles in the liquid below its boiling point, *i.e*., cavitation effect, due to dynamic stressing and
iii) Increase in the mechanical stressing, *i.e.*, internal friction of the cells (Devgun *et al*., 2010).

The microwave-assisted extraction (MAE) method involves the usage of microwaves which are electromagnetic waves whose frequencies range from approximately 300 megahertz to 1000 gigahertz. The exclusive and swift localized heating of moisture in the sample forms the basis of MAE (Devgun *et al*., 2009b).

The aim of the study was to appraise the anti-diabetic activity of extracts of *Pterocarpus marsupium* heartwood obtained by conventional and non conventional methods and thereby attempting to increase the yield of active constituents as well as efficacy of drug by using non conventional methods.

MATERIALS AND METHODS

Plant material

The *Pterocarpus marsupium* Roxb. heartwood was purchased from Yucca Enterprises, Mumbai (India) and was identified at the Department of Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (India). It was assigned reference no. NISCAIR/RHMD/Consult/-2010-11/1469/67 and a voucher specimen was deposited for further reference.

Reagents

Alloxan monohydrate was purchased from Spectrochem Pvt. Ltd., India. Gliclazide was purchased from Panacea Biotec Ltd., India. For the aqueous extractions, purified water was used and for ethanol extractions absolute ethanol (Hayman Ltd., England) was utilized.

Preparation of extracts

Conventional method: The powdered heartwood of *Pterocarpus marsupium* was extracted (aqueous and ethanol) by using various conventional methods like infusion, maceration, decoction and percolation.

Non conventional methods

Ultrasound-assisted extraction (UAE): The UAE (aqueous and ethanol) was carried out using Ultrasonic Bath (Model No. UCB-5200 D, Macro Scientific Works Pvt Ltd., India). The aqueous extraction was optimized by simplex search method.

Microwave-assisted extraction (MAE):

The MAE (aqueous and ethanol) was performed by using Microwave (Model No MG-555 f,lg, India). The irradiation time in the aqueous extraction was optimized by performing various experiments, taking microwave power constant at 100% (1350W). The extract methods are briefly given in the early publication (Devgun *et al*., 2012).

Experimental animals

Sprague-Dawley (220-250 g) male rats with average age of 10-12 weeks were used. They were procured from the in-house bred animals. These were kept in colony cages at an a temperature of 22±3ºC and standard 30-70% relative humidity and a 12 h light: dark cycle, with 12-15 air changes per hour. The Aqua guard purified water was freely available to all animals. The animals were fed with pellets of rat feed (Vetcare/Nutrilab). The Institutional Animal Ethics Committee approved the study involving rats (BIO IAEC 216-10/4). The study was done according to the latest guidelines for the care of laboratory animals.

Experimental design

Anti-diabetic activity of *Pterocarpus marsupium* heartwood was assessed in normal and alloxan induced diabetic rats.

Evaluation of plant extracts on normal healthy rats: The rats were arranged in 5 groups of 8 rats in each group. Group 1: control rats (sterile normal saline, 1 ml/100 g), group 2: standard rats (gliclazide, 25 mg/1000 g, oral), group 3-5: test rats (extracts, 250 mg/1000 g, oral by gavage) (Mukhtar, 2005). The tail vein method was used to collect the samples of blood. The Accuchek strip test was used to estimate the quantity of glucose in blood, immediately before receiving that is at 0 hr, 1 hr after and 3 hrs after receiving the vehicle/gliclazide/plant extracts. All the 40 rats used in the above experiment were given a washing period of 7 days after the completion of the above experiment, so that the rats can be optimally utilized for the subsequent experiments.

Evaluation of extracts in alloxan induced rats

A single intra-peritoneal injection of alloxan monohydrate (120 mg/1000 g) in sterile saline was used to induce diabetes. The animals were allowed for 7 days to develop diabetes. On day 7, animals showing blood glucose range of >200 mg/dl were considered as diabetic.

Acute treatment: The hyperglycemic rats were arranged in 6 groups of 8 rats in each group. Group 1: normal control, group 2: diabetic control, group 3 received standard anti-diabetic drug gliclazide, group 4-6 were treated orally with extracts. The dosage was same as that in the case of normal rats. Blood glucose levels were estimated immediately before receiving that is at 0 hr, 1 hr after, and 3 hrs after receiving the extract/vehicle/reference drug.

Sub-acute treatment: In sub-acute mode, the extract/standard drugs were administered for 10 days (o.d). The samples of blood were collected through tail vein after day 1 and 1 hour and 3rd hour on day 3rd, 7th, 10th. The Accuchek strip method was used for the quantitative estimation of glucose levels in samples.

Body weight estimation

The body weight measurements were done on day 1 before the test item administration and after the completion of 10th day of administration.

Statistical analysis

All values are expressed in as mean ± standard error (S.E). Statistical analysis was performed using student's t test and one way analysis of variance (ANOVA) followed by Dunnet's t test. A p-value of less than 0.05 was considered to be significant.

RESULTS

The significant yield was obtained by using percolation (aqueous) extraction method. The UAE (aqueous) method was optimized at 47^{o}C temperature for 26 min. The MAE (aqueous) was optimized by extracting at 100 % power and for 30 min (Devgun et al., 2012). Table 1

Table 1: Screening of 3 herbal extracts for its antidiabetic efficacy in sprague-dawley normal fasted rats. Summary of mean glucose concentrations (mg/dl). [Values are mean ± standard error (S.E.) (n = 8)]

Group	Treatment	Mean Glucose Concentration (mg/dl)			% reduction in glucose compared to control group		
		Hour 0	Hour 1	Hour 3	Hour 0	Hour 1	Hour 3
1	Control	127.7± 4.43	132.0± 4.14	129.4± 4.29	_	_	_
2	Gliclazide-standard	128.0± 0.73	85.7± 4.06$^{!}$	90.7 ± 8.50$^{!}$	-0.235	35.076	29.907
3	Plant Extract-1	119.7± 1.74	116.7± 0.99$^{\Psi}$	111.7± 1.13$^{\#}$	6.265	11.591	13.678
4	Plant Extract-2	123.4± 2.05	119.6± 2.05$^{\#}$	121.0± 3.88	3.367	9.394	6.491
5	Plant Extract-3	127.2± 1.33	108.0± 4.33$^{!}$	102.4± 1.42$^{!}$	0.391	18.182	20.865

Plant extract 1-Aqueous percolation extract; Plant extract 2-Aqueous MAE extract; Plant extract 3-Aqueous UAE extract. $^{\#}p<0.02$ when compared with the corresponding value of the control. $^{\Psi}p<0.01$ when compared with the corresponding value of the control. $^{!}p<0.001$ when compared with the corresponding value of the control.

Table 2: Screening of 3 herbal extracts for its antidiabetic efficacy in sprague-dawley alloxan-induced diabetic rats (acute and sub-acute treatment). Summary of mean glucose concentrations (mg/dl). [Values are mean ± standard error (S.E.) (n = 8)]

Group	Treatment (vehicle)	Acute treatment						Sub-acute treatment						
		Day 1 (Mean Glucose Concentration, mg/dl)			% reduction in glucose compared to Diabetic control group			Mean Glucose Concentration (mg/dl)						
		Hour 0	Hour 1	Hour 3	Hour 0	Hour 1	Hour 3	Day1 after	Day 3 after		Day 7 after		Day 10 after	
								24 hr	1 hr	3 hr	1 hr	3 hr	1 hr	3 hr
1	Normal Control	104.37 ± 1.75	103.37± 1.88	103.37 ± 1.09	-	-	-	103.62 ± 2.14	110.00 ± 0.88	106.00 ± 2.01	108.00 ± 3.38	111.62 ± 3.09	103.00 ± 2.72	111.00 ± 2.77
2	Diabetic Control	263.00 ± 7.15	256.00± 7.18	254.00 ± 8.38	-	-	-	250.37± 7.48	251.75 ± 7.75	253.37 ± 7.78	253.37 ± 7.31	257.75 ± 6.42	251.37 ± 8.05	253.37 ± 7.79
3	Gliclazide-standard	220.75 ± 1.83	214.75± 2.13$^{!}$	216.37 ± 2.42$^{!}$	16.06	16.11	14.84	220.75± 2.25$^{!}$	183.75 ± 5.44$^{!}$	182.37 ± 5.03$^{!}$	142.75 ± 5.08$^{!}$	133.00 ± 2.81$^{!}$	94.00 ± 5.87$^{!}$	93.75 ± 2.91$^{!}$
4	Plant Extract-1	241.37 ± 1.49	235.37 ± 1.47$^{\Psi}$	237.37 ± 1.51*	8.22	8.06	6.55	236.37± 1.42*	238.00 ± 2.35	234.37± 1.49$^{\#}$	233.37± 2.63$^{\Psi}$	233.75 ± 1.94$^{!}$	227.00 ± 1.92$^{\Psi}$	228.37 ± 1.41$^{\Psi}$
5	Plant Extract-2	264.37 ± 5.71	262.00 ± 7.47	263.75 ± 5.81	-0.52	-2.34	-3.84	261.62 ± 5.91	259.37 ± 6.42	256.00 ± 6.37	255.62 ± 5.65	254.00 ± 6.94	255.00 ± 6.39	253.00 ± 6.88
6	Plant Extract-3	234.00 ± 1.42	223.37 ± 2.23$^{!}$	223.62 ± 2.34$^{!}$	11.03	12.75	11.96	223.62± 2.15$^{!}$	209.00 ± 2.97$^{!}$	208.00± 2.42$^{!}$	189.37± 2.54$^{!}$	187.62 ± 2.28$^{!}$	147.37 ± 2.32$^{!}$	148.00 ± 2.65$^{!}$

Plant extract 1-Aqueous percolation extract; Plant extract 2-Aqueous MAE extract; Plant extract 3-Aqueous UAE extract. *$p<0.05$ when compared with the corresponding value of the diabetic control. $^{\#}p<0.02$ when compared with the corresponding value of the diabetic control. $^{\Psi}p<0.01$ when compared with the corresponding value of the diabetic control. $^{!}p<0.001$ when compared with the corresponding value of the diabetic control.

showed marked reduction in the blood glucose levels of the normoglycemic rats with the oral feed of percolation and UAE (aqueous) extracts. Table 2 showed that in alloxan-induced hyperglycemic rats, gliclazide, UAE and percolation (aqueous) extracts resulted in marked reduction of blood glucose levels in acute treatment whereas in sub-acute treatment gliclazide and UAE aqueous extracts led to persistent and marked reduction in the blood glucose levels. Moreover there was no apparent change in the animal body weight as compared to the normal control group after 10 days of administration of plant extracts and gliclazide (table 3).

DISCUSSION

Conventional extraction methods

Aqueous percolation yielded significant ($p<0.05$) amount of extraction yield when compared to the aqueous decoction as well as other conventional extraction methods.

Non conventional extraction methods

Ultrasound-assisted extraction: The UAE method (aqueous) was optimized by using the most widely used optimization method, which was a sequential experimental design, called as simplex optimization. Accordingly, the optimized conditions of temperature 47^{o}C and time 26 min were obtained for UAE (aqueous).

Microwave-assisted extraction: The MAE (aqueous) was optimized by extracting at 100 % power and for 30 min (Devgun *et al*., 2012).

Effect of Pterocarpus marsupium extracts on normoglycemic rats

The hypoglycemic effect of gliclazide and the three plant extracts on the normal fasted rats is shown in table 1. At the end of 3rd hour of drug administration, *Pterocarpus marsupium* percolation aqueous extract (plant extract-1) and UAE aqueous extract (plant extract-3) shows statistically significant hypoglycemic effect, whereas the

Table 3: Effect of test drugs on the weight of the Sprague-dawley alloxan-induced diabetic rats [Values are mean ± standard error (S.E.) (n = 8)]

Group	Treatment	Mean body weight (g)		Weight change (g)
		Day 1	Day 10	
1	Normal Control	232.64±2.41	273.50±6.61	+40.86
2	Diabetic Control	233.00±2.03	235.87±4.41*	+2.87
3	Gliclazide-standard	233.76±2.17	259.25±4.58	+25.49
4	Plant Extract-1	235.90±1.97	276.67±10.03	+40.77
5	Plant Extract-2	236.16±2.29	260.96±2.22	+24.8
6	Plant Extract-3	237.36±2.52	269.30±5.01	+31.94

Plant extract 1-Aqueous percolation extract; Plant extract 2 -Aqueous MAE extract; Plant extract 3-Aqueous UAE extract. *p<0.001 when compared with corresponding value of the normal control.

MAE aqueous extract (plant extract-2) shows non-significant results. There is 20.865% and 29.907% reduction in glucose with UAE plant extract and gliclazide respectively when compared to control group.

Effect of Pterocarpus marsupium extracts on alloxan-induced hyperglycemic rats

Acute treatment: In Acute treatment the oral administration of gliclazide, UAE aqueous extract (plant extract-3) and percolation aqueous extract (plant extract-1) resulted in significant reduction of blood glucose levels in alloxan-induced diabetic rats (table 2). There is 11.96% and 14.84% reduction in glucose with UAE plant extract and gliclazide respectively when compared to control group.

Sub-acute treatment: In sub-acute treatment, the oral administration of gliclazide and UAE aqueous extract (plant extract-3) led to consistent and statistically significant ($p<0.001$) decrease in the glucose levels of the blood samples of alloxan-induced hyperglycemic rats. The percolation aqueous extract (plant extract-1) also showed significant decrease in the glucose level of the blood samples but the response was inconsistent (table 2).

Effect on body weight

On the day 1 of the study, the weight of the animals in different groups did not vary significantly ($p>0.05$). At the end of 10^{th} day of the study, there was no significant difference ($p>0.05$) in the weight of the animals in the diabetic standard, plant extract 1, 2, and 3 groups in comparison to the normal control. There was no abnormal change in weight of the hyperglycemic animals after 10 days of administration of plant extracts and gliclazide (table 3). However, there was only slight increase in the weight of the diabetic control rats as compared with other groups.

In conclusion, the *Pterocarpus marsupium* heartwood aqueous extract possesses significant antidiabetic activity, thereby justifying its traditional claim and providing a rationale for the use of *Pterocarpus marsupium* to treat diabetes mellitus. The antidiabetic activity of UAE extract was found to be statistically significant and consistent as compared to gliclazide, which can be attributed to unique mechanism of UAE, which results in increased cell membrane permeability. As a result, it can be postulated that the UAE results in a better (or rather complete extract), as compared to MAE or percolation. Hence, the antidiabetic activity of the *Pterocarpus marsupium* can be enhanced by extracting the heartwood by non conventional method of UAE.

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