ANTIDIABETIC ACTIVITY OF ETHYL ACETATE EXTRACT OF *PTEROCARPUS MARSUPIUM* IN ALLOXAN INDUCED DIABETIC RATS

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Summary

Diabetes was induced by single intra-peritoneal administration of alloxan monohydrate (120 mg/kg) and blood samples were collected from retro-orbital plexus on 15th day for estimation of blood glucose level. In-vitro antioxidant activity was tested using DPPH free radical scavenging assay and amount of DPPH remaining was recorded at 517 nm. Hyperglycemia was significantly ($p<0.05$) lower by the administration of ethyl acetate extract of *pterocarpus marsupium* (750 mg/kg) and metformin. Ethyl acetate extract of *Pterocarpus marsupium* (EPM) reduced blood glucose level to 36.95% as compared to standard which was 38.66% on 7th days. Whereas it showed significant in vitro antioxidant activity scavenging DPPH radicals (IC_{70} value=74 ug/ml). In the present study suggests that the anti-hyperglycemic activity of *Pterocarpus marsupium* may be due to its free radical scavenging activity against alloxan induced free radicals.

Keywords: *Pterocarpus marsupium*, Diabetes, Antioxidant, Alloxan, DPPH.

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Introduction

For a long time, diabetes, a chronic metabolic disorder, has been treated with several medicinal plants of their extract based on folklore medicine [1]. Synthetic oral hypoglycemic agent can produce serious side effect and in addition, they are not suitable for use during pregnancy [2]. Therefore the search for more effective and safer hypoglycemic agent has continued to be an important area of active research. Furthermore, after the recommendations made by WHO on diabetes mellitus [3], investigations on hypoglycemic agents from medicinal plants have become more important.

Pterocarpus marsupium (Papilionaceae), vernacularly called Bijay sar, is a tree having height 15-30 m, compound, imparipinnate leaves and yellow flowers. It is distributed through out India, Ceylon and most of the temperate countries. The *P. marsupium* have been reported to contain flavonoids, mucilage, glycosides, saponin, tannin and epicatechin [4]. The heart wood of *p. marsupium* have been used in ancient medicine in India for diabetes, leprosy and antipyretic [5].

The hypoglycemic activities of alcoholic and aqueous extract of *p. marsupium* heart wood have reported [6] .The prophylactic action of epicatechin against alloxan induced diabetic rats have reported [7] and adrenergic type activity also reported [8]. The compound pterostilbene from heartwood of *p. marsupium* have been reported [9, 10]. The anti-hyperlipidaemic effect of flavonoids of *p. marsupium* have been reported [11]. Traditionally, it has been used in the treatment of diabetes mellitus and its use in diabetes is also reported [12].

Although literature survey reveled various scientific data of solvent extract of this plant showed anti-hyperglycemic effect but no scientific data available on ethyl acetate extract. Therefore, the present study reports the anti-hyperglycemic effect of ethyl acetate extract of heart wood of *p. marsupium* in alloxan induced hyperglycemia in rats.

Material and Methods

Plant materials: *P. marsupium* purchased from local market and authenticated by the Department of Botany, R.T.M. Nagpur University, and Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany, collection number 7451 R.T. M. Nagpur University.

Chemicals: Alloxan monohydrate was procured from S.D. Fine Chem. Ltd., Mumbai. DPPH was procured from Sigma Chemicals U.S.A. and GOD-POD (Glucose Oxidase-Peroxidase) kit was procured from Ranbaxy Laboratory Pvt. Ltd., New Delhi.

Extraction: Dried and powdered heart woods, defatted with petroleum ether and subjected to hot extraction with ethyl acetate using soxhlet apparatus. The solution was air dried giving an extract yield 5.6% w/w. The extract was suspended in water using CMC as a suspending agent for the purpose of oral administration.

Animals: The animals (Sprague-Dawley rats, 200-250g) both male and female that were used for these experiments They were kept at 25± 2℃ in a 12 h dark cycle with lights on at 07 h and fed the standard pellet rat diet (Goldmohar Brand, India) and water *ad libitum*. Institutional Animal Ethics Committee, constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved all the animal experimental protocol.

Evaluation of hypoglycemic activity: Diabetes was induced by single intra-peritoneal administration of alloxan monohydrate (120 mg/kg) [12]. The blood samples were collected from retro-orbital plexus on 15th day and blood glucose level were estimated. Rats having blood glucose level above 200 mg/dl were selected for further experiment [13] and divided in five groups of six rats each. Group I (normal rats), group II (diabetic untreated rats), group III (diabetic rats treated with 500 mg/kg metformin), group IV (diabetic rats treated with petroleum ether extract of *p.* marsupium 750 mg/kg), and group V (diabetic rats treated with EPM 750 mg/kg). All groups were treated orally once a day for 7 days.

The blood glucose levels were evaluated at regular time interval at 0, 2, 4 and 24 h after the first treatment (acute treatment) and on the 3rd and 7th day 1 h after the last treatment (chronic treatment).

In vitro antioxidant test

DPPH radical method: In order to measure in vitro antioxidant activity, DPPH free radical scavenging assay was carried out as described previously by Lee *et al.* [14]. EPM 4, 8, 12, 16, 20, and 100 ug/ml, were added to a solution of 0.05 M DPPH in CH_3OH and the mixture was shaken vigorously. The amount of DPPH remaining was recorded at 517 nm.

The antioxidant activity of EPM was expressed as IC_{70}. IC_{70} value was defined as the concentration (ug/ml) of the extract required for inhibiting the formation of DPPH radical by 70%.

Statistical analysis: All the data were analyzed by one away analysis of variance (ANOVA) followed by Newman-Keul's test for multiple comparison, $p<0.05$ was considered significant.

Results

As expected, the administration of alloxan induced significant hyperglycemia at blood glucose level>200 mg/dl in rats. This hyperglycemia was significantly ($p<0.05$) lower by the administration of EPM (750 mg/kg) and metformin, as shown by the fig, both after acute and chronic oral administration. EPM reduced blood glucose level to 36.95% as compared to standard which was 38.66% on 7th days. Furthermore, EPM showed significant in-vitro antioxidant activity scavenging DPPH radicals (IC_{70} value =74 ug/ml).

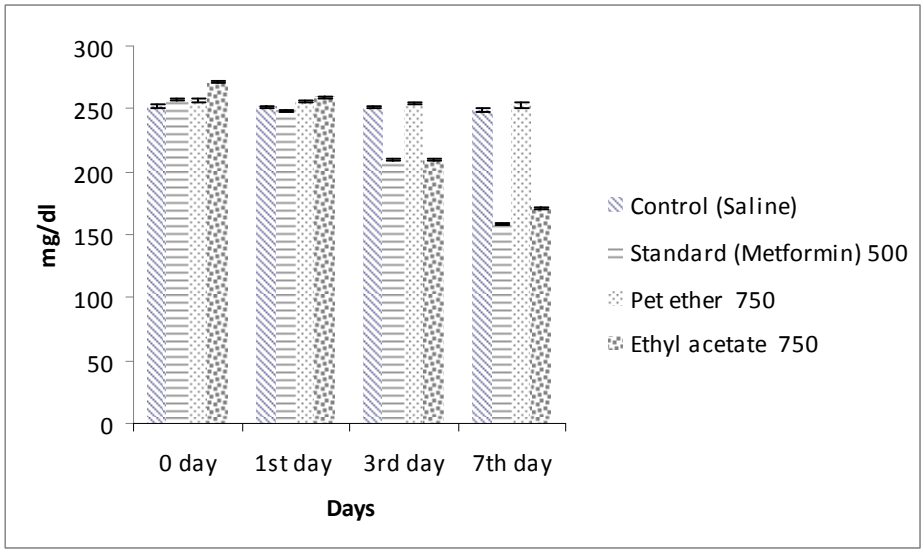
It is well known that oxygen free radicals are involved in the diabetogenic action of alloxan [15] and antioxidant have been shown to be effective in diabetes [16]. The injection of hydroxyl radical scavengers in to animal protected them against the diabetoigenic action of alloxan [17]. In the present study, evidence of the radical scavenger activity of *p. marsupium* has been given. This suggests that the anti-hyperglycemic activity of *p. marsupium* may be due to its free radical scavenging activity against alloxan induced free radicals.

Table 1. Screening of antidiabetic activity of different extract of *P. marsupium*

Treatment	Dose mg/Kg	Blood sugar level mg/dl			
		0 day	1st day	3rd day	7th day
Control (saline)	-	252.39±1.125	251.46±0.6274	250.78±0.8895	248.70±1.654
Standard (Metformin)	500	257.46±1.202	248.62±0.6710	209.10*±0.8284	158.08*±0.605
Pet. ether extract	750	256.68±1.168	255.82±1.237	254.29±0.8376	253.32±2.123
Ethyl acetate extract	750	270.86±0.9374	259.12±0.4215	209.38*±0.9667	171.09*±1.005

N=5 value are mean ± S.D., P<0.05 with respect to control

Fig. 1 Screening of antidiabetic activity of different extract of *P. marsupium*



Discussion

The antidiabetic activity of various sub-fractions of the ethyl acetate extract of the bark of *P. marsupium* was evaluated in alloxan-induced diabetic rats. The effects of these extracts evaluate their activity in controlling diabetes related metabolic disorder. *P. marsupium* control the diabetes related metabolic alterations and

controlling the glucose levels. Among the fractions tested, ethyl acetate sub-fraction was found to be more active in comparison with other sub-fractions. It can be concluded that the ethyl acetate sub-fraction of the extract of *P. marsupium* exhibits significant antidiabetic activity and corrects the metabolic alterations in diabetic rats and this activity may resemble insulin-like properties.

P. marsupium has been used as antidiabetic since time immemorial and used commercially in pharmaceutical preparations. In the *P. marsupium* extracts, many chemical constituents like pterostilbene, marsupin, pterosupsin, (-) epicatechin etc have been identified and isolated. The literature review revealed that *P. marsupium* can be used in variety of pharmacological disorders, however more investigations must be carried out to evaluate the mechanism of action of its active principles, so that it's potential can be fully utilized.

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