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Research Article

**PTEROCARPUS MARSUPIUM EXTRACTION AND
EVALUATION FOR DIABETIC NEUROPATHY****Kousar Begum^{*}, Naziya Begum, Shaik Reshma, Wajiha Fatima, Mariyanaaz Ali
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Abstract:

Herbal medicines are considered to offer gentle means of managing chronic diseases at a lower cost. Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Pterocarpus marsupium. (PM) heartwood and bark have been majorly used as antidiabetic remedies in many cultures for thousands of years. The aim of this research is to address the existing evidence on antidiabetic Neuropathy effects of the P. marsupium by tail flick method and Eddy's hot plate method by Thermal hypoalgesia. In this research work , Experimental Study Design for Diabetic neuropathy screening the animals were divided into Group-I: Rats served as normal control group. Group-II: served as diabetic/disease control. Group-III: Diabetic rats treated with PM , at a dose 50mg/kg (low dose). Group-IV: Diabetic rats treated with PM at a dose of 100mg/kg (high dose). Group V: Diabetic rats treated with Diclofenac sodium (standard drug) at 100mg/kg. Diabetic neuropathy alterations were tested using thermal hypoalgesia and Tail flick responses. By the results Pterocarpus Marsupium is having Neuro protective activity in diabetic animals.

Keywords: *Anti diabetic neuropathy, Pterocarpus marsupium, Standard drug, Tail flick method, thermal hypoalgesia method*

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INTRODUCTION:

Diabetes mellitus is one of the most common endocrine diseases in all populations and all age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion or impaired insulin action, or both. Diabetes mellitus comprises of heterogeneous group of disorders characterized by hyperglycemia, altered metabolism of carbohydrates, lipids and proteins. Diabetes mellitus is associated with complications such as nephropathy, retinopathy, neuropathy and cardiovascular disease. At advanced stages, diabetes can cause kidney failure, amputation, blindness and stroke. However, complications can be prevented or significantly delayed by exercising good control of diabetes, blood pressure and cholesterol [1].

Diabetic neuropathy refers to the damage that affects the nerves of the body in people who have diabetes. It is a progressive disease, and symptoms worsen over a number of years. People who do not control their blood sugar levels and those who have high blood pressure, high blood cholesterol, or who are overweight are more susceptible.

Neuropathy can affect any nerve in the body, but especially the nerves of the ganglia, the outside of the skull, the spinal cord, and those that impact the functioning of fundamental organs, such as the heart, bladder, intestines, and stomach. Problems can occur in the nerves that control the periphery, or outside, of the body, such as the feet and hands, those that control the automatic functions of the body, such as heart rate and digestion, or just one or a small group of nerves. Different nerves are affected in different ways.

Diabetic neuropathy may involve either the periphery, gastrointestinal, genitourinary, or all systems. Diabetic neuropathy produces symptoms in 60-70% of all diabetic persons. Neuropathic complications are divided into autonomic dysfunction and sensory dysfunction. Sensory complications include paresthesias and the loss of sensation in the extremities, leading to an increase in serious foot problems in diabetics. Autonomic complications include sexual dysfunction, and postural hypotension.

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes.” As with other microvascular complications, risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect

their predisposition to developing such complications [2].



Fig 1: Uncontrolled diabetes can lead to nerve damage [3].

Natural Plants

The term “**medicinal plant**” includes various types of plants used in herbalism (“herbology” or “herbal medicine”). It is the use of plants for medicinal purposes, and the study of such uses [4].

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions. India has about 45,000 plant species; medicinal properties have been assigned to several thousands. About 2000 are found in the literature; indigenous systems commonly employ about 500–700 [5].

History of Pterocarpus Marsupium:

Moreover, FDA has even set up an exclusive department of complementary medicine to look for their role in health and diseases. *Pterocarpus marsupium* (PM) is one such plant that has been used for over thousands of years as a treatment of different diseases. It is used in 'Ayurveda' as 'Rasayana' for the management of various metabolic disorders. It has a long history of numerous traditional and ethnobotanical applications in diverse cultures. Many tribes considered it as a cure for all ailments. As per the traditional claim heartwood of *Pterocarpus marsupium* is the potential source of drugs used as an astringent, anti-inflammatory, anthelmintic, leprosy, skin diseases, diarrhea, asthma, bronchitis and grayness of hairs. It has been scientifically reported for hypolipidemic, hepatoprotective, anti-ulcer, anti-inflammatory, and anti-diabetic activity. Extensive

phytochemical studies have been carried out for this plant. Phytochemical testing showed that the methanol extract of *P. marsupium* contains carbohydrates, glycosides, saponins, tannins and flavonoids [6].

Phytoconstituents:

Pterosupin , Pterostilbene , Liquirtigenin , Isoliquirtigenin, Kinoin , Kinotannic acid , Kino-red , Beta-eudesmol , Marsupol , Marsupinol , Pterocarpol, Marsupol, Maesupin, Carpusin(Marsupsin), (-) epicatechin, Propterol, Pterosupin, Marsupinol, Lupeol, Resin contains Kino tannic acid, Pyro catechin,.

Water Soluble Extractive: Not less than 5.00% [7]

Table 1: Physical Evaluation of Pterocarpus Marsupium [8]:

ASH VALUES: Parameter	Pterocarpus marsupium
Total Ash	0.60
Water soluble Ash	0.25
Acid Insoluble Ash	0.12
EXTRACTIVE VALUE Solvents	Pterocarpus marsupium
Pet-Ether	0.50
Chloroform	0.20
Alcohol	0.45
Water	5.65
pH VALUES: Conc.	Pterocarpus marsupium
1%	6
10%	6

Role in maintaining Blood sugar:

The blood sugar lowering effects of Pterocarpus marsupium were well known in India long before pharmacological studies of the tree were undertaken. Diabetics in the rural areas of Chattisgarh (region of Madhya Pradesh) would drink water from tumblers carved from the wood of Pterocarpus marsupium or drink water in which a piece of the wood had been immersed, and they were said to feel better⁷.

Mechanism Of Action by β -cell Regeneration

There is evidence to suggest that (-)-epicatechin is effective in β -cell regeneration. Chakravarthy et al. Demonstrated the effectiveness of a flavonoid fraction from the PM bark in reversing the β -cell population of the pancreas in alloxan induced diabetic rats. Later they showed that (-)- epicatechin given for 4 to 5 days resulted in regeneration of the β

-cell population of the islets of pancreas which were previously necrosed due to alloxan in alloxan-induced diabetic rats. Functional nature of the regenerated β -cells was identified based on the immune reactive insulin studies. However, some other investigators could not confirm the effectiveness of epicatechin in β -cell regeneration [9].

Anti-Diabetic Activity:

PM has been used as a highly potent anti-diabetic agent since ancient times. It possesses blood glucose lowering, beta cell protective and regenerative properties. Numerous experimental studies have been conducted on various animal species viz., rats, dogs, and rabbits to study the hypoglycemic effect of PM.

The results have shown that PM restored the normal insulin secretion by reversing the damage to the beta cells and by repopulating the islets. In a study, alcoholic extract were found to possess beneficial effects on blood glucose levels.

The findings of a clinical trial (flexible dose double blind multicenter randomized controlled trial) revealed that PM is an effective blood sugar lowering agent. Three phenolic compounds were evaluated for their antidiabetic potential and it was found that marsupin and pterostilben were more effective than pterospin on comparison with metformin.

Extraction Methods:

i) INFUSION

ii) DECOCTION

MACERATION: Ahmed *et al.* chopped the wood of *Pterocarpus marsupium* into small pieces and extracted in absolute ethanol for 1 week. Joshi *et al.* collected the heartwood and cut it into very small pieces. Maceration with methanol was done for 7 days. The extract was vacuum dried and stored in a refrigerator until further use. In one study, the alcohol extract of the bark of *Pterocarpus marsupium* was prepared by cold double maceration. The extract obtained was concentrated using a rotary flash evaporator and then dried in a desiccator [11].

iii) PERCOLATION

iv) HOT WATER EXTRACTION

Microscopical Evaluation of Pterocarpus Marsupium Description

Deciduous trees, to 30 m high, bark 10-15 mm, surface grey or greyish-black, rough, deeply vertically cracked, exfoliations small, irregular,

fibrous; blaze pink; exudation blood-red. Leaves imparipinnate, alternate; stipules small, lateral, cauducous; rachis 6.5-11.1 cm long, slender, pulvinate, glabrous; leaflets 5-7, alternate, estipulate; petiolule 6-10 mm, slender, glabrous; lamina 3.5-12.5 x 2-7 cm, elliptic-oblong, oblong-ovate or oblong, base obtuse or acute, apex obtuse and emarginate, margin entire, glabrous, coriaceous; lateral nerves 9-20 pairs, parallel, prominent, ascending, secondary laterals prominent; intercostae reticulate, prominent. Flowers bisexual, yellow, in terminal and axillary panicles; 10-12 mm long; bracts small, dioecious; bracteoles 2, cauducous; calyx tube campanulate, lobes short, the upper 2 often connate; corolla exerted; petals 5, all long-clawed, crisped along the margins; standard orbicular, wings oblique, obovate, auricled; keel petals oblique, small, slightly connate; stamens 10, monadelphous; filaments subequal; anthers uniform; ovary shortly stalked, inferior, tomentose, 1-celled, ovules 2; style filiform, in curved, beardless; stigma capitate. Fruit a pod, 2.5-5 cm across, orbicular-reniform, broadly winged; seed one, subreniform [8,12].

Aim:

The Aim of the study was to prepare methanolic extract of aerial parts of *Pterocarpus Marsupium* and to evaluate ant diabetic neuropathic activity.

Objectives:

To formulate methanolic extraction of *Pterocarpus Marsupium*

To evaluate different tests in Animals like Tail Flick method and Eddys Hot Plate method.

Materials:**Table 2: Materials**

MATERIAL	SOURCE
Sodium citrate	Virat labs, Hyd, India.
Diethyl ether	Finar chemicals limited, Ahmadabad.
Methanol	E-Merk, Mumbai, India.
Normal saline	Claris life sciences, Ahmadabad, India.
Formaldehyde	Finar chemicals limited, Ahmadabad, India.
Chloroform	Molychem, Mumbai, India.
Alloxan monohydrate	Sigma, St Louis, U.S.A.
Metformin	MSN Formulations, HYD, India
<i>Pterocarpus Marsupium</i>	KP labs, Hyd, India.

Equipments Used:

EQUIPMENT	SOURCE
Centrifuge	Remiequipments Pvt, Ltd, Hyd, India.
Shimadzu electronic balance	Toshvin Analytical Pvt. Ltd, India
Shimadzu UV-spectrophotometer	Toshvin Analytical Pvt. Ltd, Mumbai.
Inverted microscope	Boeckl + co, Hamburg.

METHODOLOGY:**Collection and Authentification of Plant Material**

The Aerial Parts of *P. Marsupium* were collected and authenticated.

Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool [13].

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissector for 7 days.

% Yield value of Methanol Extract from Aerial Parts of *P. Marsupim* Plant

Powder taken for extraction = 200gm

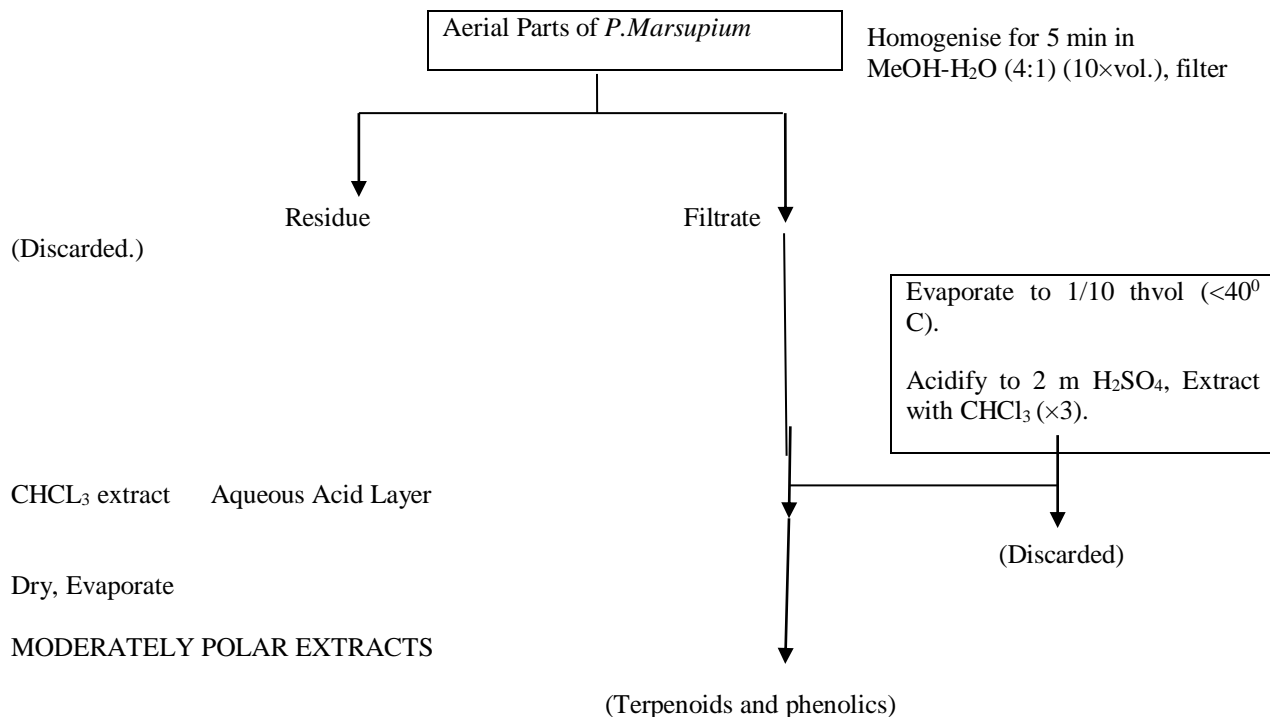
Weight of the empty china dish = 53.70gm

Weight of the china dish with extract = 73.24gm

Weight of the extract obtained =
(73.24-48.70) gm = 24.54 gm

% yield of methanol extract =(weight of extract)/(powder taken for extraction) × 100

$$= 24.54/200 \times 100 = 12.27 \%$$

Phenolic Constituents Extracts [14]**Fig 2: Phenolic Constituents Extracts****Animals:**

Healthy Adult Male wistar rats of 8-10 weeks old with Average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled ($27^{\circ}\text{C} \pm 3^{\circ}\text{C}$) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study [15].

Acute toxicity studies

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organisation for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats (130-200 g) were taken for the study and dosed once with 1000 mg/kg. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 1000 mg/kg dose to be safe. Thus, 1/10 and 1/20 doses of 1000 mg/kg i.e.

100 mg/kg and 50 mg/kg were chosen for subsequent experimentation.

Induction procedure

Diabetes mellitus or hyperglycemia was induced in rats by administration of alloxan monohydrate (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-primidinetrone) at dose of 120mg/kg intraperitoneally in normal saline. After one hour of alloxan administration the animals were given feed *ad libitum*. The animals were kept fasting overnight and blood glucose levels were estimated before and after 72hrs of alloxan treatment. Animals showing blood glucose levels of $>200\text{mg/dl}$ is considered as diabetic and were used for study[16].

Experimental Study Design for Diabetic screening

Diabetic rats were divided in to five groups with each group four animals.

Group-I: Rats served as normal control group.

Group-II: served as diabetic/disease control.

Group-III: Diabetic rats treated with *Pterocarpus Marsupium* at a dose 50mg/kg.

Group-IV: Diabetic rats treated with *Pterocarpus Marsupium* at a dose of 100mg/kg

Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg.

The treatment was given for 14 days and blood samples were collected at different intervals [16,17].

Collection of blood samples

Blood samples were collected from all the groups of animals at 0, 7, 15th day intervals through puncture of retro orbital plexus and were centrifuged at 3000 revolutions per minute (rpm) for 15 minutes. Serum was separated and stored at -20°C and then used for estimating blood glucose levels.

Experimental Study Design for Diabetic neuropathy screening

Group-I: Rats served as normal control group.

Group-II: served as diabetic/disease control.

Group-III: Diabetic rats treated with *Pterocarpus Marsupium* at a dose 50mg/kg (low dose).

Group-IV: Diabetic rats treated with *Pterocarpus Marsupium* at a dose of 100mg/kg (high dose).

Group V: Diabetic rats treated with Diclofenac sodium (standard drug) at 100mg/kg.

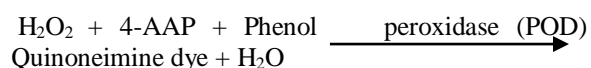
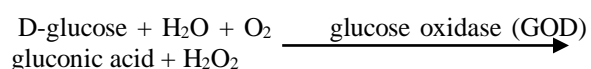
All the animals are tested for tail flick and thermal hypoalgesia Eddies plate method response to find out the peripheral neuropathy [18].

Statistical Analysis

All the values will be expressed as mean \pm standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance P value <0.05 will be considered as statistically significant [19].

GLUCOSE Method: GOD/POD method

Principle:



Procedure:

- Wavelength/filter : 505 nm (Hg 546 nm) / Green
- Temperature: 37°C / R.T.
- Light path : 1 cm

- Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Glucose Reagent L1)	1.0	1.0	1.0
Distilled Water	0.01	--	--
Glucose Standard S)	--	0.01	--
Sample	--	--	0.01

Mix well and incubate at 37°C for 10 min or at R.T. (25°C) for 30 mins. Measure absorbances of the Standard (Abs.S) and Test Sample (Abs.T) compare these against the Blank within 60 mins [20].

Tail Flick Method:

Healthy albino rats weighing about 150-200gm were taken. The animals were divided into five groups of 6 animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle. Group-II: served as diabetic/disease control. Group III, IV were treated orally with 50, 100 mg/kg body weight, and Group V Diabetic rats treated with Diclofenac sodium (standard drug) at 100mg/kg intraperitoneally respectively. They were divided into different groups, numbered and placed into individual restraining cages leaving the tail hanging out freely. The animals are then allowed to adapt in the cages for 30 minutes before testing. The lower 5cm portion of the tail was marked and immersed in a cup of freshly filled warm water of exactly 55°C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stop watch. After each determination the tail was carefully dried. The reaction was determined before oral administration of respective treatments which was recorded as zero minutes reading. After the drug was administered the reaction time was recorded at an interval of 30, 60, 90, 120 and 150 mins. The cut off time of the immersion is 15 seconds. The mean reaction time was recorded for each group and compared with the value of standard drug.

Eddy's hot plate method Thermal hypoalgesia:

Male albino mice weighing 22-25g were taken. The animals were divided into five groups of 6 animals each. Group I served as control and received drugless (0.5%, w/v, CMC; 10 ml/kg) vehicle. Group-II: served as diabetic/disease control. Group III, IV were treated orally with 50, 100 mg/kg body weight, and Group V Diabetic rats treated with Diclofenac sodium (standard drug) at 100mg/kg intraperitoneally

Respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds. The test was carried before the treatment and at 30, 60 and 90 min after administration.

Results: Authentication Results

Total phenolic content

Total phenolic content of the methanolic extract of *P. marsupium* was determined by using the Folin-Ciocalteu reagent and were expressed as GAE per gram of plant extract. The total phenolic contents of the extract were calculated using the standard curve of gallic acid ($y = 0.010x - 0.031$; $R^2 = 0.994$) (Fig. 1). The alcoholic extract of *P. marsupium* was found to contain total phenolic value of 90.9 ± 1.6 mg GAE/g of gallic acid.

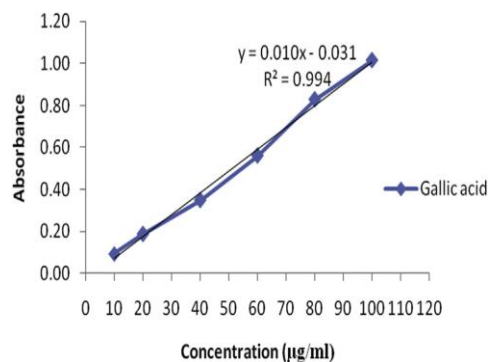


Fig 3: standard curve of gallic acid

GLUCOSE:

Table 3: Effect of *Pterocarpus Marsupium* (EPM) on serum glucose levels (mg/dl) in diabetic rats

Groups/Interval	0 th Day	7 th Day	15 th Day
Normal	83.3±4.23	79.1±5.36	77.7±5.62
Diabetic control	283.8±5.01	286.4±12.4	300.3±8.64
EPM(50mg/kg)	293.1±9.83	192.1±12.3**	100.3±12.5**
EPM(100mg/kg)	280.5±42.4	185.2±11.2***	94.2±7.2***
Metformin (450mg/kg)	271.0±13.5	80.2±6.4***	70.1±6.3**

All the values of mean±SD; n=6; ** indicates $p < 0.01$, *** indicates $p < 0.001$ vs diabetic control.

Standard calibration curve of Gallic acid for estimation of total phenolic content.

Total flavonoid content

The total flavonoid content of methanolic extract of *P. marsupium* was determined using $AlCl_3$ method. A standard curve of quercetin at different concentrations was obtained and the flavonoid content of the methanolic extract was extrapolated and calculated. The total flavonoid content of methanolic extract of *P. marsupium* was calculated as 57.33 ± 1.5 mg QE/g extract (n = 3).

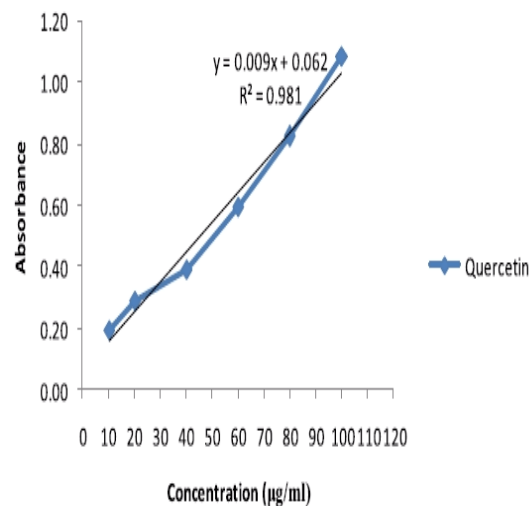


Fig 4: Standard calibration curve of Quercetin for estimation of total flavonoid content.

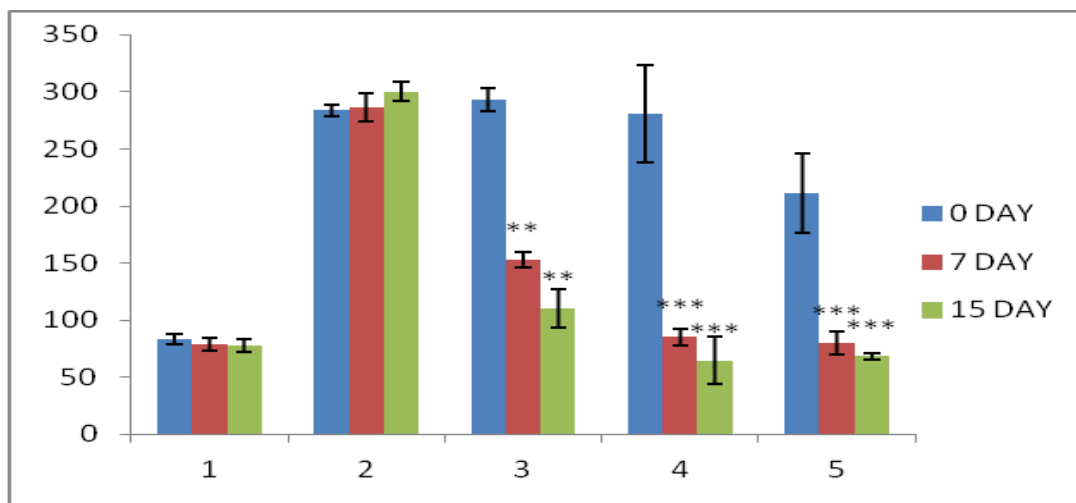


Fig 5: Effect of EPM on serum glucose levels (mg/dl) in diabetic rats

Table 4: Diabetic Neuropathy screening by tail flick response

Group	Mean latency period			
	0	30	60	90
Normal	2.18±0.12	2.31±0.20	2.43±0.27	2.49±0.32
EPM(50mg/kg)	2.79±0.20	2.94±0.34	3.10±0.45	3.19±0.40
EPM(100mg/kg)	3.11±0.36	3.28±0.28	3.42±0.1	3.55±0.20
Diclofenac sodiu(100mg/kg)	2.25±0.35	6.91±0.20*	9.74±0.34*	11.32±0.22*

All the values of mean±SD; n=6; ** indicates $p < 0.01$, *** indicates $p < 0.001$ vs. diabetic control.

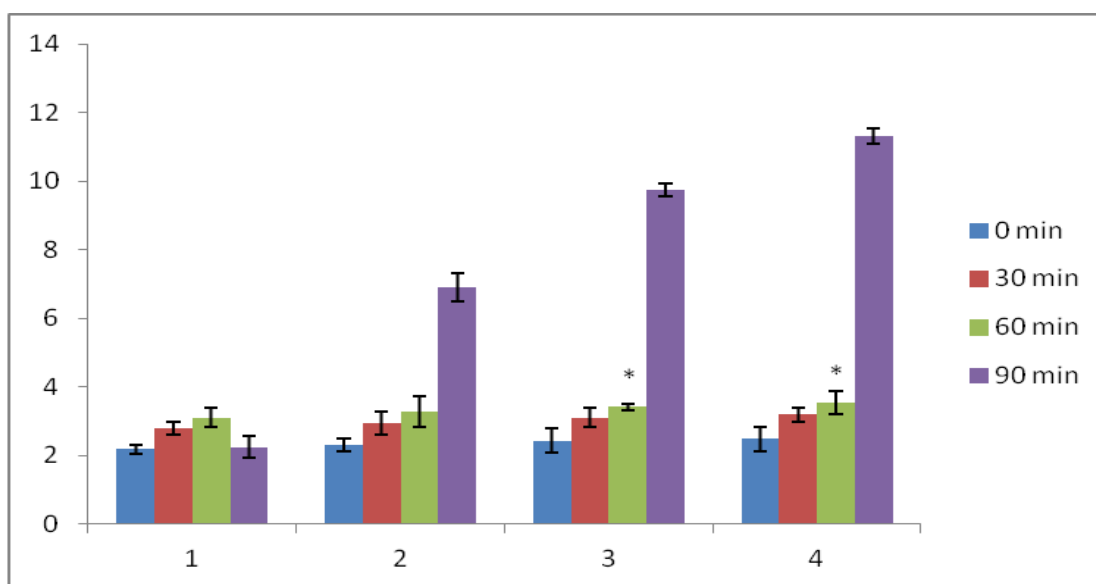
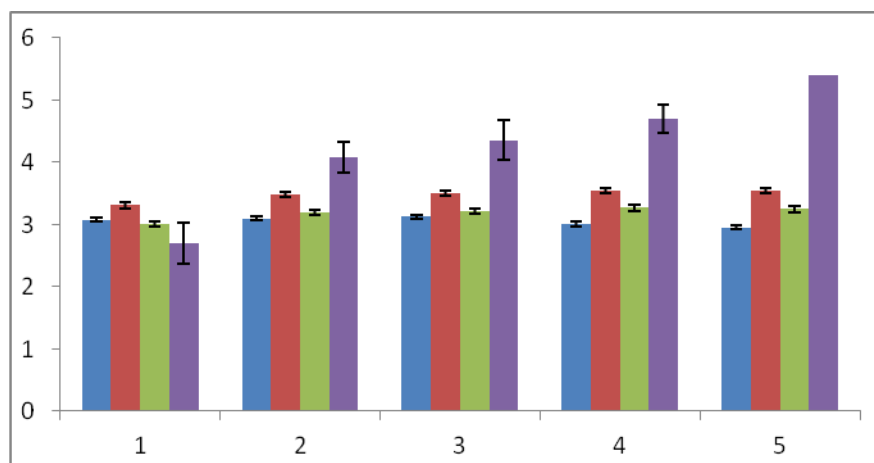


Fig 6: Effect of EPM on Diabetic neuropathy by tail flick in diabetic rats

All the values of mean±SD; n=6; ** indicates $p < 0.01$, *** indicates $p < 0.001$ vs. diabetic control.

Table 5: Diabetic Neuropathy screening by Thermal hypoalgesia response

Group	Mean latency period				
	0	30	60	90	120
Normal	3.08± 0.4	3.1± 0.33	3.13± 0.6	3.01± 0.51	2.95± 0.34
EPM(50mg/kg)	3.31± 0.29	3.48± 0.30	3.51± 0.34	3.54± 0.42	3.55± 0.25
EPM(100mg/kg)	3.01± 0.35	3.19± 0.30	3.22± 0.40	3.27± 0.37	3.25± 0.32
Diclofenac sodium (100mg/kg)	2.70± 0.40	4.08± 0.38*	4.35± 0.45*	4.70±0.19*	5.40±0.22*

**Fig 7: Effect of EPM on Diabetic neuropathy by Thermal hypoalgesia method in diabetic rats****DISCUSSION:**

The present study was aimed to evaluate the anti diabetic neuropathic effect of *P. Marsupium*. The activity was measured by estimating biomarkers like blood glucose levels, in experimental rats.

In the previous studies it was shown that alloxan monohydrate induced to diabetes mellitus. When given in a dose of 120mg/kg to rats intraperitoneally as evidenced in study. In the present study alloxan was administered in a single dose to induce diabetes mellitus in rats at the dose of 120mg/kg.

Alloxan forms an increased glucose levels that generates diabetes. Pretreatment with *P. Marsupium* produced significant decrease in glucose levels indicating the protective effect of tissue. On alloxan treatment a dose dependent decrease in glucose levels were observed.

Diabetic neuropathy alterations were tested using thermal hypoalgesia and Tail flick response as mentioned by watez *et al* that neuropathy can be tested by these experimental procedures and results in

comparison to that of the standard drug show that, *P. Marsupium* is Neuro protective in diabetic animals.

CONCLUSION:

- *Pterocarpus Marsupium* has different medicinal properties and may able to treat diabetes & diabetics complications.
- Subjected to acute oral toxicity studies and found that the *Pterocarpus Marsupium* is safe to use up to the dose of 100mg/kg .
- The *Pterocarpus Marsupium* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that methanolic extract of *Pterocarpus Marsupium* possess Antidiabetic activity and has shown significant effect when compared to Alloxan administration.
- *Pterocarpus Marsupium* had shown protection in neuropathy of diabetes and effective peripheral protection as shown by results

- It needs comprehensive investigations for developing a safe and effective drug. Further research is required to confirm the antidiabetic and antidiabetic neuropathic complications.

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