

**EVALUATION OF PTEROCARPUS MARSUPIUM EXTRACT FOR
ANTIDIABETIC ACTIVITY IN RATS****Naidu Narapusetty*, P.Kousalya, M.Govindu Naik, SK.Jani Basha, G.Anusha, U.Chandrababu**

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E.Mail: narapusetty.naidu@gmail.com**ABSTRACT**

Herbal medicines are considered to offer gentle means of managing chronic diseases at a lower cost. *Pterocarpus marsupium*. (PM) (Gammalu in Sinhala) heartwood and bark have been used as antidiabetic remedies in many cultures for thousands of years. The aim of this review is to address the existing evidence on antidiabetic effects of the *P. marsupium*. The hypoglycaemic effects, antidyslipidaemic effects, antioxidative effects and the safety of the PM heartwood and the bark have been scientifically validated using a multitude of in vitro and in vivo studies. Multiple α -cell regeneration, insulin β mechanisms responsible for hypoglycaemic effects of PM including release and insulin-like actions of some compounds isolated were identified. (-)-Epicatechin, a α -cell regeneration and β flavonoid isolated from the bark has shown insulin-like effects, effects on insulin release. Several compounds including pterostilbene and marsupsin isolated from the PM heartwood were identified as compounds with hypoglycaemic effects. The latex (gum) of the tree is a popular remedy used in Sri Lanka for diabetes even though the literature on PM does not discuss about the antidiabetic effects of the latex. Few investigations focused on the antidiabetic effects of PM latex have demonstrated strong inhibitory effects of the latex on α -amylase and α -glucosidase activities and on protein glycation. Investigations focusing on the antidiabetic effects and possible toxicity of the PM latex are essential to validate its efficacy and safety.

Key Words: Anti diabetic, neuropathy, *pterocarpus marsupium* etc.***Corresponding Author:****Mr.Naidu Narapusetty**

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INTRODUCTION

Herbal medicines are considered to offer gentle means of managing chronic diseases at a lower cost. *Pterocarpus marsupium* Roxb. (PM) (Gammalu in Sinhala) heartwood and bark have been used as antidiabetic remedies in many cultures for thousands of years. The aim of this research is to address the existing evidence on antidiabetic effects of the *P. marsupium*. The hypoglycaemic effects, antidyslipidaemic effects, antioxidative effects and the safety of the PM heartwood and the bark have been scientifically validated using a multitude of in vitro and in vivo studies. Multiple α -cell regeneration, insulin β mechanisms responsible for hypoglycaemic effects of PM including release and insulin-like actions of some compounds isolated were identified. (-)-Epicatechin, a α -cell regeneration and β flavonoid isolated from the bark has shown insulin-like effects, effects on insulin release. Several compounds including pterostilbene and marsupsin isolated from the PM heartwood were identified as compounds with hypoglycaemic effects. The latex (gum) of the tree is a popular remedy used in Sri Lanka for diabetes even though the literature on PM does not discuss about the antidiabetic effects of the latex. Few investigations focused on the antidiabetic effects of PM latex have demonstrated strong inhibitory effects of the latex on α -amylase and α -glucosidase activities and on protein glycation. Investigations focusing on the antidiabetic effects and possible toxicity of the PM latex are essential to validate its efficacy and safety. [1-5]

MATERIALS

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.

Equipments used

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.

Collection and Authentication 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.

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 vacuum desiccator for 7 days.

% Yield value of Methanol Extract from Aerial Parts of *P. Marsupium* 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 × 100 = 12.27 %.

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 °C ± 3 °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.

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-primidinetetrone) at dose of 120mg/kg intraperitoneally in normal saline²³. 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 >200mg/dl is considered as diabetic and were used for study.

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.

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

RESULTS & DISCUSSION

Table 1: Effect of *Pterocarpus Marsupium* (EPPM) 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
EPPM (50mg/kg)	293.1±9.83	192.1±12.3**	100.3±12.5**
EPPM (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**

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

Table 2: 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
EPPM (50mg/kg)	2.79±0.20	2.94±0.34	3.10±0.45	3.19±0.40
EPPM (100mg/kg)	3.11±0.36	3.28±0.28	3.42±0.1	3.55±0.20
Diclofenac sodium (100mg/kg)	2.25±0.35	6.91±0.20*	9.74±0.34*	11.32±0.22*

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

Table 3: 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
EPPM (50mg/kg)	3.31± 0.29	3.48± 0.30	3.51± 0.34	3.54± 0.42	3.55± 0.25
EPPM (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*

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

DISCUSSION

The present study was aimed to evaluate the anti diabetic, of *P. Marsupium*. The activity was measured by estimating various 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.²³ In the present study alloxan was administered in a single dose to induce diabetes mellitus in rats at the dose of 120mg/kg. The *P. Marsupium* has reported anti-microbial properties but the effect of the plant extract on antidiabetic, were not reported yet and so the plant was chosen for the study. 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. Pretreatment with *P. Marsupium* and metformin produced significant alteration in levels. 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 have 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 1000mg/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* posses Antidiabetic activity & 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 complications.

REFERENCES

1. News-Medical.Net. <http://www.news-medical.net/health/What-is-Hyperlipidemia.aspx>. Accessed on Dated 12/02/2013.
2. Nutrition MD Accessed on Dated 10/02/2013. Available from: http://www.nutritionmd.org/health_care_providers/cardiovascular/hyperlipidemia.html. Accessed on Dated 10/02/2013.
3. Brown SL. Lowered serum cholesterol and low mood. *Br.Med.J.* 1996; 313; 637-638.
4. Berlinear JA and Suzuki Y, The role of oxidized lipoprotein in atherogenesis, free radical. *Bio.Med* 1996.20: 707-727.
5. Nimmy C, Shastry CS, Prerana S, Prasanna S, Ullas D and Patel M. Anti hyperlipidemic Activity of *Costus Igneus* in Triton X-100 Induced Hyperlipidemic Rats. *Int Jour of Pharm and Chem Science.* 2012; 1(2); 813-818.