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Original Article

PTEROCARPUS MARSUPIUM HEARTWOOD EXTRACT RESTORES LEARNING, MEMORY AND COGNITIVE FLEXIBILITY IN A STZ-NA INDUCED DIABETES ANIMAL MODEL

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ABSTRACT

Objective: Study was designed to assess the *P.marsupium* heartwood aqueous extract effect on diabetes induced cognitive impairment.

Methods: Diabetes mellitus was induced by Streptozotocin (STZ) & Nicotinamide (NA) intraperitoneal route injection. Animals were divided into 7 groups for comparing the activity of *P.marsupium* at two doses 250 mg/kg & 500 mg/kg b. w against standard (Glibenclamide) & controls groups. Rats having blood glucose above 250 mg/dL were considered as diabetic. Learning & memory was tested using Morris water maze. Time taken to reach the platform (escape latencies) by animals was noted from day 1 to 8 and probe trial was conducted on day 9 to record the time spent in the different quadrants.

Results: Blood glucose levels were significantly ($p<0.001$) reduced in plant treated and glibenclamide groups when compared to diabetic controls. Also both the treated groups had decreased escape latencies in learning phase. During probe trial, test and standard treated groups spent significantly more time in target quadrant with less entries into other quadrants compared to untreated diabetic controls.

Conclusion: The results of this investigation revealed that extract of *P. marsupium* provides beneficial effects on learning and memory in diabetes rats by providing the potential antihyperglycemic action.

Keywords: Cognitive impairment, Diabetes mellitus, Diabetic neuropathy, Morris water maze, *Pterocarpus marsupium* heartwood.

INTRODUCTION

The heterogenecity of diabetes, and the various metabolic abnormalities associated with it are well known. Chronic hyperglycemia results in long term diabetic complications and tissue damage to kidney, nerve & eyes [1]. Cognitive impairment is common in patients with diabetes. Diabetes causes structural changes in brain, known as diabetic encephalopathy. Attention, thinking, mental flexibility, learning, memory, speed are affected in this brain disorder and making the subjects more susceptible to dementia. Recent studies suggest that the possible link between diabetes and dementia could be insulin resistance [2]. Insulin is believed to be the cognitive enhancer in non-diabetic subjects; however, link between hyperinsulemia and cognitive decline is still not clear in diabetic subjects. More studies are required to find the exact mechanisms on cognitive decline in diabetes patients. Aberrant insulin signaling in brain may add to these changes in diabetes [2]. Majority of the disorders like diabetes, atherosclerosis, cancer and neurodegenerative disorders are linked to oxidative stress/glycemic stress [3]. Oxidative stress leads to generation of excess free radicals which in turn leads to tissue or nerve destruction. Thus, more trials are looked-for to determine the effects of currently used or other antidiabetic compounds on cognitive impairment in diabetes by controlling the glycemic stress.

P. marsupium, more commonly seen in hilly areas of India, particularly western ghats in Kerala-Karnataka region. It is commonly known as Malabar kino or Vijayasar. This one of the most widely used plants in herbal medicine for the management of diabetes [4]. *P. marsupium* is also known to help in regeneration of pancreatic beta-cells [5]. The active antidiabetic ingredients in the aqueous extract have been identified as epicatechin and benzopyran. Many phenolic compounds such as marsupin, pterosupin & pterostilbene have been shown to have significant antidiabetic activity in STZ induced hyperglycemia in rats [6].

Therefore, the current study intended to assess the beneficial effects of *P. marsupium* heartwood aqueous extract in diabetes induced cognitive dysfunction using the Morris water maze model.

MATERIALS AND METHODS

Animals

Study accepted by the Institutional Animal Ethics Committee (IAEC), Kasturba Medical College, Mangaluru, Manipal University, Karnataka. Albino Wistar strain either sex rats weighing 100±5g were procured from Central Animal House, Kasturba Medical College, Mangaluru. All animals were fed in an animal facility with a light-dark cycle (12 to 12 hour) and were given water and standard rat food ad libitum [7].

Plant collection

Heartwood of *P. marsupium* was collected from Alva's herbal pharmacy, Moodbidri, Karnataka and identity was authenticated by Dr. Nagalakshamma, Department of Botany, St. Aloysius College, Mangaluru, Karnataka. (Voucher number: Wood/2006/745/62, National institute of science communication and information resources (NISCAIR), New Delhi).

Preparation of plant extract

One part (30 gm) of dry coarse powder of *P. marsupium* heartwood was boiled in sixteen parts (480 ml) of water for 15 min at 50°C. It was filtered through the muslin cloth and filtrate was kept for flash evaporation in rotary vacuum flash evaporator at 5 rpm (75 °C) for seven hours. Remaining residue was collected from round bottom flask and dried in heating mantle for three hours to obtain semi-solid form of extract. Extract was stored in refrigerator (-4°C) for further studies.

Chemicals

Streptozotocin and Nicotinamide were bought from Himedia Drug Company, India. Glibenclamide was bought from Cipla Pvt Ltd, Mumbai, India.

Induction of diabetes

Rats received intraperitoneal injections of NA-25 mg/kg dissolved in normal saline 15 min before an administration of STZ-50 mg/kg dissolved in 0.1M citrate buffer (pH 4.5) [8]. Only rats having a blood glucose above 250 mg/dL was chosen and divided into groups.

Experimental design

42 rats (24 diabetic rats and 18 normal rats) were used for the study. Animals were divided into seven groups (n=6) and oral administration of plant extract and Glibenclamide started on 7th day after STZ-NA injection and it was continued for 30 d.

Group I-Normal controls treated with saline (NC)

Group II-Untreated Diabetic controls (DC)

Group III-Normal rats treated with *P. marsupium* (250 mg/kg of body weight) (NC+PM250)

Group IV-Normal rats treated with *P. marsupium* (500 mg/kg of body weight) (NC+PM500)

Group V-Diabetic rats treated with *P. marsupium* (250 mg/kg of body weight) (DM+PM250)

Group VI-Diabetic rats treated with *P. marsupium* (500 mg/kg of body weight) (DM+PM500)

Group VII-Diabetic rats treated with Glibenclamide (500µg/kg of body weight)(DM+Glib)

Estimation of blood glucose

The test was done using ACCU-CHEK Active blood glucose monitor using disposable strips from Roche Diagnostics.

Morris water maze test

Cognitive function testing was started on 31st day after treatment & continued for 9 d. Learning, memory and cognitive flexibility test was performed by the methods described by Papadopoulos *et al.,* [9] & Chen *et al.,* [10]. Animals were trained for visible platform from day 1 to day 3 & from day 4 to day 8 for hidden platform. Time taken to reach the platform (TTP) by the rat was noted. On day 9 probe trials was conducted to test spatial memory by recording the time spent in different quadrants. Escape latencies (TTP) and time spent in different quadrants was recorded by video camera.

Statistical analysis

The data was expressed as mean±Standard Deviation (S. D.). Mann Whitney-U test was used for between the group's analysis and Repeated measure ANOVA was used to analyze the repeated time intervals. SPSS-16 version was used for analysis. p value<0.05 was considered as significant.

Table 1: Effect of *T. terrestris* and Glibenclamide on random blood glucose levels (mg/dL)

Group	Day 1	Day 30	p value
NC	116±7.70	121.17±8.65	0.326
DC	445.67±41.23	423.83±40.78	0.144
NC+PM250	124.33±5.35	117.67±7.55	0.186
NC+PM500	122.50±9.15	118±5.76	0.321
DM+PM250	490±21.54	249.17±25.45	0.001*
DM+PM500	488.50±22.49	215±13.39	0.001*
DM+Glib	451.67±19.22	200.83±18.41	0.001*

Each value in the table is represented as mean±SD (n=6). * indicates p value<0.05.

Table 2: Escape latencies comparison between different groups on day 3 and day 8 to determine the effect of *T. terrestris* & glibenclamide on learning and memory

Group	Day 3 (TTP in Sec)	Day 8 (TTP in Sec)
NC	7.50±1.97	2.63±0.79
DC	29.33±6.59	35.50±3.01
NC+PM250	5.33±1.21	3.20±0.40
NC+PM500	5.29±1.75	3.35±0.50
DM+PM250	5.16±0.75	4.5±0.52
DM+PM500	4.36±0.55	3.6±0.14
DM+Glib	5.08±0.80	3.16±0.40

Each value in the table is represented as mean±SD (n=6). TTP in Sec: Time taken to reach platform in seconds.

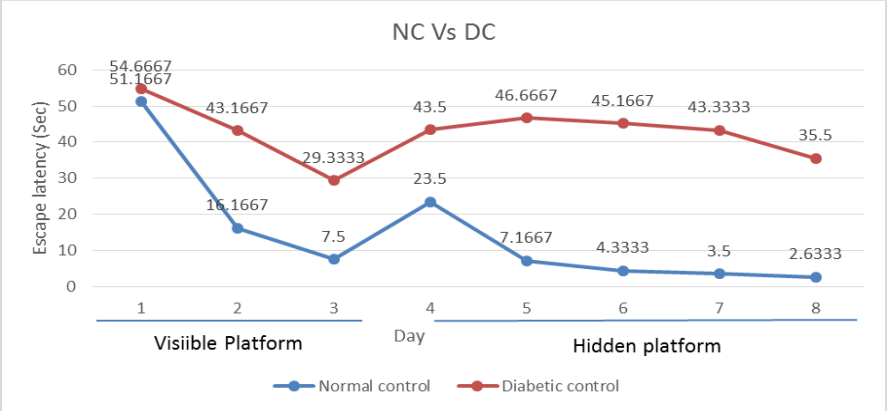

Fig. 1: Escape latencies comparison between normal controls and diabetic controls

Each value in the fig. is represented as mean±SD (n=6). NC: Normal control, DC: Diabetic control

RESULTS

Percentage yield of plant extract

5.90 grams of extract was obtained from 30 grams of plant powder. The percentage yield of *P. marsupium* extract was 19.66%.

P. marsupium effect on blood glucose

P. marsupium & Glibenclamide 30 d treatment significantly reduced (p<0.001) the blood glucose levels in diabetic rats when compared to untreated diabetic controls (table 1).

P. marsupium enhanced the spatial learning and memory-target quadrant

Escape latencies on Day 3 and Day 8 between the *P. marsupium* against standard drug group and control groups is shown in table 2. Diabetic control rats showed significant (p<0.001) increased escape latencies in finding the both platforms compared to normal control group (fig. 1). *P. marsupium* treated groups displayed significant (p<0.001) decrease in escape latencies compared to diabetic controls. (fig. 2).

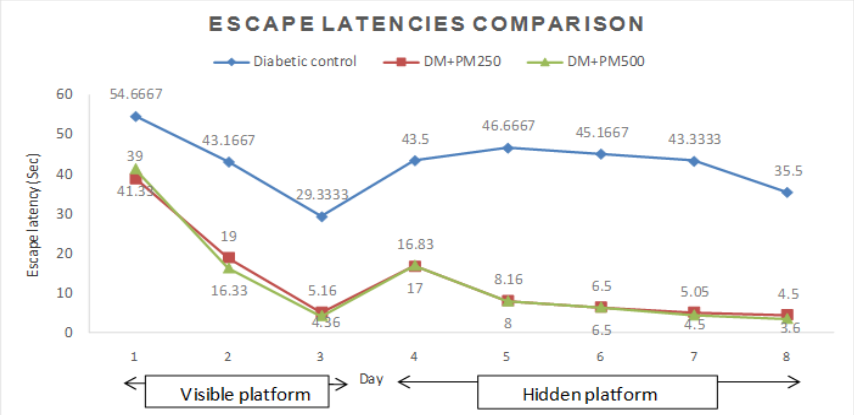

Fig. 2: Escape latencies comparison between Diabetic Controls & Diabetic rats treated with *P. marsupium* 250 & 500 mg/kg

Each value in the fig. is represented as mean±SD (n=6).

Diabetic group had significant (p<0.001) decreased inclination for the target quadrant when compared to other groups (DC-13.32±3.63, NC-31.83±2.92, DM+PM250-30.33±3.14, DM+PM500-29.5±2.94, DM+Glib-39.16±5.71, NC+PM250-35.80±4.74, NC+PM500-37.56±4.30Sec) during the probe trial on day 9 (fig. 3).

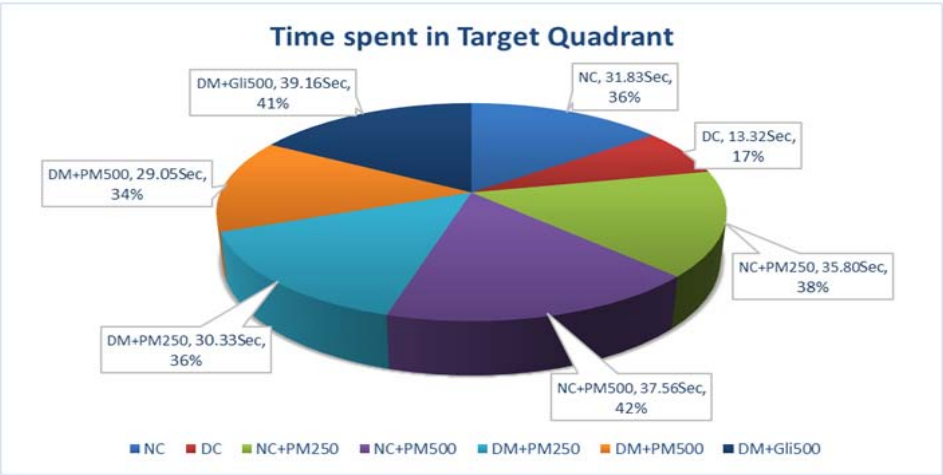

Fig. 3: Percentage of time spent in target quadrant

Six rats in each group (n=6)

P. marsupium effects on cognitive flexibility: original quadrant results

Diabetic animals spent significantly more time in the original quadrant than normal control group during the probe trial, (DC-40% & NC-19%, p<0.001). The preference of diabetic group was significantly more towards the visible platform quadrant (original quadrant) as compared to other quadrants.

Rats treated with *P. marsupium* (NC+PM250 & NC+PM500) had spent significantly (p<0.05) less time in the original quadrant. Similarly, *P. marsupium* and glibenclamide treated diabetic animals also spent less time (fig. 4) in this quadrant. However, the search of test and standard group rats for the target quadrant improved slightly, but was not complete since these groups explored in the other quadrants also as it was observed by their movement pattern.

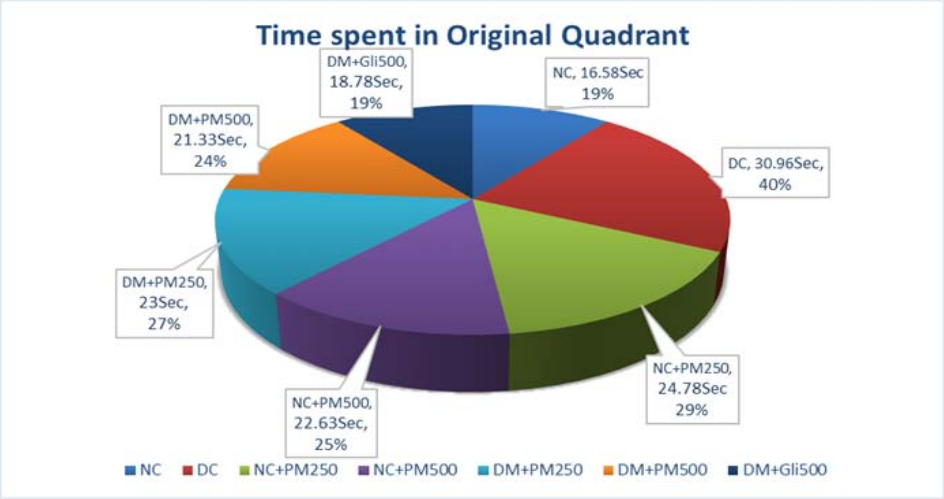

Fig. 4: Percentage of time spent in the original quadrant

Six rats in each group (n=6)

DISCUSSION

Study findings revealed encouraging effects with *P. marsupium* heartwood aqueous extract on cognitive impairment by improving learning activities, memory retention and adaptive capability, signifying better cognitive processing approaches to face new and unexpected changes after performing a task for a while, known as cognitive flexibility. *P. marsupium* & Glibenclamide treated rats displayed decreased escape latencies from day 1 to day 8. Probe trial day they concentrated on the target quadrant (hidden platform quadrant) with fewer entries into other quadrants. In difference, diabetic rats had significant increased escape latencies during learning phase & spent less time in target quadrant on probe trial. These alterations are symbolic of cognitive decline in diabetic animals. Persistent increased glucose levels and insulin resistance linked cerebrovascular changes may be the reason for these changes in diabetes.

In a study conducted by Chauhan *et al.*[11], it was reported that oral administration of methanolic extract of *P. marsupium* showed improvement on cognitive impairment in rats by using morris water maze. *P. marsupium* phytochemical tests showed the presence of several compounds like carbohydrates, glycosides, tannins, saponins & flavonoids. It is known that saponins have nootropic activities [12].

The beneficial effects of the plant extract have been attributed to its potent blood glucose lowering activity as it correlates with decreased blood glucose levels after 30days treatment in diabetic animals. In another study, Pterostilbene & marsupin were significantly reduced the plasma glucose levels of diabetic rats, efficacy was as good as metformin [13]. It was also reported that the *P. marsupium* bark flavonoid fraction can effectively reduce alloxan induced changes in the plasma glucose level and beta cell number in pancreas [14].

In diabetes, hyperglycemia induced oxidative stress can bring about many reactions that result in the production of excess free radicals (through enzymatic and non-enzymatic pathways) which in turn can lead to extensive tissue damage, neuronal degeneration & cognitive deficits. *P. marsupium* was found to have antioxidant activity, In our earlier studies the antioxidant potential of *P. marsupium* heartwood has been correlated with the presence of sufficient phenols and potent free radical scavenging activity [15]. In a study the whole aqueous extract of the stem bark of P. marsupium showed high anti-oxidant activity and protects the mitochondria against oxidative damage [16].

Inflammation plays a major role in progression of diabetic complications. In one study it was shown that aqueous extract of *P. marsupium* reduces the inflammatory cytokine TNF-alpha in type 2diabetic rats and this has an indirect effect on PPAR-Gamma expression. By decreasing TNF-α, it can up regulate the PPAR-Gamma and in turn the glucose metabolism [17]. Hence, the reversal of cognitive deficit by *P. marsupium* can be attributed to its antidiabetic, antioxidant and an anti-inflammatory property which reduces the hyperglycemia induced stress related complications in diabetic animals.

CONCLUSION

Learning and memory was widely affected in diabetic control group. Aqueous extract of *P. marsupium* heartwood (both doses i.e. 250 mg & 500 mg) along with its blood glucose lowering effects in diabetic rats, displayed beneficial effects in diabetes induced cognitive impairment by restoring the learning & memory activities. However, further investigations are required to study the exact mechanism of actions of *P. marsupium*.

ABBREVIATION

STZ-Streptozotocin, NA-Nicotinamide, TNF-α-Tumor necrosis factor α, PPAR-Peroxisome proliferator-activated receptor

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CONFLICT OF INTERESTS

Declare none

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2014;37:14-80.
2. Barbagallo M, Dominguez LJ. Type 2 diabetes mellitus and Alzheimer's disease. World J Diabetes 2014;5:889-93.
3. Biswas A, Bhattacharya S, Mahapatra SD, Debnath M, Biswas M. The Antioxidant effects of *Eupatorium triplinerve, Hygrophilatriflora* and *Pterocarpus marsupium*-A comparative study. Eur J Appl Sci 2012;4:136-9.
4. Maruthupandian A, Mohan VR. Antidiabetic, Anti-hyperlipidaemic and antioxidant activity of *Pterocarpus marsupium Roxb*. In alloxan induced diabetic rats. Int J PharmTech Res 2011;3:1681-7.
5. Chakravarthy BK, Gupta S, Gambhir SS, Gode K. Pancreatic beta-cell regeneration: A novel antidiabetic mechanism of *P. marsupium, Roxb*. Indian J Pharm 1980;12:123.
6. Dhanabal SP, Kokate CK, Ramanathan M, Kumar EP, Suresh B. Hypoglycemic activity *of Pterocarpus marsupium Roxb*. Pytother Res 2006;2:4.
7. Committee for the purpose of control and supervision of experiments on animals. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 2003;35:257-74.

8. Rabbani SI, Devi K, Khanam S. Protective role of glibenclamide against nicotinamide-streptozotocin induced nuclear damage in diabetic Wistar rats. J Pharmacol Ther 2010;1:18-23.
9. Papadopoulos P, Rosa-Neto P, Rochford J, Hamel E. Pioglitazone improves reversal learning and exerts mixed cerebrovascular effects in a mouse model of alzheimer's disease with combined amyloid-β and cerebrovascular pathology. J PLoS One 2013;8:e68612. DOI: 10.1371/journal.pone.0068612. [Article in Press].
10. Chen G, Chen KS, Knox J, Inglis J, Bernard. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. Nature 2000;408:975-9.
11. Chauhan B, Chaudhary AK. Memory enhancing activity of methanolic extract of *Pterocarpus marsupium. Roxb*. Phytopharmacology 2012;2:72-80.
12. Chintawar SD, Somani RS, Veena S, Kasture SB. Nootropic activity of Albizzia lebbeck in mice. J Ethnopharmacol 2002;81:299-305.
13. Manickam M, Ramanathan M, Jahromy MAF, Chansouria JPN, Ray AB. Anti-hyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat Prod 1997;60:609-10.
14. Chakravarthy BK, Gupta S, Gambhir SS, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium. Roxb*. Indian J Pharmacol 1980;12:123-7.
15. Vangalapati B, Manjrekar PA, Hegde A. Total phenolic content and free radical scavenging activity of *Pterocarpus marsupium* heartwood & *Tribulus terrestris* dry fruits: An *in vitro* comparative study. J Pharm Res 2014;8:610-3.
16. Mohammad I, Zafar I, Javid H, Hidayat H, Manzoor A, Asma E, *et al*. Chemical constituents and antioxidant activity of Geranium Wallichianum. Rec Nat Prod 2009;3:193-7.
17. Halagappa K, Girish HN, Srinivasan BP. The study of aqueous extract *Pterocarpus marsupium. Roxb*. on cytokine TNF-α in type 2 diabetic rats. Indian J Pharmacol 2010;42:392-6.