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 heterogeneity 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 hyperinsulinaemia 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 anti-diabetic 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 anti-diabetic ingredients in the aqueous extract have been identified as epicatechin and benzopyran. Many phenolic compounds such as marcupin, pterosupin & pterostilbene have been shown to have significant anti-diabetic 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 hours) 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 (400 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-Un-treated 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 30</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>116±7.7</td>
<td>121.17±8.65</td>
<td>0.326</td>
</tr>
<tr>
<td>DC</td>
<td>445.67±41.23</td>
<td>423.83±40.78</td>
<td>0.144</td>
</tr>
<tr>
<td>NC+PM250</td>
<td>124.33±5.35</td>
<td>117.67±7.55</td>
<td>0.186</td>
</tr>
<tr>
<td>NC+PM500</td>
<td>122.50±9.15</td>
<td>118±5.76</td>
<td>0.321</td>
</tr>
<tr>
<td>DM+PM250</td>
<td>490±21.54</td>
<td>249.17±25.45</td>
<td>0.001*</td>
</tr>
<tr>
<td>DM+PM500</td>
<td>486.50±22.49</td>
<td>215±13.39</td>
<td>0.001*</td>
</tr>
<tr>
<td>DM+Glib</td>
<td>451.67±19.22</td>
<td>200.83±18.41</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

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

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

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

\[\text{Diabetic control rats showed significant (p<0.001) increased escape latencies compared to normal control group, but was not complete since these groups explored in the other quadrants also as it was observed by their movement pattern.}\]

![Fig. 2: Escape latencies comparison between Diabetic Controls & Diabetic rats treated with *P. marsupium* 250 & 500 mg/kg](image)

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

![Fig. 3: Percentage of time spent in target quadrant](image)

Six rats in each group (n=6)

*R. marsupium* enhanced the spatial learning and memory-target quadrant

Escape latencies on Day 3 and Day 9 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](image)

![Fig. 3: Percentage of time spent in target quadrant](image)

*R. 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.
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 increased time in target quadrant on probe trial. These alterations are symbolic of cognitive decline in diabetic animals. Persistent increased glucose levels 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


