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SPATIAL AND WORKING MEMORY ENHANCING ACTIVITY OF *PONGAMIA PINNATA* LEAVES EXTRACTS IN RATS

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ABSTRACT

Memory is the ability of an individual to record sensory stimuli & retain them over short or long periods of time & recall the same at a later date when needed. Short and long term memory loss may result from deteriorating cerebral mechanisms due to different causes having impact on the quality of life. Memory enhancer can improve thinking, memory, and alertness in people with Alzheimer's disease that affect the mind. The Leaves of *Pongamia pinnata pierre* (Linn) belongs to the family Fabaceae is used as a digestive, laxative, anti-helmintic and are good for diarrhea, leprosy, dyspepsia and cough. Traditonally it is also used as Neuroprotective as it contains Chalcone derivatives.

Memory refers to retaining and recalling information over a period of time, depending upon the nature of cognitive task you are required to perform. The present study designed to assess the memory enhancing potential of *Pongamia pinnata* leaves extracts in wistar albino rats. In this study the two models has been selected under which in radial arm maze model in rats is used to evaluate working and reference memory errors and the Morris water maze is used to evaluate the retrieval of memory. The extract was administered orally in two doses (100 and 200 mg/kg p.o) for a period of 8 days for radial arm and 11 days for water maze test. Piracetam, 200mg/kg i.p, was used as standard drug treatment. Radial arm maze (RAM) was used to evaluate number of correct entries and time spent in baited arms as well as the latency to find the food. In Morris water maze used to evaluate the escape latency and time spent in target quadrant. The both the extracts of *Pongamia pinnata* at conc.200mg/kg, o.p. showed significantly increase in number correct entries and time spent in baited arms as well as increase in latency to find food, besides this in Morris water maze both the extracts i.e PPEA-200 and PPETH-200 mg/kg, o.p. showed significantly decreased in escape latency and increased in time spent in target quadrants as compared to standard (Pirecetam) and highly

significant difference when compared to control. The present study revealed that the *Pongamia pinnata* leaves extracts has potential to enhance the Memory and this might be due to presence of flavones and chalcone deriavtives such as Pongone, galbone, Pongalabol etc.

KEYWORDS: *Pongamia pinnata* leaves, Ethyl acetate and Ethanolic extract, Phytochemical screening, Memory enhancing activity.

INTRODUCTION

Memory refers to retaining and recalling information over a period of time, depending upon the nature of cognitive task you are required to perform. It might be necessary to hold information for a few seconds. For example, you use your memory to retain an unfamiliar telephone number till you have reached the telephone instrument to dial, or for many years you still remember the techniques of addition and subtraction which you perhaps learned during your early schooling.

Learning is defined as the acquisition of information and skills and subsequent retention of that information is called memory. Memory is one of the complex functions of the brain. It ultimately involves multiple neuronal pathways and neurotransmitters. Learning and memory can be conceived as both psychological process, as well as a change in synaptic neural connectivity.

Loss of memory and disturbed cognitive functions are major concerns in people afflicted with neurological diseases world-wide. Memory is the natural counter part of learning. Poor memory, low retention and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that leads to cognitive disorders. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neuro-degenerative states such as senile dementia, multi-infract dementia, Parkinson's disease, Huntington's chorea etc and Alzheimer's disease, amnesia, delirium, depression, schizophrenia etc. are the results of impairments in learning and memory Many neurotransmitters modulating learning and memory performance, some of them play an essential role in cognitive function. They are dopaminergic, glutamate, serotonergic and acetyl choline. The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence. Plants like *Bacopa monnerie, Azadirachta indica, Withania somnifera,* as well as *Ocimum sanctum,* have been investigated for their effect on cognitive function.[1]

In the present study we have selected a plant namely the leaves of *Pongamia pinnata L. Pierre* Belongs to family Fabaceae. It is native to India and distributed along Southeast Asia to the West pacific and northern Australia. All parts of the plant is used in the treatment of abscess, bronchitis, diarrhea, itches, piles, skin disease, tumors, painful rheumatic joints, ulcers, whooping cough, diabetes, blood purifier and as antiseptic to treat wound. In both the languages of Hindi and Bengali named it as 'Karanj' or 'Papar' or 'Kanji.[2]

The leaves of *Pongamia pinnta* used as an Anthelmintic, digestive and laxative, piles and wounds. Anti-inflammatory and Anti-diarrheal. *Pongamia pinnata* species contain flavonoids, alkaloids, phenolic compounds, tannins, steroids, saponins.[3]

Extensive literature survey revealed that *Pongamia pinnata* leaves possess neuroprotective activity[4] due to presence of flavones and chalcones derivatives such as Pongone, galbone, Pongalabol etc. hence the present study is undertaken to screen the leaves of *Pongamia pinnata* for its effectiveness in neuroprotective activity along with its potential for memory enhancing activity.

MATERIALS AND METHODS

Drugs and Chemicals

Pirecetam is used as a standard drug and other chemicals were obtained commercially and were of analytical grade.

Animal used

Wistar *rats* of either sex weighing 150 to 200 gm were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Acute toxicity

Acute oral toxicity studies were performed as per OECD guidelines 423, dosed each animal at the dose of 2000mg/kg b.w.p.o. The animal was observed continuously for 2hrs for gross

behavioral changes and intermittently once every 2hrs and finally at 24 and 72hrs to note any signs of toxicity including death.[5]

Behavioral models for experimental learning and memory

1) RADIAL ARM MAZE[9-10]

Rational and purpose

The radial arm-maze allows the study of spatial reference and working memory processes in the rat. In reference memory procedures, information was useful for many sessions/days and may usually be needed during the entire experiment. On the contrary, working memory procedures have a major temporal component as the information presented in the maze (arms baited) is useful for one session but not for subsequent ones; the rat has to remember the information during a delay interval (min to hours). Correct choices in the radial arm-maze were rewarded by food.[6]

Procedure

The apparatus is a fabric elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm.

Food pellets (reward) was placed at the end of the arms. The animals were trained on daily basis in the maze to collect the food pellets the session was terminate after 8 choices. Rat was exposed to the maze daily with food pellets in a fixed arm followed by respective drug treatment for the period of 7 days. The evaluation was carried out on 7^{th} day 24 hr after the respective drug treatment wherein food pellets was place in variable arm for evaluation of working memory.[7] The rat has to obtain the maximum number of rewards with a minimum number of errors.

Radial arm maze

Animal Grouping

Rats were divided into six groups (n = 6 for each group) and were housed in separate cages under controlled conditions of temperature (22 ± 2°c) and humidity (30-70). All animals were given standard diet and water ad libitum. Grouping of Animals is given below-

Group I	Animals was receive DMSO (Dimethyl sulfoxide 0.5%) for orally
Group II	Animals was receive standard drug (Pirecetam 200mg/kg i.p.)
Group III	Animals was receive lower dose of Ethyl acetate extract of *Pongamia pinnata* leaves (PPEA 100mg/kg oral)
Group IV	Animal was receive higher dose of Ethyl acetate extract of *Pongamia pinnata* leaves (PPEA 200 mg/kg oral)
Group V	Animals was receive lower dose of Ethanolic extract of *Pongamia pinnata* leaves (PPEE100mg/kg oral).
GroupVI	Animals was receive higher dose of Ethanolic extract of *Pongamia pinnata* leaves (PPEE 200mg/kg oral)

Evaluation

- The Number of errors (Entries to non-baited arms) was counted during the session.
- Time Spent in baited and non baited arm.
- Latency to find food was recorded as a measure of working memory evaluation.

Spatial learning in Water Maze

Rational and purpose

A task was developed where rats learn to swim in a water tank to find an escape platform hidden under the water. Learning is reflected on the shorter latencies to escape and the decrease on the length of the path to find the platform.

Although rodents can find the platform by using non-spatial strategies, the use of a s spatial strategy is the most efficient way to escape and young animals develop the spatial strategy after a small number of trials.[6]

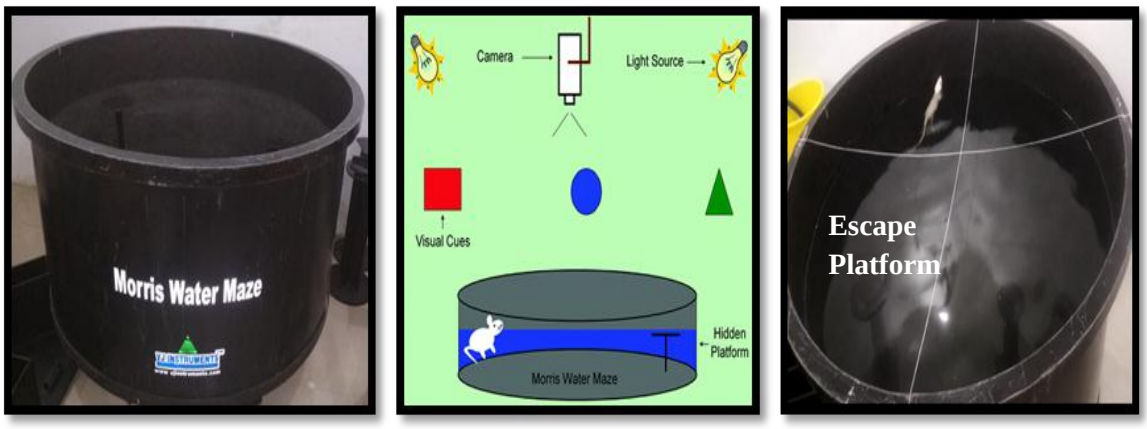
Procedure

The apparatus is a circular water tank filled to a depth of 20 cm with 25°C water. Four points equally distributed along the perimeter of the tank serve as Starting locations.

The tank is divided in four equal quadrants and a small platform (19 cm height) is located in the centre of one of the quadrants. The platform remains in the same position during the training days.

The platform remains in the same position during the training days. The rat was released into water and allowed 60-90 s to find platform. Animals usually receive 2-4 trials per day for 4-5 days until they escape onto the platform, well trained rats escape in less than 10 sec.

Afterwards the platform was removed and the rat was placed in any quadrant and allowed to explore the target quadrant for 300 sec.[11] the mean time spent in all the three quadrant was recorded. The mean time spent in target quadrant in search of missing platform was noted as an index of retrieval of memory.



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Group IV	Animal was receive higher dose of Ethyl acetate extract of Pongamia pinnata leaves (PPEA 200 mg/kg oral)
Group V	Animals was receive lower dose of Ethanolic extract of Pongamia pinnata leaves (PPEE100mg/kg oral).
Group	Animals was receive higher dose of Ethanolic extract of Pongamia pinnata leaves (PPEE 200mg/kg oral)

Evaluation

The latency to reach the escape platform was measured during the training days.

A free swim trial is generally performed after training days where escape platform was remove and animal was allow swimming for 30 sec (Time Spent in Target Quadrant) is Measured With the help of video system.[8]

STATISTICAL ANALYSIS

All the data represent as mean ± S.E.M. values. The data were analyzed by student t-test and one way analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. Statistical analysis was performed using the Graph Pad In Stat software version 5. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$. $p>0.05$ was considered as non-significant (ns) compared to Control group.

RESULTS

Estimation of *Pongamia pinnata* leaves extracts in Radial arm maze Test and water maze test

The effects of oral administration of *Pongamia pinnata* on number of correct entries in baited arms, Time spent in correct arms as well as latency to find food in the Wistar rats RAM are shown in Table 4. PPEA and PPETH at doses (100 mg/kg and 200 mg/kg) and Pirecetam (200mg/kg) all significantly increased when compared with Control (DMSO- 0.5%) in wistar rats $p< 0.05$ to $p<0.001$ and $p>0.05$ was considered non-significant (ns). Although the PPETH-200 showed no-significant difference as compare to standard and highly significant difference as compared to control.

In the second model that is water maze test, when the drug treated groups were compared with control and standard groups it showed that all four drug treated groups (PPEA-100,

PPEA-200, PPETH-100, PPETH-200) showed the significantly decreased latency to find escape platform by the animal. The Maximum activity was showed by PPEA-200 group which significantly improved learning and memory of rats, as indicated by decrease escape latency and increase in time spent in target quadrant during retrieval of memory using water maze latency to as compared to standard group. Similarly when the drug treated group screened for time spent in target quadrant with Standard and control, here also all the groups showed significant difference. And increase in time spent in target quadrant by extracts. The extract PPETH-200 showed highly significant memory enhancing activity of remaining tested groups when compared with the control and standard groups. Although the extract PPETH-200 showed no-significant difference as compare to standard and highly significant difference as compare to control.

Post hoc analysis Tukey's multiple comparisons test found that PPETH-200 mg/kg has significant difference when compared to Pirecetam standard (200 mg/kg). The Pongamia pinnata leaves extracts shwed increased in number of correct entries in baited arm as well as latency to find food and in (WM) decresed latency to find escape platform and significantly increased time spent in target quadrant. The results indicated that *Pongamia pinnata* showed significant Memory enhancing activity in Radial arm maze.

Table no. 1: Comparative evaluation of *Pongamia pinnata* leaves extracts in Radial arm maze.

		1st day			8th day		
Sr no.	**Treatment group**	**Number of correct entries in baited arm**	**Time spent in Baited arm**	**Latency to find food**	**Number of correct entries in baited arm**	**Time spent in Baited arm**	**Latency to find foo**
1	Std	8.66±0.21**	145.83±0.87**	35.83±1.49**	10.5±0.42**	170.33±1.72**	44.5±0.763
2	Control	3.5±0.22	112.33±1.5	62.67±1.78	4±0.25	137.33±0.66	77.3±2.060
3	PPEA-100	4.8±0.30**	122.16±0.70**	51.5±0.79**	6.16±0.30**	151.83±1.01**	63.5±1.11**
4	PPEA-200	5.66±0.21**	131.33±1.05**	45.83±1.07**	6.83±0.47**	161.16±1.49**	55.5±0.76**
5	PPETH100	7.16±0.40**	135.16±1.13**	42.16±1.37**	8.5±0.42**	163.33±1.40**	50±0.60**
6	PPEH-200	8±0.36**#	142.83±0.87**#	39.16±1.37**#	9.5±0.42**#	168.83±1.47**#	46±0.76**#

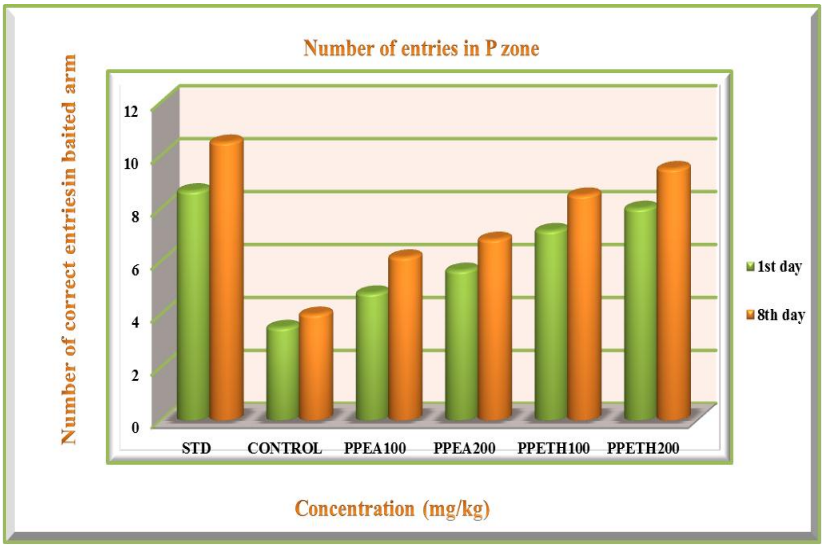

Chart no. 1: Number of correct entries in baited arms of *Pongamia pinnata* leaf extracts.

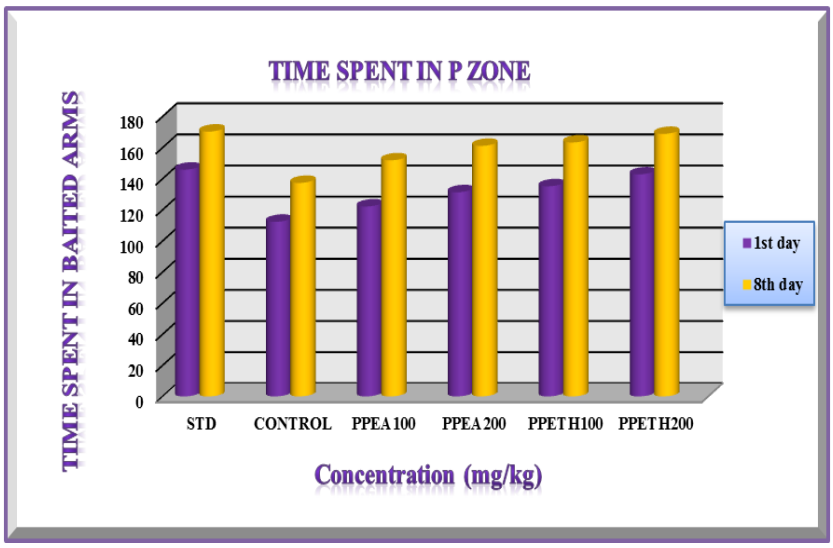

Chart no. 2: Time spent in baited arms of *Pongamia pinnata* leaf extracts.

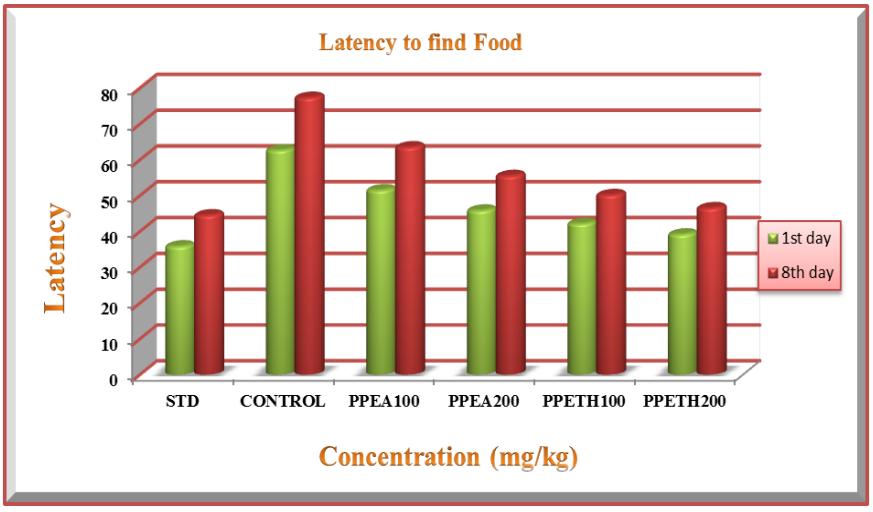

Chart no. 3: Latency to find food of *Pongamia pinnata* leaf extracts.

Table no. 2: Latency to find escape platform and time spent in target quadrant.

		1st day	6th day	10th day	5th day	9th day	11th day
Sr no.	Treatment group	Latency to find escape platform	Latency to find hidden platform	Time spent in target quadrants	Latency to find escape platform	Latency to find hidden platform	Time spent in target quadrants
1	Std	43.6±0.71**	48.83±0.4**	80.06±0.42**	33±0.55**	41.5±0.50**	92.16±90**
2	Control	87.5±0.17	89.3±0.8	51.6±0.55	74±1.26	76±1.265	56.5±0.428
3	PPEA-100	67.66±0.66**	75.3±0.76**	55.3±1.04**	53.16±0.60**	67.16±0.60**	74.66±0.98**
4	PPEA-200	61.83±0.83**	69.5±0.60**	63.3±0.70**	42.6±0.88**	57.66±1.22**	82.66±1.35**
5	PPETH100	55.83±0.79**	60.66±0.76**	77±0.93**	36.66±0.42**	46.33±0.76**	86.16±1.6**
6	PPEH-200	48±0.57**#	52.16±0.47**#	78±0.93**#	34.66±0.42**#	40.83±0.95**#	87.33±1.49**#

*The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard.*

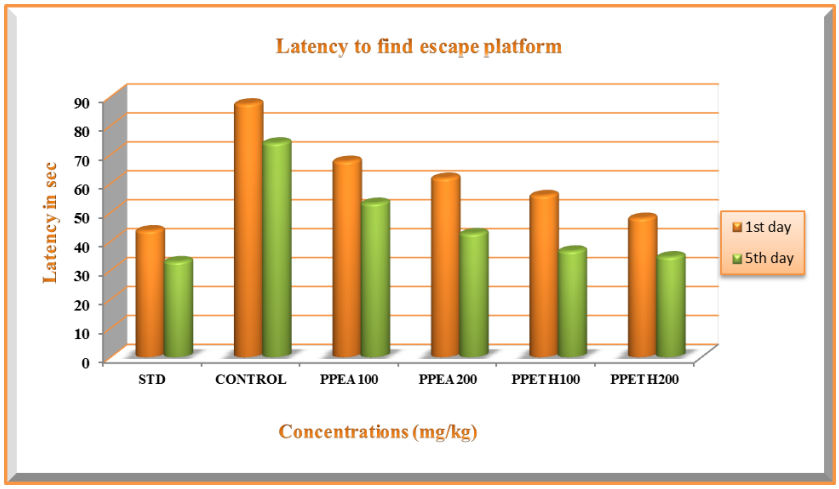

Chart no.4- Latency to find escape platform.

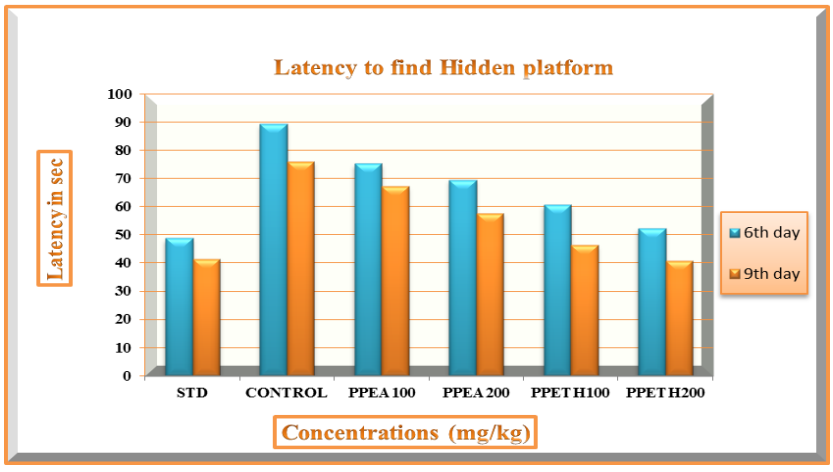

Chart no.5-Latency to find Escape platform (Hidden).

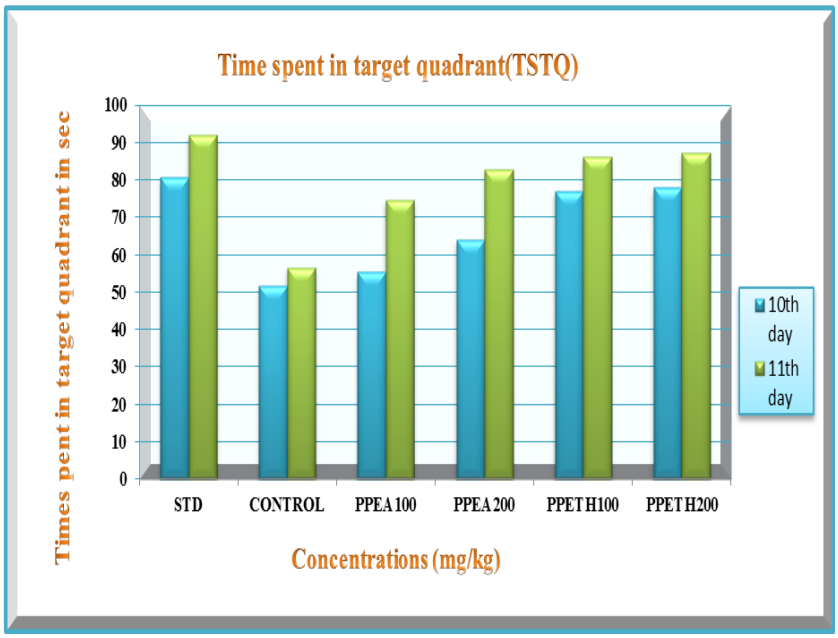

Chart no.6: Time spent in Target Quadrant.

DISCUSSION

The memory enhancing activity of *Pongamia pinnata* Ethyl acetate and Ethanolic extracts of *Pongamia pinnata* leaves was evaluated in rats by daily exposing them to the radial arm maze with the food pellet in a fix arm of maze. Food pellets were placed in a variable arm for evaluation of working memory. It is characterized by increase in latency to find the food and time spent in selected arm. The results were drawn by evaluating latency to find food, time spent & number of correct entries in baited arms.

The above result revealed that the highest dose of *Pongamia pinnata* (200 mg /kg) of extract showed highly significant memory enhancing activity, on given daily dose orally. The study reveals that the effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through ability of the animal to cause significant decrease in number of working and reference errors and increase in latency to find food and time spent in selected zone as well.

From the data obtained from the study ,it was observed that group PPETH-200 i.e. ethanolic extract at dose 200mg /kg showed maximum number of entries (**9.5±0.42**#).** The same group showed maximum time spent in baited arms (**168.83±1.47**#)** as well and the latency to find the food (**46±0.76**#)** Where as the other group i.e. PPETH-100 and PPEA-200 and PPEA-100 represent (**8.5±0.42**), (6.83±0.47**), (6.16±0.30**)** values respectively for number of entries in baited arms. **(50±0.60**), (55.5±0.76**), (63.5±1.11**)** for latency to

find food. The time spent in baited arms values are (**163.33±1.40**), (161.16±1.49**), (151.83±1.01**)** respectively. The next parameter is latency to find food and there values are as follows. All these values were compared with standard drug i.e. Piracetam at dose 200mg/kg.

The second model is water maze: The data obtained from the study revealed that the ethanolic extract at dose 200mg/kg showed decrease in latency to find escape platform. (40.83±0.95**#). The same group showed the time spent in target quadrant is (87.33±1.49**#). Where as the other groups that is PPETH-200, PPEA-200 and PPEA-100 exhibit (77±0.93**) (63.3±0.70**), (55.3±1.04**) values respectively for latency to find escape platform. The time spent in target quadrant values are (86.16±1.6**), (82.66±1.35**), (74.66±0.98**) respectively.

From the results it was revealed that both extract i.e. ethyl acetate and ethanolic showed effective memory enhancing activity. Although ethanolic extract at 200 mg/kg improved significantly learning and memory potential by using radial arm maze in rats and water maze.

CONCLUSION

Different extracts of *Pongamia pinnata* leaves shows significant Memory Enhancing activity. The ethanolic extract shows more significant activity at respective doses compared to ethyl acetate extract. This is a baseline work; further investigation is needed at molecular level.

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