# ASSESSMENT OF LEARNING AND MEMORY ENHANCING ACTIVITY OF *PHOENIX DACTYLIFERA* (DATE) IN RATS

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## ABSTRACT

**Objectives**: To assess the learning and memory enhancing activity of the ethanolic extract of *Phoenix dactylifera*(EEPD)fruit in rats using Elevated plus maze (EPM), Hebb-William maze (HWM) and Morris water maze (MWM). **Materials and methods:** *Wistar* rats (100-150 gm) of either sex, were divided into five groups (n=6). Group-I(control) animals received vehicle, group-II (scopolamine control) animals received scopolamine (0.4 mg/kg i.p), groups III, IV and V animals received ethanolic extract of *Phoenix dactylifera* fruit-100 mg/kg p.o, 200 mg/kg p.o, and piracetam (400 mg/kg i.p), respectively for 27 days, followed by scopolamine (0.4 mg/kg i.p) injection on 19th and 27th day. Assessment of transfer latency (TL), time taken to reach reward chamber (TRC) and swim latency (SL), using EPM, HWM, and MWM, respectively, was done on 16th, 17th, 19th and 27th day. Acetylcholine esterase activity, superoxide dismutase, catalase and Glutathione from brain homogenate were estimated. The data was analyzed by one way ANOVA followed by Dunnett's test. $P \leq 0.05$ was considered significant. **Result:** EEPD decreased TL, TRC and SL. EEPD decreased acetylcholine esterase activity and increased SOD, catalase and glutathione levels. **Conclusion:** Dates (fruit of *Phoenix dactylifera*) enhanced learning and memory activity.

**KEYWORDS:** Learning, Memory, EPM, HWM, MWM, Piracetam, Scopolamine.

**INTRODUCTION**

In continuation with the earlier studies done.[1] this paper presents the data for memory enhancing activity of dates. Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. Also, memory is a process involving encoding, storing, and recalling information. Thus, memory records various facts and events, make it available for further use.[2] Memory is the most important function of the brain by which experiences are recorded and can be used to adapt their responses to the environment and it is vital for survival.[3] It involves multiple neuronal pathways and neurotransmitters. Central cholinergic system plays an important role in retention of memory and cognition and is involved in attentional functions.

Many herbal remedies are used to improve memory and learning. *Phoenix dactylifera* L, family Arecaceae, called 'Nakhla' and the 'Tree of Life' by the Arabs [4] is commonly known as date palm. Different parts of the plant are traditionally used for the treatment of broad spectrum of ailments including memory disturbances, fever, paralysis, loss of consciousness, and nervous disorders.[4] Dates are good sources of energy due to their high iron, potassium, calcium, sodium, iodine and sugar contents. In addition, it is rich in vitamins and low in fat and proteins. Besides its nutritional value, date is rich in phenolic and flavonoid compounds.[5] Nutritional composition of dates includes amino acids (alanine, arginine, aspartagine, γ amino butyric acid, cysteine, cystine, glutamine, methionine, phenyl alanine etc), minerals ($Ca^{2+}$, Cu, $Fe^+$, Mg, Mn, K, P, $Na^+$, Zn, Al, Cl, Cd,), vitamins (Vit C, Vit A, Nicotinic acid, Vit $B_2$, Vit $B_1$), fatty acids (saturated – Arachidic acid, Capric acid, Caprylic acid, Lauric acid, Myristic acid, Palmitic acid etc, unsaturated – Linoleic, Linolenic, Oleic, Palmitoleic) and proteins.[6]

In India, traditionally, for newborns and infants, up to the age of 40 days to 6 months, a drop of JANAMGHUTTI is administered in the morning hours every day. The hypothesis is-Janamghutti is good for development of brain and its functions. One of the components of this is date fruit.

But till now scientifically it is not validated for learning and memory enhancing activity. Hence, present study is taken up to investigate and validate learning and memory enhancing activity of *Phoenix dactylifera*. To explore the possible mechanism of action, the brain acetylcholine esterase activity and effect of date on antioxidant enzymes is assessed.

**MATERIALS AND METHODS**

*Plant material:* Standardized ethanolic extract of *Phoenix dactylifera* fruit (EEPD), procured from Green chem. Herbal extract and formulations, Bangalore, India was used for the study. Preliminary, phytochemical analysis of the extract was carried out.[7] The plant extract was suspended in distilled water (50 mg/ml) and administered orally to rats.

*Chemicals and drugs:* Scopolamine was procured from Zydus Health Care, Bangalore. Piracetam was procured from Micro Labs Limited, Bangalore.

*Experimental animals***:** Inbred, young *Wistar* rats (8 weeks old, weighing around 100g and 150g) were used in the current study. The animals were maintained under standard laboratory conditions, i.e. room temperature of $24^0 \pm 5^0$ C; relative humidity 45-55% and natural day and night cycle. The animals had free access to standard rat pellet (Pranav Agro Industry, Bangalore), with water supplied *ad libitum* under strict hygienic conditions. All the protocols and the experiments were conducted in compliance to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Permission was taken from IAEC of Visveswarapura Institute of Pharmaceutical Sciences, Bangalore before starting the animal experiments.

*Experimental protocol: Wistar* albino rats were divided into five groups of six rats each for all the three models (EPM, HWM, MWM). Group-I animals served as control, received vehicle i.e, distilled water. Group-II, Group-III and IV animals, received scopolamine 0.4 mg/kg i.p.[8] ethanolic fruit extract of *Phoenix dactylifera*, 100 and 200 mg/kg p.o.[3] respectively. Group-V animals, received piracetam 400 mg/kg i.p.[8] The rats of group III, IV and V received the respective treatment for 15 days, followed by training session on 16th, 17th, and 18th day. On 19th day, single dose of scopolamine was administered to all the animals' except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively. The respective treatments continued for one week and on 27thday, scopolamine was administered to all the animals' except group I animals, 30 min after the respective treatment. TL, TRC, and SL were assessed 45 min thereafter respectively.

*Elevated plus maze (EPM):* Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in rats. On the first day (training session) each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) is the time

taken (in sec) by the animal to move from the open arm into any one of the enclosed arms with all its four legs. TL was recorded on the first day (training session) for each animal. The rat was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned-task (memory) was examined 24h after the first day trial and on 27th day of the treatment. Significant reduction in TL value of retention indicated improvement in memory. [8,9]

*Hebb-William maze (HWM):* Hebb-Williams maze is an incentive-based exteroceptive behavioral model useful for measuring spatial working memory of rats. In this model, changes in time taken by the animal to reach reward chamber from start box (TRC) was taken as learning and memory enhancing activity. TRC was recorded on the first day (training session) for each animal. Each animal was allowed to explore the maze for 3 min with all the doors opened, before returning to its home cage. Retention of this learned task (memory) was examined 24h after the first day trial and on the 27th day of the treatment. [8,9]

*Morris water maze (MWM):* The water maze consists of a circular tank with 100 cm diameter and a wall 20 cm above the water level. A circular platform was hidden 2 cm below the water level. The water was made opaque using titanium dioxide suspension and was kept at about 23°C during the experiment. The latency to find the platform was measured as the time of placement of the rat in the water to the time it finds the platform. If the animal fails to find the platform in any trial within 3 min it is placed on it for 10s.[10]

*Acetyl Choline esterase (AChE) activity* [11]

**Preparation of brain homogenate**

The animal was sacrificed, and the whole brain, removed from skull, was weighed and homogenized in an ice bath with 10 volume of sterile normal saline, centrifuged at 3000 rpm for 10 min and resultant cloudy supernatant liquid was used for estimation. Acetyl Choline esterase activity was estimated by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released due to the cleavage of ATC by AChE is allowed to react with the -SH reagent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which is reduced to thionitrobenzoic acid, a yellow colored anion with an absorption maxima at 412 nm. Concentration of thionitrobenzoic acid, detected using a UV spectrophotometer is taken as a direct estimate of the AChE activity.

**Antioxidant enzymes estimation**

*LPO* [12]**:** LPO was measured in brain homogenate spectrophotometrically by method of Ohkawa et al., 1979 and expressed in terms of nanomoles of thiobarbituric acid reactive substances formed per mg of wet tissue.

*SOD* [13]**:** The activity of SOD was determined in the brain homogenate spectrophotometrically at 560nm. In SOD assay ions generated, converts NBT to NBT-diformazan. This is measured as SOD activity.

*CATALASE* [13]**:** The activity of catalase enzyme was determined in brain homogenate by the method of Aebi et al (1984).

*GLUTATHIONE* **ASSAY** [14]**:** Glutathione activity was measured in brain homogenate according to method of Ellman, 1959. The assay is based on the reduction of 5, 5-dithiobis-2-nitrobenzoic acid by SH group of glutathione to 2-nitro-S-mercaptobenzoic acid per mole of glutathione.

*Statistical analysis*: All the values are expressed as mean ± SEM. The data was analyzed by one-way ANOVA, followed by Dunnett's test. $P \leq 0.05$ was considered significant.

**RESULTS**

Preliminary phytochemical analysis of the EEPD showed presence of flavonoids, saponin, tannins, steroids, phenolic compounds, proteins and vitamin C

**EPM:** Effect of *Phoenix dactylifera* on transfer latency (TL)

As shown in Table 1, scopolamine increases the time taken by rat to reach the closed arms as compared to control group, (i.e. induces amnesia). Pretreatment with *Phoenix dactylifera*, for 27 days, resulted in decrease in TL, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg). *Phoenix dactylifera* decreased TL significantly ($P<0.01$) on day 27th as compared to day 16, 17 and 19th.

**HWM:** Effect of *Phoenix dactylifera* on time taken to reach reward chamber (TRC).
As shown in Table 2, scopolamine increases the TRC as compared to control group, (i.e. induces amnesia). Pretreatment with *Phoenix dactylifera*, for 27 days, resulted in decrease in TRC, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

*Phoenix dactylifera* decreased TRC significantly (P<0.01) on day 27th as compared to day 16, 17 and 19th.

**MWM:** Effect of *Phoenix dactylifera* on Swim latency (SL).

As shown in Fig-1, scopolamine increases SL compared to control group, (i.e. induces amnesia). Pretreatment with *Phoenix dactylifera*, for 27 days, resulted in decrease in SL, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg). *Phoenix dactylifera* decreased SL significantly (P<0.01) on day 27th as compared to day 19th. Acetylcholine esterase activity

As shown in Fig-2, scopolamine increases the acetylcholine esterase activity compared to control group, (i.e. induces amnesia). Pretreatment with *Phoenix dactylifera* for 27 days, resulted in a significant (P<0.01) decrease in acetylcholine esterase activity in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

**Effect of *Phoenix dactylifera* on antioxidant enzymes**

As shown in Table 3, scopolamine increases LPO activity and decreases the SOD, Catalase and Glutathione activity. Pretreatment with *Phoenix dactylifera*, for 27 days, resulted in, significant (P<0.01) decrease in LPO and increase in SOD, Catalase, Glutathione activity, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg).

**DISCUSSION**

Traditionally, the fruit of *Phoenix dactylifera*(date) is used in the treatment of memory disturbances.[4] Also, in Indian context, date is a component of Janamghutti, administered to newborn till the age of 40 days, as it is believed to be good for development of brain and its functions. The present study was undertaken to evaluate learning and memory enhancing activity of *Phoenix dactylifera* in amnesic rats. The study was designed to investigate the effect of ethanolic fruit extract of *Phoenix dactylifera* on TL, TRC and SL using EPM, HWM, MWM respectively.

Learning is the process of acquiring information and skills, whereas, retention of that information and retrieval as and when required is called memory. Learning and memory together called as cognition. Cognitive function depends upon exteroceptive and interoceptive feedback mechanisms. Exteroceptive system is concerned with sensory stimuli such as visual, shock; whereas an interoceptive system deals with physiological condition of

the body. Numerous animal models have been developed to understand the mechanisms involved in learning and memory. Many of these assess the ability of rodents to recognize and remember a given location in space and to relate this information to a specific goal. This is often referred to a spatial learning. Elevated plus maze and Morris water maze assess the spatial learning in rodents and are the exteroceptive behavior models for assessing the short term memory.[8,9,15] Scopolamine induced amnesia in rats or mice is interoceptive behavior model, widely used to assess the memory enhancement by the new drug entity. Scopolamine increases TL, TRC and SL in rats in EPM, HWM, MWM models respectively, and thus produces amnesia in experimental animals-rats. In our study we found that EEPD reduced TL, TRC and SL in scopolamine induced amnesic rats using EPM, HWM, MWM respectively. This can be interpreted as the learning and memory enhancing activity of *Phoenix dactylifera*, which may be due to its antioxidant property.[16,17] We also found that pretreatment with EEPD for 27 days, caused decrease in LPO and increase in SOD, Catalase, Glutathione activity, in a dose dependent manner, which was comparable with Piracetam (400 mg/kg). Antioxidant properties of *Phoenix dactylifera* was attributed to phenolic, α-tocopherol, flavonoids, saponins, tannins, steroids and vitamin C.[5,6,13,17] Phenolic profile of date demonstrated the presence of cinnamic acids (ferulic, sinapic and couramic acids and their derivaties such as dactyliferic acid), flavonoid glycosides (luteolin, methyl luteolin, quercetin, methyl quercetin) and flovanols (catechin, epicatechin).[5] Several studies have shown that flavanoids and other fruit and vegetable-derived phytochemicals have beneficial effect on learning and memory.[8,9,15] Decreased cholinergic firings in the brain, increased oxidative stress, hypercholesterolemia, and neuroinflammatory reactions are some of the reasons for decline in memory.[18] Central cholinergic system plays an important role in learning and memory. Estimating the acetylcholine esterase activity provides valuable information on cholinergic function, which can correlate with cognitive function.[11] In our studies, we found that, EEPD decreased acetylcholine esterase activity in brain homogenate. As a result, there is accumulation of acetyl choline in brain(hippocampal region in cortex), hence triggering of cholinergic firings, thereby enhanced memory and cognition. AChE inhibitors play an important role in the nervous system disorders owing to their potential as pharmacological and toxicological agents. Recently, AChE inhibitors like tacrine and rivastigmine are used in the treatment of Alzheimer's disease.[11]  As found in this study, extract of date fruit facilitates retention of learned task, therefore it may be considered as potential AChE inhibitor or anticholinesterase agent.

**Table 1: Effect of ethanolic extract of *Phoenix dactylifera* fruit on Transfer latency (TL) in rats using elevated plus maze.**

| S. No | Treatment | TL on 16th Day (sec) | TL on 17th Day (sec) | TL on 19th day (sec) | TL on 27th day (sec) |
|-------|-----------|------------------------|------------------------|------------------------|------------------------|
| 1 | Control | 56.17±5.199 | 53.83±4.277 | 9.150±0.8918 | 7.988±0.42 |
| 2 | Scopolamine 0.4 mg/kg i.p | 65.50 ±8.401 | 63.67±2.940 | 55.66±2.963 | 49±2.633 |
| 3 | EEPD 100mg/kg p.o | 52.05±6.475 | 50.17±3.754 | 46.83±4.22 | 43.5±4.37* |
| 4 | EEPD 200mg/kg p.o | 50.67±6.736 | 42.17±5.540 | 6.032±0.81** | 4.903±0.59** |
| 5 | Piracetam 400 mg/kg i.p | 38.36±10.72** | 35.50±4.137** | 4.378±0.47** | 2.838±0.11** |

n=6. Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett's test

*$P<0.05$v/s scopolamine,      **$P<0.01$v/s scopolamine,

**Table-2: Effect of ethanolic extract of *Phoenix dactylifera* fruit on time taken to reach reward chamber (TRC) by rats using Hebb-William maze.**

| S. No | Treatment | TRC on 16th day (sec) | TRC on 17th day (sec) | TRC on 19th day (sec) | TRC on 27th day (sec) |
|-------|-----------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1 | Control | 131.66±12.34 | 101.83±7.842 | 62.33±6.09 | 28.33±4.01 |
| 2 | Scopolamie 0.4 mg/kg i.p | 142.16±11.731 | 123.6±9.58 | 117.33±8.35 | 110.83±9.43 |
| 3 | EEPD 100 mg/kg p.o | 137.66±10.95 | 120±7.071 | 110±4.282 * | 108.66±4.23* |
| 4 | EEPD 200 mg/kg p.o | 91.16±13.36** | 58.5±9.698** | 40.16±6.81** | 19.166±4.23** |
| 5 | Piracetam 400 mg/kg i.p | 68.83±4.078** | 47.16±4.31** | 20.66±3.52** | 7±1.15** |

n=6.  Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett's test.
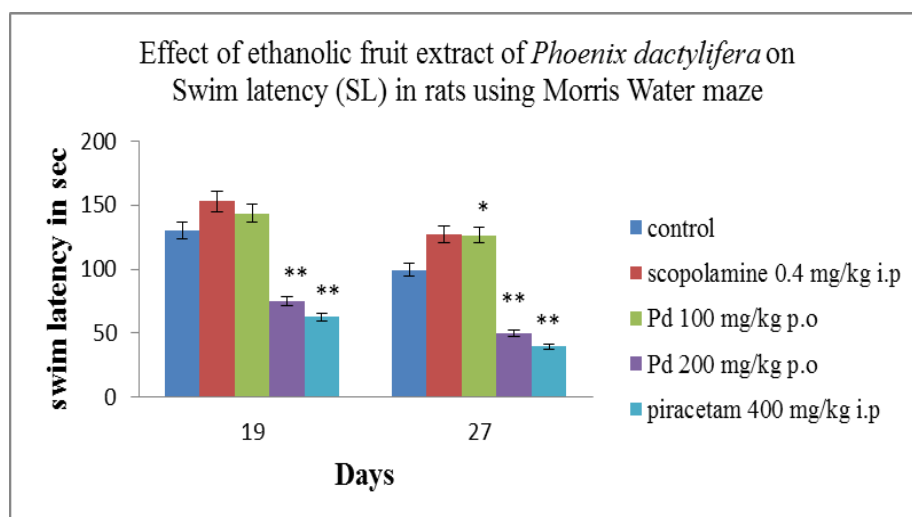
*$P<0.05$v/s scopolamine, **$P<0.01$v/s scopolamine,

**Table-3:  Effect of ethanolic extract of *Phoenix dactylifera* fruit on antioxidant enzyme.**

| S. No | Treatment | LPO (n moles /g of tissue) | GSH (n moles /mg of protein | SOD (lmoles/mg of protein) | CAT (U/mg of protein) |
|-------|-----------|----------------------------|-----------------------------|----------------------------|-----------------------|
| 1 | Control | 46.28±0.315 | 4.142±0.119 | 0.593±0.001 | 0.370±0.0007 |
| 2 | Scopolamine 0.4 mg/kg i.p | 55.42±0.618 | 2.078±0.048 | 0.034±0.035 | 0.157±0.0007 |
| 3 | EEPD 100mg/kg p.o | 42.27±0.627[*] | 4.95±0.065[**] | 0.407±0.007[**] | 0.457±0.0012[**] |
| 4 | EEPD 200mg/kg p.o | 32.71±0.501[**] | 5.46±0.100[**] | 0.477±0.006[**] | 0.542±0.0016[**] |
| 5 | Piracetam 400 mg/kg i.p | 30.86±0.257[**] | 6.221±019[**] | 0.611±0.004[**] | 0.603±0.0009[**] |

n=6. Values are expressed as mean ± SEM, one way ANOVA followed by Dunnett's test.
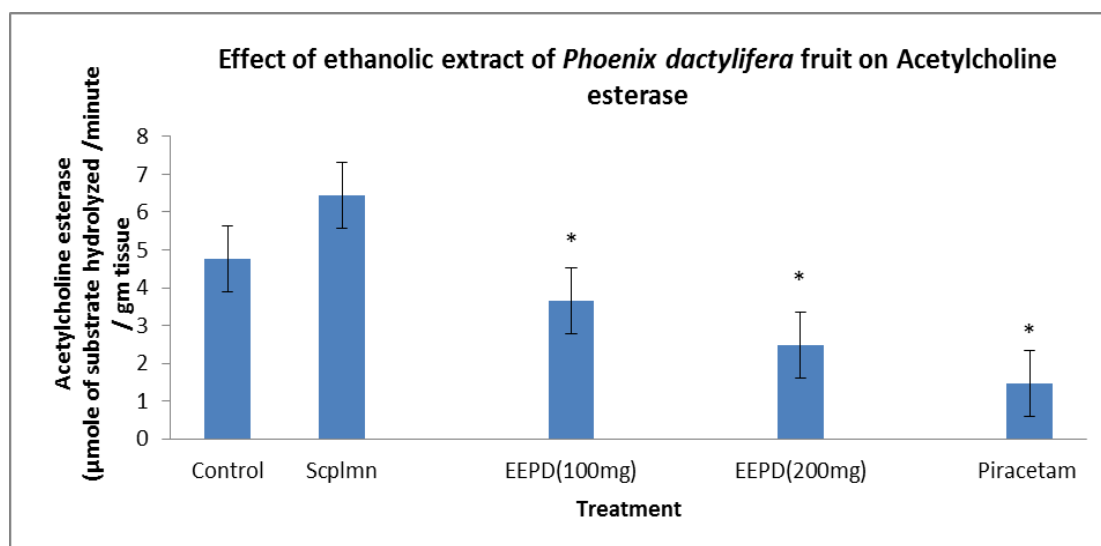
*$P<0.05$v/s scopolamine, **$P<0.01$v/s scopolamine

n=6 Values are expressed as mean ± SEM,.  one way ANOVA followed by Dunnett's test.
*P<0.05v/s scopolamine, **P<0.01v/s scopolamine,

**Fig-1: Effect of ethanolic extract of *Phoenix dactylifera* fruit on Swim latency (SL) in rats using Morris Water maze.**



n=6. Values are expressed as mean ± SEM,   one way ANOVA followed by Dunnett's test.
*P<0.01v/s scopolamine control.

**Fig-2: Effect of ethanolic extract of *Phoenix dactylifera* fruit on Acetylcholine esterase.**

**CONCLUSION**

*Phoenix dactylifera* fruit i.e. date, improves learning and memory, which, may be due to flavonoids present in it.

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