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Anti-alzheimer activity of date fruit

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Phoenix dactylifera (Arecaceae) a cousin of lily is cultivated primarily as a fruit. *P. dactylifera* (PD) is found to possess useful medicinal properties, such as anti-viral, gonadotropic and anti-tubercular. The present study was undertaken to investigate the effect of *P. dactylifera* fruit (Date) on cognitive functions in mice. A total of 180 young mice divided in 36 groups were employed in the present study. PD fruit was administered orally in three doses (5, 10, 20 mg w/w) for seven successive days to different groups of mice. The learning and memory parameters were assessed using elevated plus maze and passive avoidance apparatus. PD fruit showed significant improvement in the memory of animals as reflected by increased step down latency and decreased transfer latency. It also reversed the amnesia caused by scopolamine (0.4 mg/kg, i.p.) and diazepam (1mg/kg, i.p.). Furthermore, PD fruit reduced significantly the central (brain) cholinesterase activity in mice. Since diminished cholinergic transmission appears to be responsible for the development of dementia in Alzheimer patients, PD fruit may prove to be a useful medicine on account of its multifarious beneficial effects. PD fruit appears to be a promising candidate for improving memory and it could be worthwhile to explore the potential of this fruit (Date) clinically in the management of Alzheimer's disease.

Key words : *Phoenix dactylifera*, Date palm

INTRODUCTION

Alzheimer's disease (AD) is characterized by progressive memory loss, cognitive impairment and personality defects accompanied by diffuse structural abnormalities in the brain (Parle *et al.*, 2004 a; Dhingra *et al.*, 2005). This disease affects as on today, more than 30 million patients worldwide. Slow death of brain cells particularly cholinergic neurons, extra neuronal deposits of β-amyloid plaques and intra neuronal fibrillary tangles are the main features of AD. Cholesterol levels appear to be intimately associated with the development of amyloid plaques in humans (Puglielli *et al.*, 2003; Sayre *et al.*, 1997; Refolo *et al.*, 2000; Sparks *et al.*, 2000). Several studies are pouring in showing a strong connection between high cholesterol and high incidence of AD (Puglielli *et al.*, 2003; Sayre *et al.*, 1997; Refolo *et al.*, 2000; Sparks *et al.*, 2000). Therefore, such substances which reduce oxidative stress, protect the brain cells from inflammatory lesions and facilitate cholinergic transmission can be therapeutically used to manage patients of AD.

Phoenix dactylifera L. commonly known as Date palm belongs to the family Arecaceae. It is a cousin of lily and is cultivated primarily as a fruit. Different parts of this plant are used in Indian systems of medicine for the treatment of broad spectrum of ailments including anemia, asthma, cancer, diarrhea, fever, piles, stomachache, toothache, tuberculosis and urogenital ailments. In the light of above, present study was undertaken to investigate the effect of *P. dactylifera* fruit on cognitive functions in mice.

MATERIALS AND METHODS

Plant material :

Fresh fruits of *Phoenix dactylifera* were collected during the month of April 2008 from the local market of Hissar, (Haryana), India. Fruits were dried under shade, sliced into pieces and pulverized using mechanical grinder. The powdered form was stored in an air tight container. This powder was used in further experiments.

Animals :

All the experiments were carried out using male, Swiss mice procured from disease free small animal house of C.C.S. Haryana Agricultural University, Hissar, (Haryana), India. Young (3-4 months old) mice weighing around 20g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12h each) light-dark cycle. Food given to mice consisted of wheat in the form of dalia boiled in water with small amount of salt and refined oil. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. The experimental protocol was approved by the Institutional Animals Ethics Committee and the care of laboratory animals was taken as per the guidelines of CPCSEA,

Ministry of Forests and Environment, Government of India (Registration number - 436). Volume of oral administration and i.p. injection was 0.1ml/10g of mouse

Acute toxicity studies :

Acute oral toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). Swiss mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The PD fruit was administered orally at a dose of 5 mg/animal initially and mortality was observed for 3 days. If the mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses, such as 50, 100 and 2000 mg/animal.

Exteroceptive behavioral models:

Elevated plus maze :

Elevated plus maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and end point for testing memory was followed as per the parameters described by the investigators working in the area of psychopharmacology (Reddy and Kulkarni, 1998; Parle and Dhingra, 2003). The elevated plus maze for mice consisted of two open arms (16×5 cm^2) and two covered arms (16×5×12 cm^3) extended from a central platform (5×5 cm^2), and the maze was elevated to the height of 25cm from the floor (Dhingra *et al*., 2004). On the first day (*i.e*. seventh day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency was defined as the time (in sec) taken by the animal to move from the open arm in to one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The animal was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task (memory) was examined 24 h after the first day trial (*i.e.* eighth day, 24h after last dose). Significant reduction in TL value indicated improvement in memory.

Passive avoidance paradigm :

Passive avoidance behavior based on the negative reinforcement was used to examine the long term memory (Parle *et al*., 2004b). The apparatus consisted of a box (27× 27× 27 cm^3) having three walls of wood and one wall of plexiglass, featuring a grid floor (made up of 3mm stainless steel rods set 8mm apart), with a wooden platform (10 ×7× 1.7 cm^3) in the centre of the grid floor (Parle and Singh, 2004). The box was illuminated with 15W bulb during the experimental period. Electric shock (20V, AC) was delivered to the grid floor. Training (*i.e*. seventh day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the centre of the grid floor (Parle and Singh, 2004). When the mouse stepped down placing all his paws on the grid floor, shocks were delivered for 15 seconds and step down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor. Animals showing SDL between 2-15 seconds during the first test were used for the second session and the retention test. Second session was carried out 90 min. after the first test. During second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 seconds and were subjected for retention test. Retention (memory) was tested after 24h (*i.e*. eighth day, 24h after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor observing the upper cut off time of 300 seconds (Parle *et al*., 2005).

Biochemical estimations :

Collection of blood and brain samples :

The animals were sacrificed by cervical decapitation under light anesthesia on the seventh day 90 min after administration of last dose of PD fruit. Immediately after the decapitation, the trunk blood was collected. Then, whole blood and brain was carefully removed from the skull. The collected blood was centrifuged at 3000 rpm for 15 min so as to separate serum. The serum was used for estimation of cholesterol levels. For preparation of homogenate, the whole brain was weighed and transferred to glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% sodium chloride solution. The homogenate was centrifuged at 3000rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain cholinesterase activity.

Estimation of brain cholinesterase :

Cholinesterase activity was measured by the method of Ellman *et al*. (1961) with a slight modification (Voss and Sachsse, 1970). The cloudy supernatant liquid (0.5 ml) was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB solution (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH

8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tubes, two drops eserine solution was added. 1ml substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both tubes and incubated for 10 minutes at 30^0 C. The solution in the tube containing eserine was used for zeroing the colorimeter. The resulting yellow colour is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per minute of the sample was read at 420 nm (Dhingra *et al.*, 2006).

***Estimation of serum total cholesterol* :**

CHOD – PAP method (Allain *et al.*, 1974) was used for the estimation of serum total cholesterol. In this method, the blank sample, standard sample and test sample were pipetted into the respective reaction vessels using a micro – pipette. For the blank sample, 20 µl distilled water and 1000µl working reagent were mixed. For the standard sample, 20µl standard cholesterol and 1000µl working reagent were mixed. These mixtures were incubated for 10 min at 37^0C. The absorbance was read at 510 and 630 nm (Filters 1 and 2) against the blank sample by using auto analyzer (Erba Mannheim, Chem.–5, Plus V2).

Experimental design :

A total of 180 young mice were employed in the present study. Each group comprised of a minimum of 5 animals.

Group I:

Control group for young mice. Food was administered orally for seven successive days. TL was recorded after 90 min of fruit administration on day 7 and retention was examined after 24 h (*i.e.* on 8th day).

Group II:

Positive control for young mice. Piracetam (400mg/kg i.p.) was injected to young mice for 7 successive days. TL was recorded after 60min of i.p. injection on day 7 and retention was examined after 24h (*i.e.* on 8th day)

Groups III, IV and V:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally along with diet for 7 successive days. TL was recorded after 90 min of fruit administration on day 7 and retention was examined after 24 h (*i.e.* on 8th day).

Group VI:

Scopolamine (0.4mg/kg) was injected intraperitoneally on day 7 in to young mice and TL was recorded 45 min after injection. Retention was examined after 24 h (*i.e.* on 8th day).

Group VII:

Piracetam (400mg/kg i.p.) was injected to young mice for seven successive days. At 60 min after injection of piracetam on 7th day, scopolamine was injected. TL was noted after 45 min of injection of scopolamine and retention was examined after 24 h (*i.e.* on 8th day).

Groups VIII, IX and X:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally by admixing with diet for 7 successive days. Scopolamine (0.4 mg/kg) was injected intraperitoneally to young mice at 90 min after administration of PD fruit on day 7. TL was recorded after 45 min after injection and retention was examined after 24 h (*i.e.* on 8th day).

Group XI:

Diazepam (1mg/kg) was injected intraperitoneally on day 7 in to young mice and TL was recorded 45 min after injection. Retention was examined after 24 h (*i.e.* on 8th day).

Group XII:

Piracetam (400mg/kg i.p.) was injected to young mice for seven successive days. At 60 min after injection of piracetam on 7th day, diazepam (1mg/kg) was injected. TL was noted after 45 min of injection of diazepam and retention was examined after 24 h (*i.e.* on 8th day).

Groups XIII, XIV and XV:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally along with diet for 7 successive days. Diazepam (1mg/kg) was injected intraperitoneally to young mice at 90 min after administration of PD fruit on day 7. TL was recorded after 45 min after injection and retention was examined after 24 h (*i.e.* on 8th day).

Group XVI:

Control group for young mice. Food was administered orally for seven successive days. Shock was delivered for 15s after 90min of food administration on day7, and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XVII:

Positive control for young mice. Piracetam (400mg/

kg i.p.) was injected to young mice for 7 successive days. Shock was delivered for 15s after 60min of i.p. injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Groups XVIII, XIX and XX:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally along with diet for 7 successive days. Shock was delivered for 15s after 90min of PD fruit administration on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XXI:

Scopolamine (0.4mg/kg) was injected intraperitoneally in to young mice and shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XXII:

Piracetam (400mg/kg i.p.) was injected to young mice for seven successive days. At 60 min after injection of piracetam on 7th day, scopolamine (0.4 mg/kg) was injected. Shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Groups XXIII, XXIV and XXV:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally by admixing with diet for 7 successive days. Scopolamine (0.4 mg/kg) was injected intraperitoneally to young mice at 90 min after administration of PD fruit on day 7. Shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XXVI:

Diazepam (1mg/kg) was injected intraperitoneally in to young mice and shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XXVII:

Piracetam (400mg/kg i.p.) was injected to young mice for seven successive days. At 60 min after injection of piracetam on 7th day, diazepam (1mg/kg) was injected. Shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Groups XXVIII, XXIX and XXX:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally by admixing with diet for 7 successive days. Diazepam (1mg/kg) was injected intraperitoneally to young mice at 90 min after administration of PD fruit on day 7. Shock was delivered for 15s after 45min of injection on day 7 and SDL was recorded after 24 h. (*i.e.* on 8th day).

Group XXXI:

Control group for young mice. Food was administered orally for seven successive days. The blood and brain samples were collected for estimation of brain cholinesterase activity and total cholesterol levels after 90 min of food administration on day 7.

Group XXXII:

Donepezil (1mg/kg) an anticholinestarase agent (standard drug) was injected to young mice, 60min before dissecting the animals for estimation of cholinesterase levels.

Group XXXIII:

Simvastatin (5mg/kg) a cholesterol lowering agent (standard drug) was given orally to young mice for seven successive days. The animals were dissected for estimation of cholesterol levels after 90min of drug administration on day 7.

Groups XXXIV, XXXV and XXXVI:

PD fruit (5mg, 10mg and 20mg/ animal, respectively) was administered orally by mixing with diet for 7 successive days. The animals were dissected for estimation of cholesterol levels and cholinesterase activity after 90min of PD fruit administration on 7th day.

Statistical analysis :

All the results were expressed as mean ± standard error (SEM). Data were analyzed using Dunett's test.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Acute toxicity studies :

Phoenix dactylifera (PD) did not produce any mortality even at the highest dose (200mg w/w, p.o) employed. Both the doses of 5mg and 100mg were found to be non-toxic. The doses 5mg, 10mg and 20mg w/w were selected for further psychopharmacological and biochemical studies.

Effect on TL using elevated plus maze :

TL of first day (7th day of drug treatment) reflected learning training behavior of animals whereas TL of next day reflected retention of information or memory.

PD (5mg, 10mg and 20mg) administered orally for seven days showed remarkable reduction (P<0.01) in TL of 7th and 8th day indicating significant improvement in learning and memory (Fig. 1).

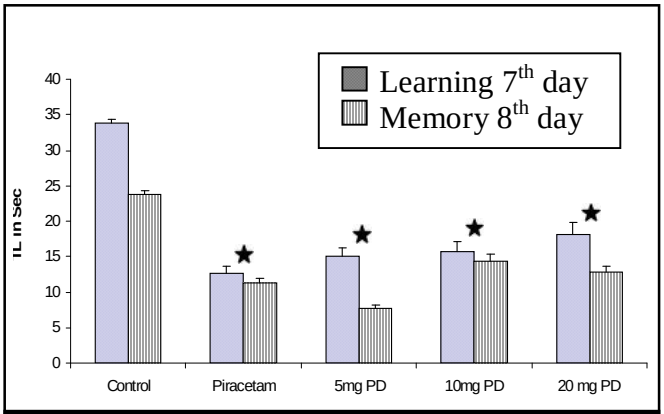

Fig. 1 : Effect of PD fruit on transfer latency (TL) of young mice using elevated plus maze Values are in mean ± SEM (n = 5). ★ denotes p<0.01 as compared to control group of young mice. (One – way ANOVA followed by Dunnett's *t*-test)

Scopolamine hydrobromide (0.4mg/kg, i.p) and Diazepam (1mg/kg, i.p) injected before training significantly increased (P<0.01) TL indicating impairment in memory. All the three doses of PD successfully reversed (P<0.01) memory deficits induced by Scopolamine and Diazepam (Fig. 2 and 3). Piracetam

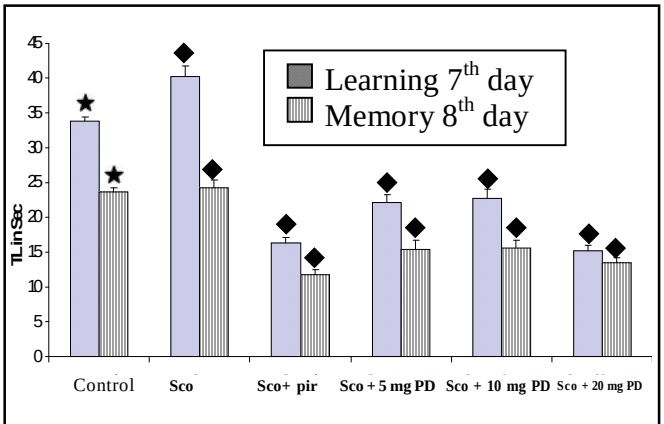

Fig. 2 : Effect of PD fruit on scopolamine induced amnesia of young mice using elevated plus maze. Values are in mean ± SEM (n = 5). ★denotes p<0.01 as compared to control group of young mice. (One – way ANOVA followed by Dunnett's *t*-test.). ◆ denotes p<0.01 as compared to scopolamine alone group

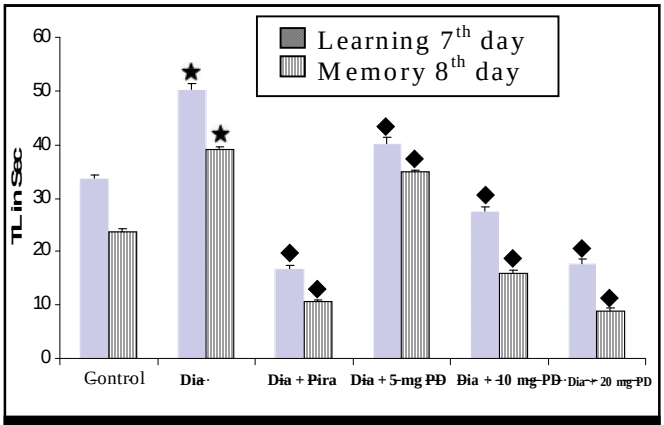

Fig. 3 : Effect of PD fruit on Diazepam induced amnesia of young mice using elevated plus maze. ★denotes p<0.01 as compared to control group of young mice. (One – way ANOVA followed by Dunnett's *t*-test.). ◆denotes p<0.01 as compared to diazepam alone

(used as a positive control) at the dose of 400mg/kg, i.p improved learning and memory of young mice and reversed the amnesia induced by scopolamine and diazepam.

Effect of PD on step down latency (SDL) using passive avoidance paradigm :

SDL on second day/8th day of PD fruit treatment reflected long term memory of animals. PD fruit 5mg, 10mg and 20mg exerted significant effect on SDL (242 ± 13.9, 270 ± 16.7, 254 ± 16.5 sec) of young mice as compared to the control (35 ± 0.7 sec) group. This remarkable enhancement of SDL value indicated improvement in memory (retention capacity) of young mice. Whereas, scopolamine (0.4mg/kg, i.p) and diazepam (1mg/kg, i.p) significantly (P<0.01) decreased SDL (16.8 ± 1.06 and10.6 ± 0.4 sec) as compared to the control group of young mice indicating impairment in memory (amnesia). PD (5mg, 10mg and 20mg) administered for 7 days successfully reversed amnesia induced by both scopolamine (101.6 ± 1.8, 106±4.3, 109 ± 4.8 sec) and diazepam (SDL values were 300 ± 0,127.8 ± 3.6, 163 ± 4.3 sec). Piracetam (400mg/kg, i.p.) for 7 successive days showed improvement in memory of young mice (SDL130.4 ± 2.9 sec) and reversed amnesia produced by scopolamine (SDL 58.2 ± 1.5 sec) and diazepam (SDL 118 ± 1.7 sec) (Fig. 4, 5 and 6)

Effect on brain cholinesterase activity :

PD (10 mg/ animal, p.o) did not produce any effect on cholinesterase activity in young mice. However, 5 mg and 20 mg of PD produced marked reduction (Fig.7) in brain cholinesterase activity as compared to the control

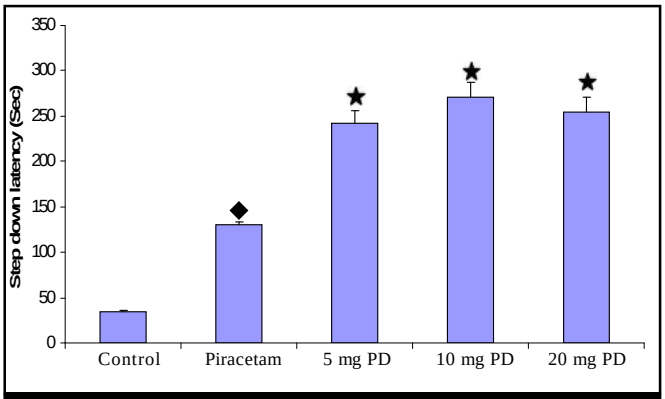

Fig. 4 : Effect of PD fruit on step down latency (SDL) of young mice using passive avoidance paradigm. Piracetam 400 mg/kg, i.p. was used as a standard drug. ★ denotes p<0.01 as compared to control group of young mice. (One – way ANOVA followed by Dunnett's *t*-test.)

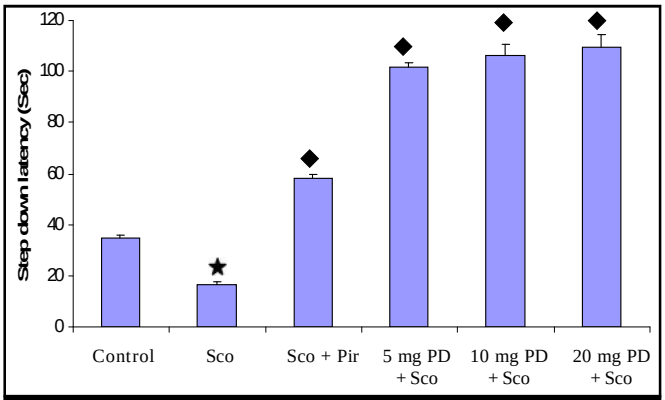

Fig. 5 : Reversal of scopolamine (0.4mg/kg, i.p) induced amnesia by PD fruit (5mg, 10mg, 20mg/mice) in young mice using passive avoidance paradigm. ★ denotes p<0.01 as compared to control group. ◆ denotes p<0.01 as compared to scopolamine alone group (One – way ANOVA followed by Dunnett's *t*-test.)

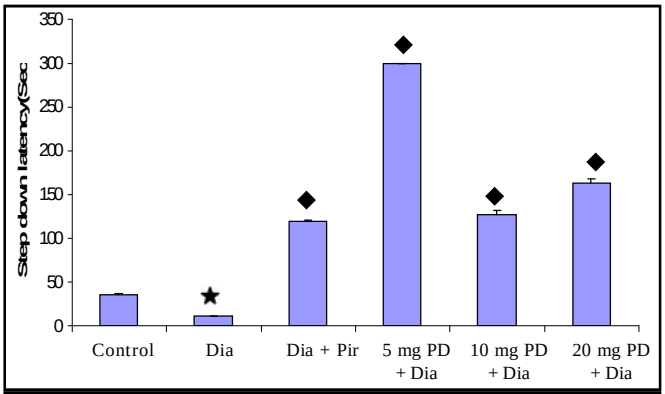

Fig. 6 : Reversal of diazepam (1mg/kg,i.p) induced amnesia by PD fruit 5mg,10mg,20mg/mice) in young mice using passive avoidance paradigm. ★ denotes p<0.01 as compared to control group of young mice. ◆ denotes p<0.01 as compared to diazepam alone group (One – way ANOVA followed by Dunnett's *t*-test.)

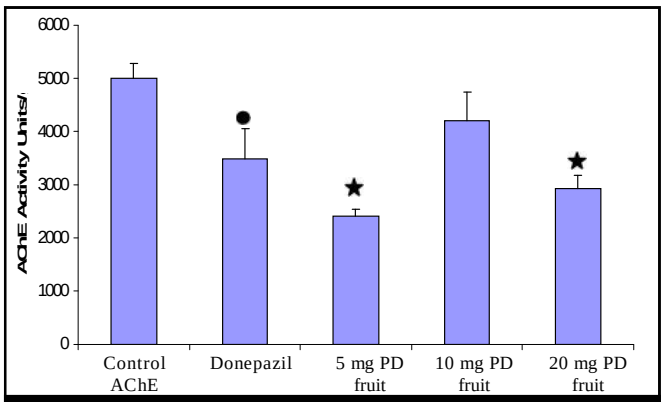

Fig. 7 : Effect of PD fruit on brain cholinesterase activity of young mice. Values are in mean ± SEM (n = 5). ● denotes p<0.05 as compared to control group of young mice; ★ denotes p<0.01 as compared to control group of young mice

group. The percentage reductions in cholinesterase activity were 51.96% (P<0.01) at the dose of 5mg and 41.32% (P<0.01) at the dose of 20 mg PD fruit. Donepezil (1mg/kg, i.p.), used as a standard drug showed reduction of brain cholinesterase activity in young mice as expected.

Effect on total cholesterol level :

5 mg and 20 mg of PD, fruit when administered for seven consecutive days produced significant reduction in total cholesterol levels (p<0.05). This reduction in total cholesterol level was comparable to simvastatin a well known cholesterol lowering agent. (Fig. 8).

Alzheimer's disease (AD) is a genetically heterogeneous neurodegenerative disorder, which is slow in onset but relentless in progress. It is characterized by aphasia, apraxia and agnosia with loss of memory as the

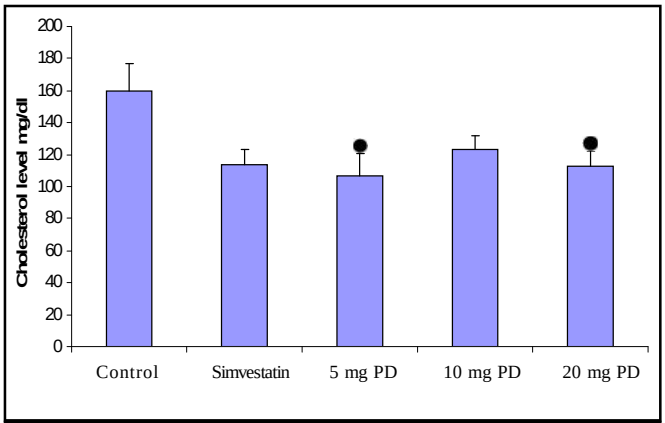

Fig. 8 : Effect of PD fruit on serum cholesterol levels of young mice. Values are in mean ± SEM (n = 5). ● denotes p<0.05 as compared to control group of young mice; (One – way ANOVA followed by Dunnett's *t*-test.)

main symptom (Parle *et al.,* 2004a). This disease affects more than 30 million patients worldwide. As there are no satisfactory medicines available for therapeutic management of Alzheimer's disease, neurobiologists all over the world are looking for new directions and alternative strategies for the management of this disease of senior citizens. In the light of above, we focused our studies on exploring the potential of *Phoenix dactylifera* (PD) fruit in reversing memory deficits.

Oxygen free radicals and other by products of oxidative metabolism have been shown to be neurotoxic. Oxygen free radicals are implicated in the process of age related cognitive decline and may be responsible for the development of AD in elderly persons. Anti-oxidant rich diets improved cerebellar physiology and learning ability of aged rats. In the present study, PD fruit (commonly known as Date) administered orally for 7 days successively improved the memory of mice as reflected by diminished TL and enhanced SDL values as compared to the control group. PD fruit extract has been reported to possess antioxidant property, by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function (Vayalil, 2002). Furthermore, PD fruit contains high concentrations of Vitamins C, BI and B2, which possess potent free radical- scavenging activity. Thus, improvement of memory by PD fruit may be due to its anti-oxidant property

PD fruit successfully reversed scopolamine and diazepam induced amnesia, when administered for seven days. Acetylcholine is considered as the most important neurotransmitter responsible for creating and maintaining long term memory. (Ghelardini *et al.*,1998). Selective loss of cholinergic neurons and increased acetyl cholinesterase (enzyme responsible for degradation of Ach) activity was also reported to be a characteristic feature of senile dementia of the Alzheimer's type (Agnolli *et al.,* 1983). In the present study, PD fruit showed elevation of acetylcholine levels by significant reduction of acetylcholinesterase activity in the brains of treated young mice.

Other histological features of AD include deposition of inter neuronal Aβ plaques and intraneuronal neuro fibrillary tangles (Sayre *et al*., 1997) Abnormal accumulation of cholesterol levels increase Aβ in cellular and most animal models of AD; and the drugs that inhibit cholesterol synthesis lower Aβ deposits in these models (Puglielli *et al*., 2003). Number of studies point out that high level of cholesterol contributes to the pathogenesis of AD (Koudinov and Koudinov 2001; Fernandes *et al*., 1999). Interestingly the animals which were treated with PD fruit showed reduction in total cholesterol levels. Hypocholesterolemic activity exhibited by PD fruit in the present study may be preventing the accumulation of α-amyloid plaques and intraneuronal neuro fibrillary tangles. Therefore, it seems that PD fruit may prove to be a useful anti – Alzheimer agent.

Conclusion :

In the present study, the multifarious beneficial effects of PD fruit *viz.*, hypocholesterolemic activity, anti-oxidant property, anti-cholinesterase activity, and memory improving effect in young mice reveal that this fruit (Date) may be looked upon as a useful brain tonic. In the light of above, it is worthwhile to explore the potential of this fruit in the management of Alzheimer's disease.

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