ABSTRACT:
Background: Alzheimer’s disease (AD) is affecting approximately 46 million people worldwide. Date fruits (Phoenix dactylifera L.) are important fruits which have been cultivated in the Middle East for over 6000 years or more. For the native arabs, dates are an integral part of food. They possess many useful properties such as antioxidant, neuroprotective, anti-stress and anticholesteromic which are essential for an effective Antialzheimer drug. Hence, based on above observations we hypothesize that date fruits can offer potential benefits for the treatment/prevention of AD.

Methods: Scopolamine and streptozotocin were used to induce memory loss in experimental mice. The prepared aqueous extract was analyzed for various chemical constituents by using standardized methods. Its antioxidant activity was established using DPPH free radical method. Scopolamine and streptozotocin were used to induce memory deficit. Different doses of the extract such as 100, 200 and 400 mg/kg were selected for the study. The potential of the extract in exhibiting nootropic activity was explored by using elevated plus maze in mice treated with the extract for a period of 30 days. At the end of treatment period and 1 hour after last dose, the transfer latency was recorded. In order to analyze the retention of this learned-task, the transfer latency was recorded again 24 h later. The effect of the extract in reversing scopolamine and streptozotocin was also carried.

Results: The results of in vitro tests proved that DFE to be an antioxidant whereas in vivo study indicated DFE had nootropic activity in absence of cognitive deficit and was also successful in preventing the chemically induced memory deficits in experimental mice. The mechanism by which DFE showed these effects could be attributed to its antioxidant, neuroprotective properties, its choline content or activation of acetylcholine system in brain.

Conclusions: In the light of above, it may be worthwhile to explore the potential of this fruit in the management of Alzheimer’s disease patients.

Keywords: Date fruits, Amnesia, Nootropic activity, Piracetam, Scopolamine, Alzheimer’s disease.

INTRODUCTION:
Alzheimer’s disease (AD) is one of the leading neurodegenerative diseases affecting elderly persons. As per the World Alzheimer Report 2015, there is an estimated 46 million people worldwide living with dementia and this number will almost double every 20 years. The expected global costs of dementia is around US$ 818 billion[1]. More than 100,000 people die due to this disease every year and prevalence increases with age and may reach nearly 30 to 50% in those with age more than 85 years. With an increasing ageing population, AD has become a significant healthcare issue, which is likely to gain in prevalence in coming years if urgent remedial steps are not taken. But till to date no permanent cure is available. Epidemiological studies on Asian population reveal AD as largely a hidden problem and the number is increasing, especially in rapidly developing and heavily populated regions [2,3].

Fruits and vegetables have been implicated in preventing or reducing the risk of many chronic diseases. The potential health benefits of fruits and vegetables have been partially attributed to their polyphenols contents, in particular flavonoids that have received much attention in the literature over the past decade for its biological effects. Several studies have shown that plant derived flavonoids; phenolic antioxidants attenuate neuronal cell death induced by oxidative stress. Other studies suggest that supplementation with antioxidants may delay the development of AD. Recent studies have reported that palm date fruit (Phoenix dactylifera L.) might be a good source of these active components. Also, it was found that palm date has a potent ability to suppress free radicals. Recalling the various pathological mechanisms underlying AD, such as oxidative stress, cholesterol and Aβ on one hand and composition, antioxidant, anti stress and neuroprotective properties of date fruits on the other hand, it was appealing to explore and investigate the beneficial effects of date fruits in amelioration of induced memory deficits in laboratory mice [5-7]. Dates of date palm tree (Phoenix dactylifera L.) are popular among the population of the Middle Eastern
Countries. The fruit is composed of a fleshy pericarp and seed which constitutes between 10% and 15% of date fruit weight. It is an important plantation crop for many countries from North Africa to the Middle East including many states of the Arabian Gulf Cooperation Countries (GCC). Date fruits are still considered by many people in this part of the world as a staple food. They are rich in simple sugars such as glucose and fructose (65–80%), and a good source of fibers and some essential minerals, but low in fat and protein with no starch [8-9].

Hence, based on above observations we had hypothesized that date fruits can offer potential benefits for the amelioration of AD.

**MATERIALS AND METHODS**

**Drugs and chemicals**

- Piracetam (Nootropil syrup, UCB) was used as a standard reference drug, a well-established nootropic drug.
- Scopolamine hydrobromide [Sigma Aldrich, USA], STZ (Sigma aldrich, USA)
- Glucose, cholesterol, acetylcholinesterase enzyme estimation kits were procured from Life technologies, INVITROGIN, USA.
- Acetylthiocholine iodide, 5, 5’-dithiobis-2-nitrobenzoic acid, DPPH (2, 2, diphenyl-1-picryl hydrazil radical), vitamin C (ascorbic acid) and vitamin E (α-tocopherol) were obtained from Fluka Chemie (Buchs, Switzerland).
- All the other chemical agents used were of analytical grade.

**Animals**

Mice were procured from research centre, college of pharmacy, King Saud University, Riyadh, KSA. Animal studies were performed after obtaining necessary permission from Institutional Animal Ethics Committee.

After procuring, the mice were acclimatized for 7 days and housed in groups of six under standard husbandry conditions with relative humidity of 45-55% and light/dark cycle of 12 hours. All the mice were fed with synthetic standard pellet diet and were supplied ad libitum water under strict hygienic conditions

Mice were fasted for 3 hrs prior to vehicle/standard/extract administration and during the experiments. All experiments were carried out during the light period (8:00 to 16:00 hour).

**Experimental design**

**Extraction of Date fruits for preparation of date fruit extract (DFE)**

Fresh fruits of sukari dates (P. dactylifera L.) were purchased from the local market and samples of these dates were kept frozen for future reference. Date fruits were separated from seeds and washed to remove any adhering dust particles and dried at room temperature. The aqueous extract of the date fruit was prepared by grinding the pulp in a mechanical set with distilled water. It was centrifuged at 4°C for 20 minutes at 4000g, and the supernatant was collected, lyophilized and stored at -20°C until use. Thus prepared date fruit extract (DFE) was used for the study. Every time fresh solution of the fruits was prepared before administration.

**Preliminary phytochemical screening of DFE**

The preliminary phytochemical investigations were carried out with date fruit extract for qualitative identification of phytochemical constituents present in the extract by following standard methods [21].

**Detection of carbohydrates**

To one ml of filtrate, 2 ml of Fehling’s solution A and 2 ml of Fehling’s B solution was added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

**Detection of Alkaloids**

0.5 ml DFE was dissolved in 10 ml of dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids:

To one ml of filtrate, 2 ml of Mayer’s reagent was added in a test tube. Formation of yellow cream precipitate indicates the presence of alkaloids.

**Detection of flavonoids**

To a small quantity of residue, add lead acetate solutin. Yellow colored precipitate is formed.

**Detection of Tannins and Phenolics**

To 2-3 ml of DFE was added with lead acetate solution. White precipitate is formed.

**Detection of Alkaloids**

To one ml of filtrate, 2 ml of Fehling’s B solution was added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of alkaloids.

**Detection of antioxidants activity of DFE**

DPPH is a stable free radical having maximum absorption at 517 nm that accepts an electron or hydrogen atom to become a stable diamagnetic molecule. Ability of the extract to neutralize the DPPH free radicals was studied. In the presence of the extract capable of donating an H atom its free radical nature is lost hence the reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm (22-24).

One ml of 100 1M DPPH in methanol was mixed with equal volume of the different concentrations of the extract in phosphate buffer (pH 7.4), mixed well and kept in dark for 20 min. The absorbance was read. Blank was also carried out to determine the absorbance of DPPH only. From these observations IC50 value for the extract was calculated. Percentage inhibition of the radicals was calculated according to the following equation, % inhibition = [(Ab - As)/Ab] *100, where Ab is control absorbance, As- sample absorbance.

Vitamin C 1mM and 1mM vitamin E were used as positive control.

**Evaluation of DFE for nootropic activity in mice using elevated plus-maze Apparatus**

The Elevated Plus maze (EPM) made up of wood and had...
two open arms and two closed arms, criss crossing each other in the form of a plus. The dimensions of the closed arms were 25 cm × 10 cm × 20 cm and that of open arms were 25 cm × 10 cm with a central platform of 10 X 10 cm area. The entire maze was elevated to a height of 90 cm above the floor level in a quiet sound attenuated dimly lit dark room. All the parameters were recorded using a web cam fixed above EPM to the roof and connected to a computer for recording and offline analysis (17, 18, and 25). Albino mice (18-22 g) of either sex were divided following groups each containing six. They were fasted for 3 hrs prior to the administration but water was supplied *ad libitum*.

**Effect of DFE for nootropic activity in streptozotocin (STZ) induced amnesia in mice**

Albino mice (18-22 g) of either sex were divided into following groups each containing six. They were fasted for 3 hrs prior to the administration but water was supplied *ad libitum*. [28-30]

**Procedure**

1. Blood was removed retroorbitally from all mice after 60 min of respective treatment. Centrifuged to separate serum.
2. 0.4ml serum is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100 μl of DTNB.
3. Solution A: 5.22g of K2HPO4 and 4.68g of NaH2PO4 are dissolved in 150 ml of distilled water.
4. Solution B: 6.2g NaOH is dissolved in 150 ml of distilled water. Solution B is added to solution A to get the desired pH (pH 8.0 or 7.0) and then finally the volume is made up to 300ml with distilled water.
5. DTNB Reagent: 39.6 mg of DTNB with 15 mg NaHCO3 is dissolved in 10 ml of 0.1M phosphate buffer (pH 7.0) and then finally the volume is made up to 300ml with distilled water.

**Solution A:** 5.22 g K2HPO4 and 4.68 g NaH2PO4 are dissolved in 150 ml of distilled water.

**Solution B:** 6.2 g NaOH is dissolved in 150 ml of distilled water. Solution B is added to solution A to get the desired pH (pH 8.0 or 7.0) and then finally the volume is made up to 300 ml with distilled water.

**Procedure**

1. Blood was removed retroorbitally from all mice after 60 min of respective treatment. Centrifuged to separate serum.
2. 0.4 ml serum is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100 μl of DTNB.
3. The contents of the cuvette are mixed thoroughly and
absorbance is measured at 412 nm in a colorimeter. When absorbance reaches a stable value, it is recorded as the basal reading.

5. 20 ml of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 mins at intervals of 2 mins. Change in the absorbance per minute is thus determined.

**Calculations**
The enzyme activity is calculated using the following formula: $R = \frac{5.74 \times 10^{-4} \times A}{CO}$

Where,
- $R$ = Rate in moles of substrate hydrolyzed / minute / gm tissue
- $A$ = Change in absorbance / min
- $CO$ = Original concentration of the tissue (mg / ml).

**STATISTICAL ANALYSIS**
All the results were expressed as mean ± Standard error. The data were analyzed using ANOVA followed by tukey’s multiple comparison post hoc test. $p < 0.05$ were considered as significant. Graphpad prism demo version software was used for this purpose.

**RESULTS**

**Preliminary phytochemical investigation**
The aqueous extract of date fruits (DFE) was subjected for phytochemical screening and found to contain carbohydrates, flavonoids, and traces of phenolic compounds.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Present/Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Tannins and Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: “+” Present, “−” Absent

**Determination of antioxidant activity of DFE**
Antioxidant activity of the extract was determined using DPPH Radical Scavenging. The decrease in DPPH absorption in the presence of varying concentrations of extract was monitored and it was noticed that the extract showed a dose dependent decrease in the absorbance of DPPH radical. From this study, the IC50 value for the extract was found to be 3.5 mg/ml. The results of this study revealed the antioxidant properties of the extract and substantiate the results elsewhere [6]. These results indicate that antioxidant in date fruit is quite potent and implicate the presence of compounds with potent free-radical-scavenging activity.

**Effect of DFE on transfer latency in elevated plus maze**
Effect of DFE on inflexion ratios in mice were recorded with elevated plus maze apparatus. Piracetam 200 mg/kg and DFE with three different dose levels i.e. 100, 200 and 400 mg/kg, treated groups have shown decrease in transfer latencies leading to increase in inflexion ratios when compared to control. But statistically significant effect ($P < 0.05$) was observed with high doses i.e. 200 and 400 mg/kg, of DFE groups only indicating a dose dependent nootropic like effect. Piracetam also has increased the inflexion ratio very significantly ($P < 0.01$, Fig 1).

**Effect of DFE on transfer latency in scopolamine induced amnesic mice**
The effect of the vehicle, scopolaminel, DFE (100, 200 and 400 mg/kg) and piracetam (250 mg/kg) were evaluated at the end of treatment period. The scopolamine (2 mg/kg) control group showed a significant ($P < 0.01$) increase in TL values on the acquisition as well as on the retention days (decrease in inflexion ratio) as compared to control mice, indicating an impairment in learning and memory. In the acquisition as well as retention trial DFE demonstrated dose dependent decrease in the TL (increase in inflexion ratio) as compared to the scopolamine control group which was found to be statistically significant ($P < 0.01$). Piracetam (250 mg/kg p.o.) exhibited marked decrease ($P < 0.01$) in TL in comparison with the scopolamine control group. However, DFE at the dose levels 300 and 600 mg/kg showed a decrease in the TL, which is comparable to that shown by piracetam ($P < 0.01$). Scopolamine treated group had shown decrease in inflexion ratio when compared to control group indicating induction of amnesia. Piracetam and DFE treated groups showed significant dose dependent reversal of scopolamine-induced amnesia (Table 2).
Table 2: Effect of DFE on inflexion ratio in scopolamine induced amnesic mice

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Inflexion ratio (IR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.421 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>Scopolamine 1 mg/kg alone</td>
<td>0.233 ± 0.050</td>
</tr>
<tr>
<td>3</td>
<td>Piracetam 250 mg/kg + Scopolamine 1 mg/kg</td>
<td>0.791 ± 0.061</td>
</tr>
<tr>
<td>4</td>
<td>DFE 100 mg/kg+ scopolamine 1 mg/kg</td>
<td>0.581 ± 0.1464</td>
</tr>
<tr>
<td>5</td>
<td>DFE 200 mg/kg+ scopolamine 1 mg/kg</td>
<td>0.651 ± 0.1017</td>
</tr>
<tr>
<td>6</td>
<td>DFE 400 mg/kg+ Scopolamine 1 mg/kg</td>
<td>0.704 ± 0.08469</td>
</tr>
</tbody>
</table>

Statistical analysis by one-way ANOVA followed by Dunnett’s t’ test. Values are expressed as mean ± S.E.M (n = 06). ns - Statistically non-significant, *p < 0.05, **p < 0.01, ***p < 0.001 when compared to scopolamine alone group. ##p < 0.05, ###p < 0.01 when compared to normal control.

Effect of DFE on transfer latency in STZ induced amnesia model

In this model, STZ had induced amnesia by decreasing transfer latency and increasing IR as compared to the control group. All different dose levels of DFE (100, 200 and 400 mg/kg) treated groups had exhibited statistically significant reduction in transfer latency leading to increase in IR. Thus reversal of STZ induced amnesic effect was observed. Piracetam (250 mg/kg) treated group also exhibited statistically significant (P<0.001) increase in the IR. DFE at 400 mg/kg showed highly significant (P<0.01) activity which was comparable to standard piracetam(Fig. 2). This indicates that DFE (400 mg/kg) had significant memory enhancing activity.

FIG 2: Effect of DFE on Inflexion ratio in STZ induced amnesia model

Values are expressed as mean±SEM. *P<0.05, **P<0.01 and ***P<0.001 statistically significant as compared to STZ control group. ###p < 0.01 when compared to normal control

(Statistical analysis by one-way ANOVA followed by Dunnett’s t’ test. n = 6)

Effect of DFE on acetyl cholinesterase (AChE) inhibitory activity, cholesterol and glucose

Vehicle treated group had shown 5.416x10-7 μmol/min of acetyl cholinesterase (AChE) activity. Prior treatment with piracetam and different doses of DFE 100, 200, 400 mg/kg had showed decreased acetyl cholinesterase activity 4.623 x10-7, 5.623 x10-7, 5.227x10-7 and 5.106x10-7 respectively. A significant effect was produced by piracetam (17.72%) but not with DFE when compared to control group. Neither of the treatment groups, produced any significant on cholesterol or glucose (Table 3).

Table 3: Effect of DFE on AChE activity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>AChE Activity (μmol/min)</th>
<th>Serum cholesterol levels (mg/dl)</th>
<th>Serum glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5.416x10-7 ± 0.00333 x10-7</td>
<td>65.7 ± 7.50</td>
<td>84.5 ± 6.62</td>
</tr>
<tr>
<td>2</td>
<td>Piracetam 250 mg/kg</td>
<td>4.623 x10-7 ± 0.2397x10-7</td>
<td>68.7 ± 4.77</td>
<td>76.7 ± 9.18</td>
</tr>
<tr>
<td>3</td>
<td>DFE 100 mg/kg</td>
<td>5.623 x10-7 ± 0.1992x10-7</td>
<td>59.2 ± 2.11</td>
<td>77.5 ± 5.51</td>
</tr>
<tr>
<td>4</td>
<td>DFE 200 mg/kg</td>
<td>5.227x10-7 ± 0.1992x10-7</td>
<td>64.5 ± 1.48</td>
<td>88.7 ± 4.66</td>
</tr>
<tr>
<td>5</td>
<td>DFE 400 mg/kg</td>
<td>5.106x10-7 ± 0.1992x10-7</td>
<td>66.4 ± 2.45</td>
<td>89.6 ± 5.76ns</td>
</tr>
</tbody>
</table>

n=6 in each group. Data is expressed as mean±SEM. Statistical analysis by one-way ANOVA followed by Dunnett’s t’ test. ns - not significant vs control group.

DISCUSSION

Alzheimer’s disease is a neurogenerative disorder associated with a decline in cognitive abilities. Despite the severity and high prevalence of this disease, the allopathic system of medicine is yet to provide a satisfactory antidote. Hence, the present study focused on exploration of the memory-enhancing activity of the date fruits in a various chemical-induced amnesia models.

In this study the exteroceptive model was used for evaluating the nootropic activity (memory enhancing) of DFE on learning and memory processes, which was indicated by decreased transfer latency and increased inflexion ratio in elevated plus maze (EPM). The interoceptive models used were amnesia induced by scopolamine, streptozotocin, which was indicated by prevention of fall in transfer latency and inflexion ratio in EPM (Table 2, Figures 1, 2 and 3). The present study suggests that DFE possesses memory enhancing activity in view of its decreased transfer latency and increased inflexion ratio in EPM. This suggests that the DFE has pronounced nootropic effect which was comparable to nootropil in the study. DFE also exhibited a facilitatory effect on the retention of memory in scopolamine and streptozotocin induced amnesic mice.

It is a well-established fact that cholinergic neuronal systems play an important role in the cognitive deficits associated with AD, ageing and neurodegenerative diseases. In our study, too scopolamine induced amnesia which is evident from the increased transfer latency in EPM. As stated before, prior treatment with DFE resulted in the reversal of scopolamine effect, indicating an activation of cholinergic systems.
system by DFE. However, in the antiChE studies, DFE per se did not affect AChE activity (Table 3). Hence, the beneficial effect of DFE may only be attributed to its choline content or cholinomimetic potential [27].

STZ has been implicated in production of oxidative stress in brain. This oxidative stress leads to the induction of amyloid β, hyperphosphorylation of Tau protein, apoptosis. Thus mimicking the pathological features of AD [28], DFE successfully prevented the induction of memory impairment by STZ in mice as evident from the restoration of inflexion ratios in EPM (Figure 2). This may be contributed to the ability of DFE in scavenging the oxidative free radical and thereby preventing the induced tissue damage. DFE has shown potential antioxidant activity in DPPH free radical scavenging assay. In addition DFE has phenolic and flavonoids compounds which are proved antioxidants (Table 1). There are also various reports establishing the antioxidant potential of dates. These results suggest that their antioxidant potential helped in preventing STZ induced memory deficit.

Thus from these observations it may be inferred that DFE can offer a potential benefit in ameliorating the Alzheimer's disease by its probable potentiality of activation of cholinergic system and/or free radical scavenging ability which can offer neuroprotection in the prevention or management of this disease. Though this study was not exhaustive but it proves our hypothesis. However further studies are required to explore the possible effect of DFE on AChE in different parts of the brain, amyloid beta plaques, involvement of other neurotransmitters such as glutamate, gamma aminobutyric acid (GABA) and catecholamines.

CONCLUSION

In the present study, we have focused upon exploring the potential of date fruit extract in improving memory in the laboratory mice as well as reversing the chemically induced memory deficits in experimental mice. The results of in vitro tests proved that DFE to be an antioxidant and the results of the in vivo study concluded that DFE has nootropic activity in absence of cognitive deficit and also was successful in preventing the chemically induced memory deficits in experimental mice. The mechanism by which DFE showed these properties can be attributed to its antioxidant, neuroprotective properties, its choline content or activation of acetylcholine system in brain. In the light of above, it may be worthwhile to explore the potential of this fruit in the management of Alzheimer's disease patients.

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