Consumption of fig fruits grown in Oman can improve memory, anxiety, and learning skills in a transgenic mice model of Alzheimer’s disease

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Alzheimer disease (AD) is one of the most common forms of dementia in the elderly. Several reports have suggested neurotoxic effects of amyloid beta protein (Aβ) and role of oxidative stress in AD. Figs are rich in fiber, copper, iron, manganese, magnesium, potassium, calcium, vitamin K, and are a good source of proanthocyanidins and quercetin which demonstrate potent antioxidant properties. We studied the effect of dietary supplementation with 4% figs grown in Oman on the memory, anxiety, and learning skills in APPsw/Tg2576 (Tg mice) mice model for AD. We assessed spatial memory and learning ability, psychomotor coordination, and anxiety-related behavior in Tg and wild-type mice at the age of 4 months and after 15 months using the Morris water maze test, rota-rod test, elevated plus maze test, and open-field test. Tg mice that were fed a control diet without figs showed significant memory deficits, increased anxiety-related behavior, and severe impairment in spatial, position discrimination learning ability, and motor coordination compared to the wild-type control mice on the same diet, and Tg mice fed on 4% fig diet supplementation for 15 months. Our results suggest that dietary supplementation of figs may be useful for the improvement of cognitive and behavioral deficits in AD.

Keywords: Alzheimer’s disease, Figs, Oman, APPsw/Tg2576 mice behavior study, Water maze, Rota-rod test

Introduction

The prevalence of Alzheimer’s disease (AD) is markedly increasing worldwide, and represents one of the biggest challenges for most societies throughout the world.1 AD is characterized by progressive cognitive and functional impairment in areas such as problem solving, orientation/spatial navigation, attention, language, perceptual, learning and memory skills.2,3 The pathology of AD is characterized by the presence of senile plaques mainly composed of fibrillar amyloid-β (Aβ) peptide4 and neurofibrillary tangles composed of paired helical filaments of hyperphosphorylated tau.5,6 Plaques and tangles are present mainly in brain regions involved in learning and memory, such as the cortex and hippocampus. Current preventive and therapeutic approaches for AD are still in contention. Several agents have failed in clinical trials because they only provide symptomatic benefits from cognitive impairment and do not slow down the disease progression. An agent that cannot only improve cognitive functions but also prevent neuronal death in AD brain is urgently required.

An important theory of aging suggests that the generation of free radicals or reactive oxygen species can lead to cell and tissue damage paralleled by alterations
in the function of genetic apparatus, resulting in aging and neurodegeneration in particular. This has prompted renewed interest in the role of dietary antioxidants as a potential treatment strategy for neurodegenerative diseases. Several studies have shown inhibition of Aβ plaque formation \textit{in vitro} and \textit{in vivo} by compounds from natural sources.\textsuperscript{9–11} Positive responses against Aβ toxicity have been reported using the \textit{Ginkgo biloba} extract EGb 761.\textsuperscript{12,13} Many studies have claimed that reversal in age-related memory declines might be accomplished by increasing the intake of diet having high antioxidant activity.\textsuperscript{14–16}

The fig (\textit{Ficus carica} L.) is one of the oldest known fruit tree species. Due to its nutritional value and health benefit, its importance is likely to continue worldwide.\textsuperscript{17} The Mediterranean region and the Middle East countries are an important center of fig growth from time immemorial.\textsuperscript{18} Figs have been proven to have high amounts of minerals and vitamins compared with other common fruits and beverages.\textsuperscript{19} Fruits of \textit{F. carica} can be eaten fresh or dried or used as jam. Figs are sweet, having purgative, aphrodisiac properties, and beneficial for inflammations and paralysis.\textsuperscript{20,21} It contains the highest concentrations of polyphenols,\textsuperscript{22} and includes relatively high amounts of crude fiber.\textsuperscript{23} Figs are an excellent source of phenolic compounds, such as proanthocyanidins.\textsuperscript{22} Actually, red wine and tea, two well-publicized sources of phenolic compounds contain lower amounts of phenols than figs.\textsuperscript{24} The leaves are being used traditionally in the treatment of jaundice.\textsuperscript{25} \textit{F. carica} has been reported to have excellent radical scavenging and antioxidant activities.\textsuperscript{26,27} Figs are free from fat and cholesterol, and contain high amount of amino acids and other nutrients.\textsuperscript{28–31} In traditional medical system, figs are said to be beneficial for various ailments including cardiovascular disease, respiratory problems, ulcers, warts, etc.\textsuperscript{30–35} Figs have been reported to exhibit antioxidant,\textsuperscript{26} antibacterial, antifungal,\textsuperscript{36} antispasmodic, antiplatelet,\textsuperscript{37} antipyretic,\textsuperscript{38} anti-HSV,\textsuperscript{39} hemostatic,\textsuperscript{40} hypoglycemic,\textsuperscript{41} anticancer,\textsuperscript{42,43} hepatoprotective,\textsuperscript{44} antituberculosis,\textsuperscript{45} hypolipidemic activities.\textsuperscript{46} Though all the above benefits of figs have been well documented, the neuroprotective effect of the same have not been well studied. Hence, the current study was designed to find out the effect of long-term dietary supplementation of figs (4%) on AD-like behavior in APPsw/Tg2576 (Tg mice) mice model of AD.

**Materials and methods**

**Collection and preparation**

Fresh fig fruits were collected from Al-Jabal Al-Akhdar farms, Oman. The flesh were isolated manually and were rinsed with water and dried for 18 hours in a drying cabinet at 40°C, and stored at room temperature. The dried fruits were crushed and extracted with acetone (1:1 ratio, weight to volume) under agitation at room temperature. After 48 hours, the extract was then filtered and the filtrate was evaporated to dryness in a drying cabinet at 40°C and stored. After that, the samples were ground into fine powder using a coffee grinder.

Total phenolics of figs were measured by the modified Folin–Ciocalteu assay as previously described by Berker \textit{et al.}\textsuperscript{47} The concentration of phenolics in fig extracts was expressed as gallic acid equivalents (GAE) and the total phenolic content was found to be 419.134 ± 0.462 (mg of GAE/100 g).

**Diet preparation for the animals**

The ground figs were sent to USA to prepare the diet for the mice. The diet was prepared by mixing the figs (4%) with regular diet as per National Institute Health, USA protocol by Research Diet Inc., NJ, USA. Rodent diet with 60 kcal% fat and 4% fig fruits extract were made (Table 1) and used for the study.

**Animals and treatment**

Twelve transgenic female (Tg mice) and six wild control (non-transgenic) mice (Taconic farm, NY, USA) were used. Animals were quarantined for 7 days after shipping and individually housed in plastic cages in an animal room, which was maintained at a temperature of 22 ± 2°C, a relative humidity of 50 ± 10%, and a 12-hour light/dark automatic light cycle. Tap water was offered \textit{ad libitum} throughout the study.

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study. The study was approved by the Animal Care and Use Committee of the Sultan Qaboos University, Oman (SQU/AEC/2010-11/3).

All these animals were free from pathogens and viruses. Experimental period commenced from the age of 4 months. The animals were divided into three groups: Group 1: wild-type (non-transgenic) control of the Tg mice fed with regular diet, Group 2: AD transgenic mice also fed with regular diet, and Group 3: AD transgenic mice fed with 4% fig fruit customized diet. Influence of fig-supplemented diet on cognitive and behavioral function after 15 months, assessed by using the Morris water maze test (for spatial memory and learning ability), T-maze test (for position discrimination learning ability), rota-rod test (for psychomotor coordination), elevated plus maze test and open-field test (for anxiety-related behavior).

Open-field test
Locomotor activity was measured in the open field of a white Plexiglas chamber (45 × 45 × 40 cm³). Illumination in the chamber was adjusted in between 50 and 100 lux approximately. The mice were placed in the same environment as that of the chamber 30 minutes before exposure to the open field. Each mouse was placed individually at the center of the open field, and locomotion was recorded for 60 minutes. Horizontal locomotor activity was judged according to the distance the animal moved. The inner 30% of the open field was defined as the center in the current study.48

Rota-rod test
Motor coordination and motor learning were measured by means of rota-rod tests. The rotarod consisted of a rotating cylinder (diameter 4.5 cm) with a speed controller. The mice were placed on top of the cylinder with the coarse surface for a firm grip. The rotarod was accelerated from 5 to 20 rpm and maintained at the indicated speed for 5 minutes, and then the mice were subjected to tests at successively higher speeds. A cutoff time of 5 minutes and an inter-trial interval of 60 minutes were used. The time spent on the rod without falling down was recorded.49
Morris water maze test

The water maze consisted of a metal pool (170 cm in diameter \times 58 cm tall) filled with tap water (25°C, 40 cm deep) divided into four quadrants. In the center of one quadrant was a removable escape platform below the water level and covered with a non-toxic milk powder. The pool was divided into four quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. For each animal, the location of invisible platform was placed at the center of one quadrant and remained there throughout training. The mice must memorize the platform location in relation to various environmental cues, and there was nothing directly indicative of the location of the escape platform in and outside of the pool. Therefore, the placement of the water tank and platform were the same in all acquisition trials. Each mice was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it found and climbed onto the platform. During the training session, the mice subject was gently placed on the platform by an experienced investigator when it could not reach the platform in 60 seconds. In either case, the subject was left on the platform for 15 seconds and removed from the pool. The time for animals to climb onto the hidden platform was recorded as escape latency or acquisition time. To determine the capability of the animals to retrieve and retain information, the platform was removed 24 hours later and the rat was released into the quadrant diagonally opposite to that which contained the platform. Time spent in the region that previously contained the platform was recorded as retention time. In each trial, the animal was quickly dried with a towel before being returned to the cage. All tests were carried out at the end of the experimental period following 4% fig fruit dietary supplementation.

Elevated plus maze test

The elevated plus maze was made of black Plexiglas. The apparatus consisted of four arms (30 cm \times 7 cm²) elevated 50 cm from the floor and placed at right angles to each other. Two of the arms had 20 cm high walls (enclosed arms), whereas two had no walls (open arms). The illumination at the center was adjusted to 40 lux. For the test, the mice was initially placed at the center of the platform and left to explore the arms for 5 minutes. The number of...

Figure 2  Supplementation with 4% figs ameliorated the decline in spatial memory and learning ability of Tg mice. (A) Escape latency or the time to reach the platform in the Morris water maze test at age of 4 months. (B) Escape latency or the time to reach the platform in the Morris water maze test after treatment of figs diet for 15 months. (C) Retention time in the Morris water maze test at the age of 4 months. (D) Retention time in the Morris water maze test after treatment of figs diet for 15 months. Data are presented as mean ± SD, n = 6/group. *P < 0.05 compared to wild-type mice.
entries into closed arms and the time spent in open arms was recorded. Entry into arm was scored as an event if the animal placed all four paws in the arm.48

Left-right discrimination learning
The T-maze (length of stem 64 cm, length of arms 30 cm, width 12 cm, and height of walls 16 cm) was made of clear Plexiglass and filled with water (23 ± 1°C) at a height of 12 cm. A platform (11 × 11 cm²) was submerged 1 cm below at the end of the target arm. During the first two trials, platforms were placed on both arms to test turning preferences. Afterwards, only the least chosen arm, if any, was reinforced, with approximately the same number of mice being reinforced on either side. Tg and control mice were placed in the stem of the T-maze and swam either to the left or the right until finding the submerged platform up to a maximum of 60 seconds. The animals were gently guided to the platform if they failed to find it. After reaching the platform, the mice remained on it for 20 seconds and then placed back in the maze for up to a maximum of 48 trials, except for a 10-minute rest period after each 10-trial block. A mice was considered to have achieved criterion after five consecutive errorless trials. The reversal learning phase was then conducted 2 days later; with the protocol repeated except that the mice were trained to find the escape platform on the opposite side. Escape latencies and errors were recorded.51

Statistical analysis
Data analysis was done by using the Graph pad Prism software. All values are mean ± SD. One-way analysis of variance and post hoc Tukey’s multiple comparison tests were used to determine statistical significance between treatment groups. Differences between treatment groups were considered significant if $P < 0.05$.

Results
Improvement in the impaired motor coordination in AD Tg mice exposed to a fig-rich diet
In this study, we characterized the behavioral properties of the Tg and wild-type control mice, with and without fig fruit supplementation. In the open-field test, the locomotor activity of Tg mice was significantly impaired compared to the wild-type control
mice after 15 months (Fig. 1A and B). Similarly, the motor coordination of the Tg mice on the rotarod was significantly lower compared to wild-type controls after 15 months (Fig. 1). Supplementation with 4% figs to Tg mice restored their locomotor activity in both the open-field and rota-rod test for 15 months (Fig. 1C and D).

**A 4% fig-rich diet improved spatial memory in AD Tg mice**

The cognitive ability of the Tg mice was assessed by applying the Morris water maze test. Wild-type control mice after 15 months were given the task of learning how to find the hidden platform in the Morris water maze, and their performance was found to improve in an experience-dependent manner. In contrast, the Tg mice after 15 months showed a significantly delayed latency to finding the hidden platform compared with the wild control mice (Fig. 2A and B). Supplementation with figs in the diet of Tg mice for 15 months improved the escape latency in finding the platform in a dose-dependent manner. The escape latency in the 4% figs diet group was significantly different to transgenic mice not fed with figs (Fig. 2C and D). These data indicate that 4% fig dietary supplementation in Tg mice may improve spatial memory.

**Figs supplementation reduces anxiety in AD Tg mice**

Our data also shows that the level of anxiety in Tg mice was significantly reduced in mice receiving 4% fig dietary supplementation. The Tg mice receiving the 4% fig diet, and the non-transgenic controls showed a greater preference for the percentage of time spent in open arm (Fig. 3A and B) and a lower percentage of entries into the closed arms in the elevated plus maze after 15 months compared to Tg mice receiving the control diet (Fig. 3C and D).

**Figs improve position discrimination learning ability in AD Tg mice**

We also showed that figs supplementation improved position discrimination learning ability in Tg mice (Fig. 4). The time to reach platform and number of errors were significantly increased after 15 months in Tg mice receiving control diet compared to wild-type control mice. On the other hand, the time to reach platform and the number of errors were significantly reduced in 4% fig-supplemented Tg mice (Fig. 4A–D).

**Discussion**

Normal aging is a complex process involving progressive and deleterious changes in cellular function accompanied by behavioral deficits, including
impaired cognitive and motor performances.\textsuperscript{52} On the other hand, AD is a major human neurodegenerative disease and the most common cause of dementia among the elderly. Increasing evidence suggests that functional food and diet could reduce the oxidative stress and hence the inflammation associated with AD and aging in general.\textsuperscript{53,54} Several studies have suggested that fruit consumption can play an important role in the prevention of age-related neurodegenerative diseases associated with an imbalance in oxidative stress formation and reduced endogenous total antioxidant capacity.\textsuperscript{55–58} We therefore hypothesized that adequate intake of figs may be beneficial in humans and animal models of disease.

The main goal of this study was to examine the effects of long-term administration of dietary supplementation of figs on spatial memory, learning ability, and anxiety in Tg mice model of AD. It was reported that between 6 and 10 months, the insoluble Aβ\textsubscript{42} and Aβ\textsubscript{40} are easily detected in every animal. But they are histopathologically minimal because only isolated Aβ cores can be identified. By 12 months, diffuse plaques are evident. Furthermore, from 12 to 23 months of age APPS\textsubscript{w} animals have shown well detectable amyloid beta plaques and with strong memory deficit.\textsuperscript{57,58} Based on this, we have aimed for 15 months dietary supplementation of figs fruits in AD Tg mice.

The results clearly demonstrated that dietary supplementation with figs significantly improved learning and memory deficits, motor coordination, and less anxiety. Our study is the first to show the neuroprotective effects of a fig-rich diet on cognitive and behavioral deficits in the Tg mice model for AD using a battery of psychometric measures. Our results also show that dietary supplementation with figs improved reversal learning of left–right discrimination.

We observed a tendency of the figs diet to improve the performance over the acquisition phase of the spatial learning, as was indicated by the residual decrease of latencies in the Tg mice. The fig diet also improved the performance during the probe test. That is, figs-fed animals spent more time in the target quadrant and made more annulus crossings than the animals fed with the control diet. As well, Tg control mice displayed significantly higher percentages of entries into the closed arms of the elevated plus maze (EPM) and spent less time in the open arms of the open-field test than wild control mice. These behaviors were related to an anxiogenic effect induced by deposition of Aβ. Interestingly, dietary supplementation of figs decreased the number of entries into the closed arms and spent more time in the open arms of the EPM, which shows figs might have antianxiolytic effect. We have found the Omani figs extract might be able to inhibit the fibrillization of Aβ under in vitro conditions (unpublished data). There are reports about the memory-enhancing effects of hexane extract of F. carica leaves on interceptive behavioral models.\textsuperscript{59} Phytochemical screening of the extract confirmed the presence of flavonoids (quercetin) which have been claimed to be responsible for CNS active moiety were reported in recent studies,\textsuperscript{60–64} which also support our current findings.

In conclusion, our study showed that the beneficial effects of figs in attenuating the cognitive and behavioral disturbances in AD Tg mice model might be due to the presence of high antioxidant active constituents. Further extensive studies (preclinical and clinical) are warranted to determine the exact mechanism of action offered by figs on AD.

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Disclaimer statements
Contributors
All authors are involved in the MS study designing, analyzing, drafting, and reviewing.

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Conflicts of interest
None.

Ethics approval
The study was approved by the Animal Care and Use Committee of the Sultan Qaboos University, Oman (SQU/AEC/2010-11/3).

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