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Research Article
Soaked Almonds Exhibit Vitamin E-dependent Memory Protective Effect in Rodent Models

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Abstract

Background and Objective: It is believed in South-Asian traditions that a small dose of over-night soaked almonds taken in empty stomach have the potential to improve memory. This study aimed to investigate the comparative efficacy of whole and soaked almonds together with vitamin E estimation, given with or without food, for their memory protective effects in different animal models. **Methodology:** In study 1, different groups of mice (n = 9-10) were fed with three different doses (3, 6 and 12 g kg^{-1}) of almonds (whole, soaked and blanch-control) for 14 days. Memory protection was assessed using Morris water maze (MWM) in scopolamine-induced amnesia. Afterwards, mice were sacrificed and acetylcholinesterase (AChE) inhibition in hippocampus and frontal cortex was estimated. In study 2, High Fat Diet (HFD) was given to rats (n = 8) for 6 weeks for inducing memory impairment, together with different doses (1, 2 and 4 g kg^{-1}) of almonds (whole, soaked and blanch-control). Learning ability was tested through MWM performance in the last week. Besides, HPLC analysis was performed to see the effect of soaking on vitamin-E content of almonds. **Results:** Almond supplementation prevented scopolamine-induced amnesia in mice and improved learning ability in HFD-fed rats, respectively. Soaking led to an increase in vitamin-E content of almonds. Soaked almonds, consumed without food, protected memory and enhanced learning ability at a lower dose than the whole almonds in both models. With a dose-dependent trend, soaked almonds without food were found to be more effective in improving MWM performance and inhibiting AChE in hippocampus and frontal cortex. **Conclusion:** It is concluded that overnight soaking which enriches the vitamin-E content of almonds, effectively ameliorates memory impairments at low doses when consumed in empty stomach.

Key words: Acetylcholinesterase, vitamin-E, Morris water maze, mice, rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Human life expectancy has increased. People now live long enough to experience chronic metabolic and neurological diseases which their aged bodies are not sufficiently capable to deal with. Among the neurological disorders, Alzheimer's Disease (AD), the major cause of dementia in elderly, appears to be a serious threat[1]. The AD is a complex disorder, while, the efficacy of the available treatments is limited[2], with multiple side effects; hence, natural products are warranted that could target multiple pathways and have fewer side effects[3-5].

Edible nuts are bestowed with these properties and they appear to be important natural remedies for improving cognitive performance, particularly in old age[6]. Among edible nuts, folkloric recommendations have placed huge emphasis on almonds (*Prunus dulcis*) for improving memory. Specifically, the traditional recommendation in South Asian culture to enhance memory is to consume a handful of almonds (about 10 g) in empty stomach after overnight soaking and peeling. As far as this mode of consumption (soaking) is concerned, our laboratory has used it on human[7] and animal studies[8] for features related to cardiovascular diseases, not memory. This process of soaking *per se* has been shown to change the chemical composition of certain food items[9]. However, data are missing regarding effects of soaking on almonds and its memory-enhancing potential.

The process of soaking, breaks dormancy and the seed prepares to germinate, which is facilitated by certain chemical changes like catabolic conversion of complex carbohydrates to simpler ones and enrichment of vitamin[9]. Almonds are seeds with high vitamin-E content[10]. Research over the past decade has particularized the memory protective potential of vitamin-E, be it in Alzheimer's disease, or other age-associated memory impairments[11]. The radical scavenging potential of vitamin-E is particularly important in this context, since one of the contributing molecular mechanisms of dementia includes oxidative stress causing neurological dysfunction and degradation. It is hypothesized that soaking increase almond's vitamin-E content, thereby enhancing the memory effects.

When testing potential treatment options in AD, the significance of appropriate animal models to depict important aspects of AD cannot be overstated. For example, in AD, there is cholinergic hypo-function in the brain. Subsequently, scopolamine-induced amnesia mice model is frequently used to depict brain's cholinergic decline that occurs in AD patients[12]. To overcome cholinergic hypo-function, inhibition of the acetylcholinesterase (AChE) enzyme, to reduce acetylcholine's breakdown, is among the predominant mechanisms of most of the AD-therapies approved by FDA[2]. A second animal model that can reflect AD-like learning disability is the High Fat Diet (HFD) rat model[5,13]. Hence, both of these models were used to assess the short term and long-term effects of almonds on learning and memory.

Overall, it is aimed to extensively elaborate on the memory-effects of different modes of almond consumption, using two different animal models: Scopolamine-induced amnesia model that involves 2 weeks of almond consumption and HFD rat model involving 6 weeks of almond consumption.

MATERIALS AND METHODS

Animals and diets: Mice, aged 4-6 months, with C57Bl/6 background and adults of Sprague-Dawley rats (180-200 g) of either gender, housed in plastic cages with sawdust at The Aga Khan University animal house (at 23-25°C) were used. Animals were given tap water *ad libitum*. All the mice received standard diet. All rats received HFD comprising cholesterol-cholate-butter fat as described by Jamshed *et al.*[5], except the normal diet group, which received standard diet. The experiments conducted were in accordance with the guidelines for care and use of laboratory animals provided by The National Research Council[14]. Protocol of this study was approved by the Ethical Committee for Animal Care and Use, The Aga Khan University, Karachi, Pakistan (Approval No. 008-Ani-BBS-13).

Chemicals: Physostigmine, scopolamine, electric eel acetylcholinesterase, acetylthiocholine iodide 5, 5-dithiobis (2-nitrobenzoic) acid and chemicals in HPLC analysis were obtained from Sigma Chemical Company, St. Louis, MO, USA. Drug solutions were made fresh on the day of experiment.

Almond administration: The almonds used were imported from California Sheld (Karmal), purchased from the local market. Knowing how difference in size of animal affects the metabolic rate, the formula recommended by FDA[15] was employed for dose translation of almonds from humans to mice and rats. Smaller animals have higher metabolism and therefore higher unit doses (per kilogram) compared to larger animals and/or human. Correspondingly, human doses of 14, 28 and 56 g day^{-1} (or roughly 14, 28 and 56 almonds day^{-1}) were translated into equivalent mice doses of 3, 6 and 12 g kg^{-1}, respectively, while 10, 20 and 40 g human doses were converted into rat doses of 1, 2 and 4 g kg^{-1}, respectively.

Almonds were given in any of the three forms: Whole, soaked or blanched. Soaking was done by keeping almonds in tap water overnight (10 h) and removing the skin in the morning. To control the effects of skin removal *per se*, blanching was carried out by boiling almonds in tap water (3 min) and removing the skin. In both soaked and blanch-control almond groups, only the kernel was administered after the skin-removal. The protocol for almond administration was similar to that described in the earlier study[8]. Briefly, weighed almonds were given without food in which there was 60 min fasting for mice and 90 min for rats. Once the almond was consumed, food was given back after 30 min. In the "With food" groups, this protocol (of fasting) was not followed and almonds pieces were given without food-withdrawal. The placebo group represented diseased-control and received diet-flour 6 g kg^{-1} instead of almonds.

Study design

Scopolamine-induced amnesia mice model: Two studies were designed in which effects of short-term dosing of almonds were observed. In study 1, multiple doses of whole and soaked almonds were compared (Table 1). In study 2, comparative effects of almond supplementation with food and without food were observed (Table 2).

Protocol of Ahmed and Gilani[16] was followed, in which mice were injected with scopolamine (2 mg kg^{-1} i.p.), except for the normal control group that received saline injection instead of scopolamine. Physostigmine (0.2 mg kg^{-1} i.p.) served as positive control. The flow of study and behavioral tests are described in Table 3. After the behavioral investigations, animals were sacrificed and brains were isolated for AChE inhibitory assay in hippocampus and frontal cortex.

High Fat Diet (HFD)-induced model of memory impairment in rats: Again, two studies (study 3 and 4) were designed, in which effects of long-term dosing of almonds were observed. In study 3, multiple doses of whole and soaked almonds were compared (Table 4). In study 4, comparative effect of almond administration with food and without food was observed (Table 5). Protocol of Jamshed and Gilani[8] was followed. Briefly, normal group was fed with normal diet, whereas other group was fed with a cholesterol-cholate-butter fat-containing

Table 1: Design of study 1, comparing whole, soaked and blanched almonds in scopolamine-induced amnesia mice model

Groups (n = 9-10)	Interventions
1	Saline
2	Placebo
3	Physostigmine
4	Whole almonds (3 g kg^{-1})
5	Whole almonds (6 g kg^{-1})
6	Whole almonds (12 g kg^{-1})
7	Soaked almonds (3 g kg^{-1})
8	Soaked almonds (6 g kg^{-1})
9	Soaked almonds (12 g kg^{-1})
10	Blanched almonds (3 g kg^{-1})
11	Blanched almonds (6 g kg^{-1})
12	Blanched almonds (12 g kg^{-1})

Table 2: Design of study 2, comparing soaked almonds with and without food, in scopolamine-induced amnesia mice model

Groups (n = 9-10)	Interventions
1	Saline
2	Placebo
3	Physostigmine
4	Soaked almonds (3 g kg^{-1})
5	Soaked almonds (6 g kg^{-1})
6	Soaked almonds (12 g kg^{-1})
7	Soaked almonds (3 g kg^{-1}) (with food)
8	Soaked almonds (6 g kg^{-1}) (with food)
9	Soaked almonds (12 g kg^{-1}) (with food)

Table 3: Progression of behavioral studies on scopolamine-induced amnesia mice model

Days	Intervention
1-6	Only almonds
7	Almonds+open field
8	Almonds+acclimatization for Novel Object Recognition (NOR)
9	Almonds+NOR with scopolamine
10-13	Almonds+Morris Water Maze (MWM)
14	Almonds+MWM with scopolamine

Table 4: Design of study 3, comparing whole, soaked and blanched almonds on high fat diet-induced rat model of memory impairment

Groups (n = 8)	Interventions
1	Normal diet
2	High fat diet
3	Whole almonds (1 g kg^{-1})
4	Whole almonds (2 g kg^{-1})
5	Whole almonds (4 g kg^{-1})
6	Soaked almonds (1 g kg^{-1})
7	Soaked almonds (2 g kg^{-1})
8	Soaked almonds (4 g kg^{-1})
9	Blanched almonds (1 g kg^{-1})
10	Blanched almonds (2 g kg^{-1})
11	Blanched almonds (4 g kg^{-1})

Table 5: Design of study 4, comparing soaked almonds with and without food, in high-fat diet-induced rat model of memory impairment

Groups (n = 8)	Interventions
1	Normal diet
2	High fat diet
3	Soaked almonds (1 g kg^{-1})
4	Soaked almonds (2 g kg^{-1})
5	Soaked almonds (4 g kg^{-1})
6	Soaked almonds (1 g kg^{-1}) (with food)
7	Soaked almonds (2 g kg^{-1}) (with food)
8	Soaked almonds (4 g kg^{-1}) (with food)

HFD. Almonds were given for 6 weeks. During the memory analysis through MWM, almond administration along with HFD continued.

Behavioral paradigms

Open Field (OF) test: The OF test was performed both in mice and rats for assessing locomotor performance. The parameters observed were line crossing, rearing and grooming, as described by Okoli *et al.*[17].

Novel Object Recognition (NOR): The NOR was performed only in mice for assessing memory, as described by Dere *et al.*[18]. Briefly, 5 min acclimatization was carried out first, in which mice were individually placed in a wooden box to get familiarized. The following day, a 5 min acquisition trial with two identical objects was conducted after scopolamine administration (2 mg kg^{-1} i.p.). Following 3 h, a 4 min test trial was conducted in which one object was replaced with a novel object. The time of exploration of familiar (Tf) and novel object (Tn) were recorded after analyzing the recordings. The Discrimination Index (DI) or the difference between the time spent exploring novel and familiar objects was calculated as Eq. 1:

$$\frac{Tn\text{-}Tf}{Tn+Tf} \quad (1)$$

Morris Water Maze (MWM): It was performed for assessing memory[19] as described by Ahmed and Gilani[16], in both mice and rats. Briefly, for mice, after the completion of training trials, on 5th day, the trained mice were injected with scopolamine (2 mg kg^{-1} i.p.); then mice were allowed to swim for 90 sec and the time spent to reach the platform was measured. The rats, on the other hand, were allowed to swim for 7 days: Two trials on first day and one trial in the remaining 6 days, as described by Jamshed *et al.*[5].

Acetylcholinesterase (AChE) Activity: After behavioral examinations, mice brains were isolated for *in vivo* AChE inhibition testing[20] in frontal cortex and hippocampus, as described by Ahmed and Gilani[16].

Vitamin-E (α-tocopherol) estimation: The α-tocopherol content in whole, soaked and blanched almonds was estimated by HPLC method[21,22].

Statistical analysis: The software Graph PrismPad 4 was used for statistical analysis and for plotting graphs. One-way ANOVA followed by Dunnett's post-test was applied to calculate the statistical significance. The p-values <0.05 were considered significant and the values were represented as Mean±SEM.

RESULTS

Memory-restoring effect of soaked almonds on scopolamine-induced amnesia in mice: The OF test showed no locomotor dysfunction among mice from any group. The NOR memory test showed similar effects ($p>0.05$) among all the groups treated with almonds in study 1. Figure 1 shows MWM in which, whole almonds showed amnesia-prevention only at the highest dose tested (12 g kg^{-1}; $p<0.01$), while soaked almonds were efficacious even at a lower dose of 6 g kg^{-1} ($p<0.01$).

The AChE inhibition assay, overall, validated the findings of MWM. Both in hippocampus (Fig. 2a) and frontal cortex (Fig. 2b), soaked almonds at 6 and 12 g kg^{-1} markedly inhibited AChE enzyme ($p<0.01$), similar to physostigmine. Whole almonds significantly inhibited AChE ($p<0.01$) only at the highest dose of 12 g kg^{-1} in frontal cortex but not in the hippocampus. Blanched almonds had no effect on behavior and/or enzyme inhibition ($p>0.05$) at both the tested doses.

Memory-restoring effect of soaked almonds on HFD-induced memory impairment in rats: In study 3, using HFD model, the OF test indicated no prior locomotor disability, as indicated by the observation that rats from all the groups performed in a similar manner ($p>0.05$).

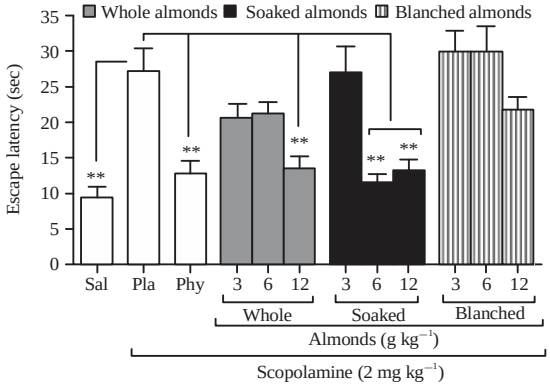

Fig. 1: Effect of soaking on efficacy of almonds in preventing scopolamine-induced amnesia in mice

Sal: Saline, Pla: Placebo, Phy: Physostigmine. Almond doses (3, 6 and 12 g kg^{-1}) given for 2 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 9-10 (**p<0.01)

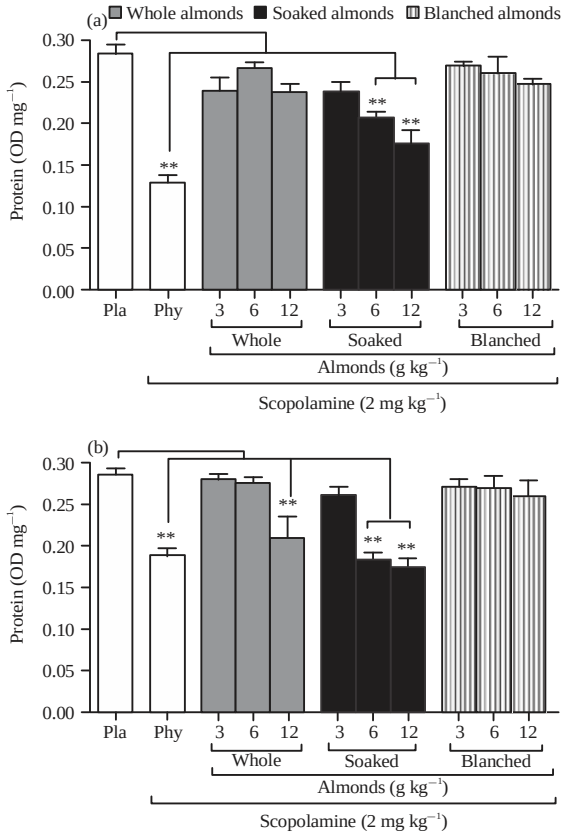

Fig. 2(a-b): Effect of soaking on almonds efficacy in inhibiting acetylcholinesterase enzyme in scopolamine-induced amnesic mice (a) Hippocampus and (b) Frontal cortex

Pla: Placebo, Phy: Physostigmine. Almond doses (3, 6 and 12 g kg^{-1}) given for 2 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 5 (**p<0.01)

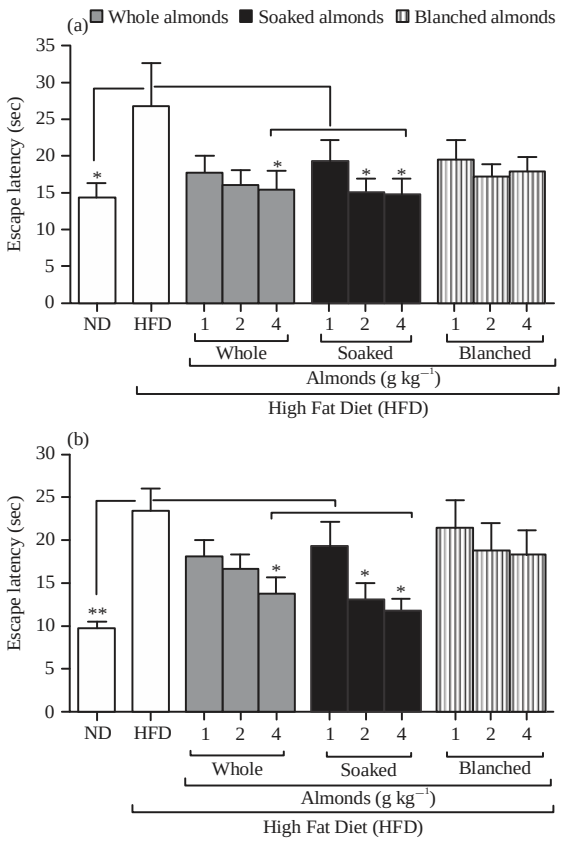

Fig. 3(a-b): Effect of soaking on almonds' efficacy to improve learning ability in High Fat Diet (HFD)-fed rats on Morris water maze (a) Day 6 and (b) Day 7

ND: Normal diet, Almond doses (1, 2 and 4 g kg^{-1}) given for 6 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 8 (*p<0.05 and **p<0.01)

Further, it can be seen that in 6 weeks, HFD led to noticeable learning disability ($p<0.05$) as shown by significantly increased escape latency (Fig. 3). In the first 5 days of 6 week, there was no observable difference among the performances of rats from all groups. At 6 day (Fig. 3a) and 7 day (Fig. 3b), soaked almonds were effective in preventing slowness of learning from HFD at low (2 g kg^{-1}) and larger (4 g kg^{-1}) doses, while whole almonds were effective ($p<0.05$) only at the highest tested dose of 4 g kg^{-1}.

Effect of soaking on vitamin-E content of almonds: The HPLC analysis showed that vitamin-E (α-tocopherol) concentration was 119.4 mg kg^{-1} for whole almonds, 259 mg kg^{-1} for soaked almonds and 189 mg kg^{-1} for blanched almonds. In other words, there was 117% increase in vitamin-E content in soaked almonds and 58% increase in blanched almonds, as compared to the whole almonds.

Effect of food on almonds efficacy in scopolamine-induced amnesia in mice: The OF test showed that all mice in study group 2 were physically fit for further behavioral testing. The NOR test showed no difference in performances of almond treated groups ($p>0.05$).

Figure 4 shows that efficacy of almonds, particularly at low dose (6 g kg^{-1} soaked) is markedly compromised in the presence of food as can be seen from the higher escape latency of groups consuming almonds with food compared to 'Without food'.

The *ex vivo* AChE is inline with these findings as the memory improving effect is compromised by food accompanying almonds. In both, hippocampus (Fig. 5a) and frontal cortex (Fig. 5b) of these mice, profound inhibition of

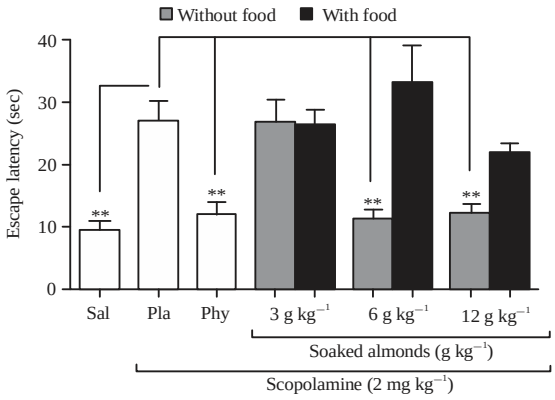

Fig. 4: Effect of food on efficacy of soaked almonds to prevent scopolamine-induced amnesia in mice

Sal: Saline, Pla: Placebo, Phy: Physostigmine. Almond doses (3, 6 and 12 g kg^{-1}) given for 2 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 9-10 (**p<0.01)

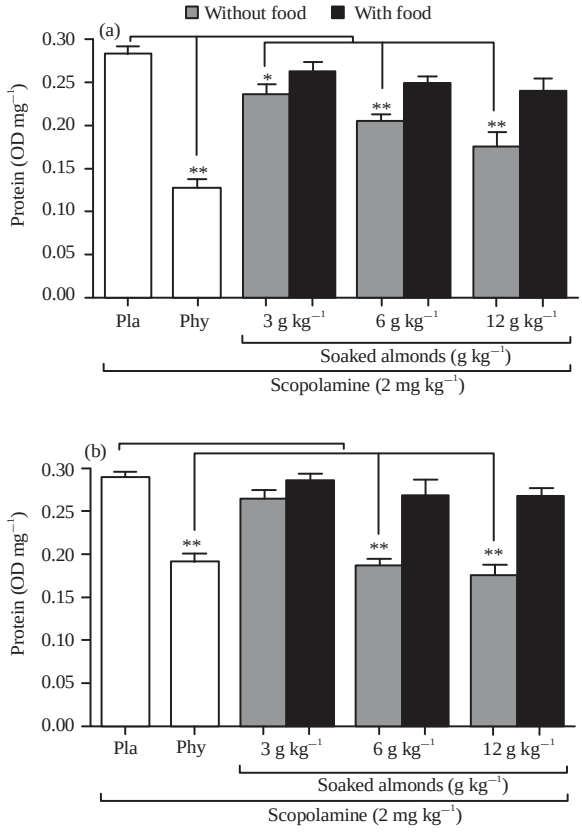

Fig. 5(a-b): Effect of food on efficacy of soaked almonds to inhibit acetylcholinesterase enzyme in scopolamine-induced amnesic mice (a) Hippocampus and (b) Frontal cortex

Pla: Placebo, Phy: Physostigmine. Almond doses (3, 6 and 12 g kg^{-1}) given for 2 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 5 (**p<0.01)

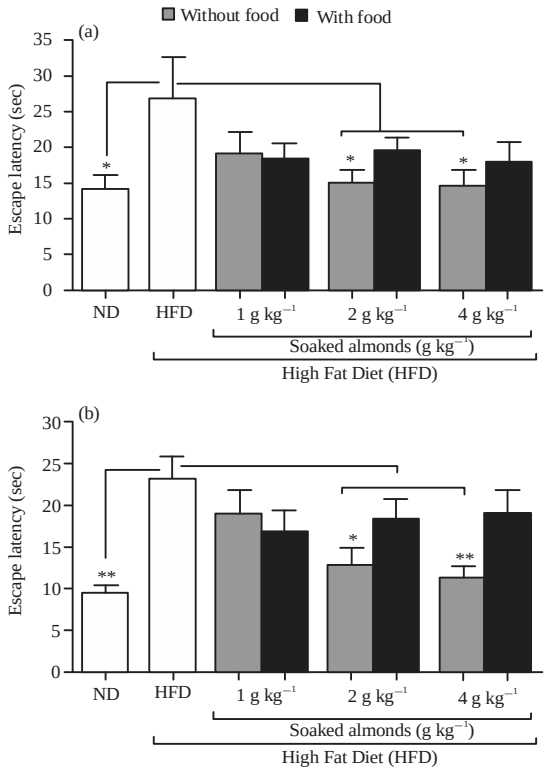

Fig. 6(a-b): Effect of food on efficacy of soaked almonds to improve learning ability in High Fat Diet (HFD)-fed rats on Morris water maze (a) Day 6 and (b) Day 7

ND: Normal diet, Almond doses (1, 2 and 4 g kg^{-1}) given or 6 weeks. One-way ANOVA followed by Dunnett's test was applied. All values are Mean±SEM, n = 8 (*p<0.05 and **p<0.01)

AChE was observed by physostigmine as well as by all the three doses of soaked almonds (3, 6 and 12 g kg^{-1}) only when given without food (p<0.01).

Effect of food on almond's efficacy in HFD-induced memory impairment in rats: The OF test on rats of study group 4, using the HFD model, indicated no locomotor abnormality in any group. In the 6th week of treatment, MWM showed no marked difference in the performances of rats from different groups, in the first 5 days. On day 6 (Fig. 6a) and day 7 (Fig. 6b), HFD induced some slowness in learning ability. The two high doses of soaked almonds (2 and 4 g kg^{-1}), only when given without food, effectively prevented this impairment (p<0.05) on day 6 and 7 (2 g kg^{-1} at p<0.05 4 g kg^{-1} at p<0.01).

DISCUSSION

Almonds alleviate memory loss in two different animal models was observed. Interestingly, overnight soaking

enhanced the vitamin-E content of almonds improving their efficacy compared to whole or blanched almonds. Even a low dose of soaked almonds was effective but only when given without accompanying food. While exploring the probable underlying mechanisms, it was also elaborated inhibition of AChE in hippocampus and frontal cortex by almonds.

This study was distinctive in a number of ways: Two species of experimental animals was used to test hypotheses of the present study, also tested the effect of soaking on almonds' content and showed vitamin-E enrichment to escalate efficacy in terms of neuronal function and that food can hinder the bio-effectiveness of almonds.

To the best of our knowledge, this is first study to explore the memory improving effects of almonds in different animal models. Although Kulkarni *et al.*[23] have reported some preliminary findings of memory improvement by almond administration but there were some limitations of that study that warranted further exploration. For example, the study design was weak in the sense[23] that used Elevated Plus Maze (EPM) for memory evaluation, even though EPM is primarily meant to assess anxiety-like behaviors[24]. Secondly, it was found non-reproducibility of method of almond paste administration mentioned therein. Interestingly, another study[25] described administration of almond suspension to the scopolamine-induced amnesic rats but the fact that such an oral administration would cause distress to the animal was considered.

The study conducted by Kulkarni *et al.*[23] and Batool *et al.*[25] addressed only one mode of almond consumption in one model. Since, there is no single animal model that could truly represent AD because of its complexity[26,27], this study investigated effects of different doses of various modes of almond consumption in two different animal models.

Batool *et al.*[25] observed a reduced cholinergic functioning with scopolamine and noticed that almonds not only reversed that but also attenuated scopolamine-induced amnesia in rats. Results of present study are in line with these findings but it was observed AChE inhibition by whole almonds at the highest dose tested (12 g kg^{-1}) only in the frontal cortex (Fig. 2). Moreover, Kulkarni *et al.*[23] also reported reduction in AChE levels by almonds but they observed AChE levels in brain homogenates, rather than estimation in specific brain components like hippocampus and frontal cortex.

Previously, soaked almonds were tested in a clinical trial on coronary artery disease patients[7] but the study did not explore change in almond constituents due to soaking. This was perhaps because the efficacy of soaked and un-soaked almonds seemed to be similar on cardio-metabolic parameters. It is now known that vitamin-E has almost negligible beneficial effect on the cardiovascular system[28].

Current study addresses the literature gap by measuring one of key components in almonds, vitamin-E[29]. The soaking-induced increase in vitamin E (α-tocopherol) in this study is in line with previous reports where a similar increase in various constituents has been reported for other food items like cereal grains[9]. Soaking leads to seed germination, which is evident by sprouting. This also results in conversion of complex nutrients like carbohydrates to simpler forms and degradation of anti-nutrients like phytates[8]. Since almonds are known to possess the highest amount of vitamin-E among nuts[10], soaking was expected to increase the potential of almonds against memory impairment, based on increased α-tocopherol level observed in this study. Interestingly, vitamin-E has previously shown effectiveness against AD[30], as well as mild cognitive impairment[31], which explains enhanced effect of soaked almonds in this study.

Oxidative stress, resulting from endogenous overproduction of reactive oxygen species (ROS) is injurious to all body cells but particularly to the post-mitotic neuronal and glial cells. With increasing age, the cells' competence to combat oxidative threats is compromised. This signifies the role of natural and dietary antioxidants like vitamin-E, which come to rescue in the fight against progressively detrimental ROS[11].

It was observed that, it was the soaked almonds, not the blanched almonds that led to improvement in memory. This indicated that, it is not just the removal of almond-skin but also the process of removing the skin that has positive effects on the role of almonds in memory improvement. Although blanched almonds also showed some increase in α-tocopherol but this mode of almond consumption did not lead to any improvement in memory, indicating the blanching might bring some chemical changes in the almond that prevent bioavailability of α-tocopherol (vitamin-E). This also indicated that vitamin-E is just one of the components that enhance soaked almonds' memory-improving potential. Future investigations into the effects of soaking and blanching on the bioavailability of vitamin-E and other constituents are warranted. The efficacy of soaked almonds that are noteworthy at lower dose is dependent on its consumption in empty stomach.

The duration of 60 and 90 min for mice and rats, respectively, before administering almonds is likely to ensure the stomach emptying to mimic the consumption style in humans, as rodents, like mice and rats, eat relatively constantly as opposed to human, who eat in intervals. Once the almond was consumed, food was given back after 30 min to ensure maximum absorption of almonds' content in gastrointestinal tract without probable hindrance from any food material. The presence of food compromised the memory enhancing effects

of almonds was observed. This was not unusual as several studies on digestive models have shown that the accompanying food matrix limits the bio-accessibility of almond constituents from GI tract[32]. This indicated that there is greater absorption of the almond constituents when stomach is empty. These results are in line with the previous study in which almonds when given in empty stomach showed enhanced efficacy in cardiovascular parameters[8]. It appears that fasting or empty stomach enhances bioavailability of almond contents, which warrants further investigations.

All animals were equivalent in terms of locomotor abilities, as indicated by the OF tests, highlighting that almond administration does not cause any locomotor disabilities which could influence the results in NOR or MWM. In other words, the results obtained in the memory testing were purely based on memory performance and not on locomotor competencies of animals.

The reason why almonds failed to show effect in NOR is probably because almonds may require a minimum of 14 days of administration to exhibit any memory improvement. Second reason could be the fact that NOR paradigm has less motivation than MWM[18]. In other words, MWM may be more vigorous than NOR in terms of assessing memory. The reversal of amnesia by the positive control, physostigmine can be intriguing as it did not require long-term administration like almonds do, which is not surprising as food or natural products in their crude form usually takes more time in their action compared to single chemical entity. However, further studies are needed to explore the mechanisms involved.

CONCLUSION

This study provided evidence that overnight soaking elevated the vitamin-E content of almonds thereby improving its efficacy in memory impairment in rat and mice model of dementia and memory impairment. Low doses of soaked almonds, when given in empty stomach, showed greater efficacy than other forms and modes of almond administration. This is mediated possibly through AChE inhibitory actions in the brain along with raised vitamin-E, a well-known natural anti-oxidant, although additional mechanisms cannot be ruled out. By extension, it can be hoped that almonds can target dementia of AD through multiple mechanisms, an aspect commonly seen in natural products. This study is likely to offer safer remedy when compared to established chemical drugs, which are extraordinarily expensive and possess multiple side-effects, yet with limited efficacy. Based on the findings in this study, recommendation can be made to use almonds in empty stomach after overnight soaking, thus allowing lower dose resulting in cost-effectiveness, an important component when life-long supplementation is warranted.

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