RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n1.2871
Majalah Kedokteran Bandung. 2023;55(1):1–6

Majalah Kedokteran Bandung (MKB)

Received: July 4, 2022
Accepted: October 25, 2022
Available online: March 31, 2023

Ajwa Date (*Phoenix dactylifera* L.) Extract to Prevent Alzheimer's Disease in Rat Model

Rizka Muthia Nuruddin,[1] Brian Wasita,[2] Adi Magna Patriadi Nuhriawangsa[3]
[1]Postgraduate Program of Nutrition Science, Universitas Sebelas Maret, Surakarta, Indonesia
[2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[3]Animal Husbandry Study Program, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

Abstract

Alzheimer's disease (AD) is the most common disease of aging characterized by increased extracellular deposits of amyloid-β (Aβ) in the brain. Globally, the number of people affected by AD has increased from 35.6 million in 2010 to 46.8 million in 2015. Ajwa dates contain phenolic compounds that can protect against inflammation and oxidative stress in the brain. This study aimed to analyze the effect of the dose and duration of Ajwa date extract administration on IL-6 levels and SOD activity in rats induced with 400 μg/day homocysteine to trigger Alzheimer's Disease. This was a laboratory experimental study with a pretest-posttest group design conducted at the Laboratory of the Center for Food and Nutrition Studies (PSPG), Universitas Gadjah Mada, Yogyakarta, Indonesia from December 2020–January 2021. A total of 48 rats were divided into one control group, one untreated group, and 4 treatment groups that received different doses of Ajwa Date Extract (ADE) for 21 and 28 days in rats. The results showed that the administration of ADE (Ajwa date extract) for 21 and 28 days could reduce IL-6 levels but did not have the same effectiveness as donepezil. The administration of 800 mg/kg BW ADE for 28 days can increase SOD activities with the same effectiveness as donepezil. Ajwa date extract can be proven to have beneficial effects to prevent Alzheimer's disease and can be used to prevent decreased antioxidant and increased inflammation. Thus, further studies to explore the potential clinical use of the extract to manage Alzheimer's Disease may be beneficial.

Keywords: Ajwa date, alzheimer, rats

Introduction

Alzheimer's is a neurodegenerative disease characterized by increasing extracellular deposits of amyloid-β (Aβ) and protein TAU in the brain that causes memory loss.[1,2] The number of people with dementia, part of Alzheimer, worldwide has increased to 11.2 million from 2010 until 2015. This case is also expected to double every 20 years. Besides affecting the human brain's memory, Alzheimer's disease (AD) can also affect cognitive impairment, emotions, decision-making, behavioral problems, and poor taste and smell.[1,3]

The pathogenesis of AD is related to oxidative stress and inflammation, which causes nerve damage and nerve death by apoptosis or necrosis.[4,5] Inflammation causes high production of cytokines such as IL-1, IL-6, and TNF-α, it causes plaque formation and neuronal dysfunction in AD.[6] IL-6 cytokine expression was found in the early stages of amyloid-β (Aβ) plaque formation in the brain.[6] Research in animal models has proven that IL-6 regulates of central nervous system pathways for cognitive function.[7] Research conducted by Al-Yahya et al.[6] and Essa et al.[9] on rats proved that the IL-6 levels of Alzheimer's rats were higher than the control group.

High superoxide dismutase (SOD) activity shows antioxidants in healthy humans. The decreased SOD activity in the elderly is evidence that free radical levels are increasing; this is related to degenerative conditions such as AD.[17] High levels of oxidative stress are also influenced by high plasma malondialdehyde levels and age. The increasing age of a person is followed by increased production of free radicals.[18] Alzheimer's severity can be affected by decreased SOD activity in the body.[10]

Antioxidants are known to reduce the

Corresponding Author:
Rizka Muthia Nuruddin
Postgraduate Program of Nutrition Science, Universitas Sebelas Maret, Surakarta
Email: rizkamuthia@student.uns.ac.id

formation of oxidative stress and neuronal damage in AD.[5,11,12] Phenolic acid, an antioxidant, can protect cells from damage and has a preventive role against oxidative stress.[6,13] Dates have phenolic compounds that can protect against inflammation and oxidative stress in the brain.[6,13,14] The dates' phenolic acids, flavonoids, and antioxidants vary from various varieties.[2] Research on Ajwa dates proves that Ajwa dates are rich in phenolics and flavonoids, which affect cognition. Ajwa dates have the highest total phenolic content of the 12 types of dates studied at 22.11 mg/100 g.[13]

In experimental animal models, date fruits have been demonstrated to exhibit neuroprotective properties.[6,15,16] A study by Subash et al.[2] in Alzheimer's rats with date supplementation showed reduced oxidative stress and increased antioxidant enzymes. Another study by Essa et al.,[6] dates administration in Alzheimer's rats, can reduce inflammatory cytokines A 400 mg/kg dose is an effective dose in preventing memory and cognitive deficits in Alzheimer's rats. This study used data extract with a higher dose and shorter time than other studies. This study aimed to analyze the effect of the dose and duration of Ajwa date extract administration on IL-6 levels and SOD activity in Alzheimer's rats.

Methods

This study was an experimental laboratory method with a pretest-posttest group design. It was conducted at the Laboratory of Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta, from December 2020–January 2021. Sprague Dawley rats were used as experimental animals. The rats' model of Alzheimer's was prepared to refer to the study of Mahaman et al. using Homocysteine (Hcy) induction with modification.[11] Study by Rizma concluded that Hcy injection of 400 g/day for 14 days in Alzheimer's rats model showed pyramidal cell loss and amyloid bodies as a sign of Alzheimer's.[2,3] Determination of sample size using the Frederer formula with simple random sampling. Forty-eight rats, weighing approximately 150–200 grams, were randomly divided into six groups as follows; (1) normal, healthy untreated rats, (2) control, Hcy, (3) Hcy and administrated donepezil 1 mg/kgBW orally, (4) Hcy and ADE 200 mg/kgBW, (5) Hcy and ADE 400 mg/kgBW, (6) Hcy and ADE 800 mg/kgBW. DL-Homocysteine (Hcy) was injected respectively through vena caudalis with a dose of 400 µg/kgBW once a day. All treatments were given to two different groups for 21 days and 28 days. They maintained a 12:12 light: dark cycle and were given access to food and water ad libitum. The standard feed used is Comfeed AD II. The Health Research Ethics Committee of Medical Faculty approved the experimental protocol at Sebelas Maret University, number 172/UN27.06.6.1/KEPK/EC/2020. The rat model measured superoxide dismutase activity and IL-6 levels after 21 days and 28 days through blood sampling. The result was computed and analyzed with SPSS. Fresh Ajwa dates (P. dactylifera L.) were obtained from Al-Madina Al-Munawwarah, KSA. Extraction of P. dactylifera conducted in the Laboratory of Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta. Ajwa dates extract was prepared using the previously described protocol with slight modification.[13,15] The edible part of Ajwa dates was manually separated, then extracted by ethanol 70% (1:2 ratio, weight to volume) on a shaking homogenizer.[13,15] The edible part of Ajwa dates was manually separated, then extracted by ethanol 70% (1:2 ratio, weight to volume) on a shaking homogenizer. [13,15] The resultant extract was filtered using filter paper and evaporated under low pressure at 45°C using a rotary evaporator. Significant differences among the mean of the 21-day and 28-day groups were determined using Independent Sample T-Test. The significant difference between the mean of the extract date treatment groups was determined using the one-way ANOVA with the Post Hoc Tukey test. A level of $p<0.05$ was accepted as statistically significant. The data from the SOD and IL-6 levels were shown in mean ± standard deviation (SD).

Results

The study showed that the levels of IL-6 at 21 days and 28 days did not have a significant difference ($p>0.05$), namely 87.5±2.2 pg/mL at 21 days and 90.8±2.5 pg/mL at 28 days (Table 1). The ADE (Ajwa Date Extract) group with 21 days also did not significantly differ in IL-6 levels within 28 days of treatment. The lowest IL-6 level was in the normal group and the higher IL-6 level was in the control group. The levels of IL-6 in the ADE treatment group showed that the higher the dose, the lower the levels of IL-6 in both 21- and 28-day treatment.

There was a significant difference ($p<0.05$) in the mean levels of IL-6 between the treatment

Table 1 Effect of Ajwa Date Extract on IL-6 Level

Experimental Group	IL-6 (pg/mL)		p[a]
	21-days	28-days	
Normal	61.2±1.2[a]	66.9±3.5[ac]	p>0.05
Control	123.4±1.8[b]	134.5±4.3[b]	
Donepezil	68.3±2.3[c]	68.3±3.4[c]	
ADE 200mg/kgBW	103.3±2.8[d]	107.9±3.2[d]	
ADE 400mg/kgBW	90.6±4.2[e]	89.0±5.0[e]	
ADE 800mg/kgBW	78.0±2.2[f]	78.2±2.5[f]	
p[b]	p<0.05	p<0.05	

[a]: Independent sample t-test; [b]: One way ANOVA test

groups, both 21- and 28-days treatment. The ADE 200, 400, and 800 groups showed lower IL-6 levels than the control group, with a significant difference. It shows that Ajwa date extract can reduce IL-6 levels compared to IL-6 in Alzheimer's rats. ADE groups 200, 400, and 800 showed higher levels of IL-6 than the normal and donepezil group with a significant difference. This indicates that the levels of IL-6 in the Ajwa date extract group were high and could not be equivalent to the levels of IL-6 in normal rats and donepezil rats.

The study showed that SOD activity at 21 days and 28 days had no significant difference (p>0.05), namely 57.8±2.4% at 21 days and 56.3±2.8% at 28 days (Table 2). The group administration of ADE at 21 days also did not significantly differ in SOD activity for 28 days. The highest SOD activity was in the control group, and the lowest was Alzheimer's group. There was a significant difference in SOD activity in each treatment group (p<0.05), both at 21 days and 28 days (Table 2). The ADE group had significant differences between groups, and this shows that the different doses of ADE had different effects on SOD activity, and the higher the ADE dose could increase the SOD activity.

There was a significant difference (p<0.05) in the average SOD activity between groups at 21 days of treatment. The ADE 200 group showed a higher SOD activity than the control group, but this value did not show a significant difference from the ADE 200 SOD activity with the control group did not differ. This shows that the administration of Ajwa date extract at a dose of 200 mg/kgBW for 21 days still could not increase SOD activity compared to Alzheimer's rats. In contrast, the SOD 200 and 400 groups showed higher SOD activity than the control with a significant difference. The ADE 200, 400, and 800 groups showed lower SOD activity than normal and donepezil, with a significant difference.

There was a significant difference (p<0.05) in the average SOD activity between groups at 28 days of treatment. ADE groups 200, 400, and

Table 2 Effect of Ajwa Date Extract on SOD Activity

Experimental Group	SOD (%)		p[a]
	21-days	28-days	
Normal	87.1±3.3[a]	91.2±3.8[a]	p>0.05
Control	26.4±5.3[bd]	15.4±3.1[b]	
Donepezil	78.3±3.7[c]	76.1±3.7[cf]	
ADE 200mg/kgBW	30.8±3.8[d]	29.0±7.5[d]	
ADE 400mg/kgBW	53.3±4.0[e]	54.4±8.4[e]	
ADE 800mg/kgBW	70.5±2.7[f]	71.7±5.3[f]	
p[b]	p < 0.05	p < 0.05	

[a]: Independent sample t-test; [b]: One way ANOVA test

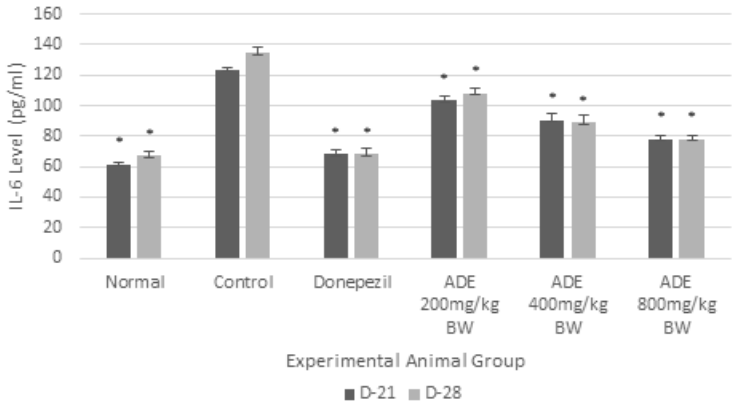

Figure 1 Effect of Ajwa Date Extract on IL-6 Level

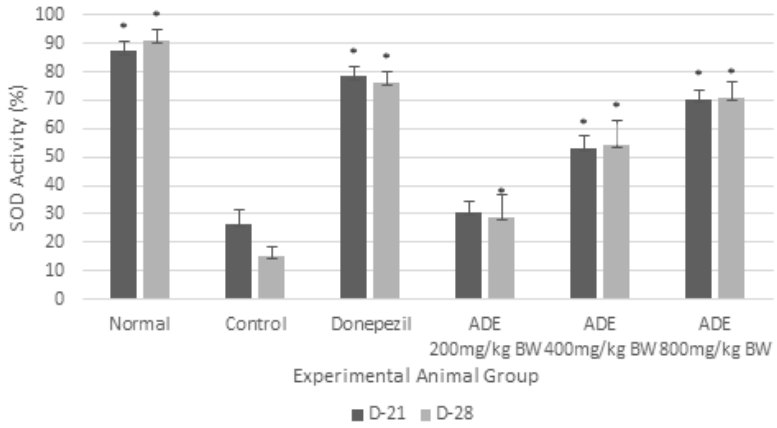

Figure 2 Effect of Ajwa Date Extract on SOD Activity

800 showed higher SOD activity than the control with a significant difference. The ADE 200, 400, and 800 groups showed lower SOD activity than normal with a significant difference. This shows an increase in SOD activity because the administration of Ajwa date extract for 28 days has not had the same effectiveness as healthy mice but has been able to increase beyond the SOD activity of Alzheimer's mice. The ADE 200, 400, and 800 groups showed lower SOD activity than donepezil, but the ADE 800 groups showed insignificant SOD activity. This shows that the administration of Ajwa date extracts at a dose of 800 mg/kgBW has the same effectiveness as the drug donepezil in increasing SOD activity for 28 days. The higher dose of Ajwa date extract, namely the ADE treatment, showed increased SOD activity at 21 and 28 days.

Discussion

Inflammation is one of the main factors contributing to aging and the development of age-related neurodegenerative diseases such as Alzheimer's.[2] According to Essa et al.[6] IL-6 cytokine expression was found in the early stages of amyloid-β (Aβ) plaque formation in the brain.Inflammation is formed due to the excessive production of cytokines such as IL-1, IL-6, and TNF-α, this causes plaque formation and neuronal dysfunction in AD.[6,8] The proinflammatory cytokine IL-6 is associated with important memory and learning cellular mechanisms. Research in animal models further proves that IL-6 regulates central nervous system pathways for cognitive function.[7]

Research showed that the higher the dose of

ADE, the lower levels of IL-6. This is in line with the study of Al-Yahya et al., who observed that giving dates for 21 days at a dose of 250 and 500 mg/kgBW can decrease the expression of pro-inflammatory cytokines (IL-6, IL-10, and tumor necrosis factor).[9] Further research by Essa et al. showed that supplementation with 4% date palm in transgenic mice significantly reduces the inflammatory cytokine IL-6 compared to control mice.[6]

The study's results in the 21-day group showed that the ADE group had significant differences from the donepezil group. This can be concluded that the ADE administration can reduce IL-6 levels to prevent AD but has not had the same effectiveness as donepezil. Dates have solid anti-inflammatory characteristics.[9,19] This mechanism is described by the presence of enzymatic modulation that releases signals from the antioxidant defense system in inflammatory situations.[19] Dates have active compounds that can inhibit the production of inflammatory hormones, such as prostaglandins and thromboxane.[9,19,20] Physiological changes in inflammatory defenses may involve the overproduction of several mediators such as reactive oxygen species (ROS), reactive nitrogen species (RNS), cyclooxygenase (COX), and cytokines, which are associated with the development of various disorders, including AD.

SOD activity is an intracellular antioxidant enzyme that is important in protecting cells against oxidative stress disorders. Antioxidants can reduce the formation of oxidative stress and neuronal damage in AD.[11,12,15] The severity of Alzheimer's can be affected by decreased SOD activity in the body.[10] Research showed that the higher the dose of ADE, the higher the SOD activity. This is in line with the Research of Pujari et al.,[15] which showed that the higher the dose of date extract is given, the more SOD activity significantly increases.It was found that ischemia caused a decrease in SOD activity, but the administration of date extract at doses of 100 and 300 mg/kgBW could significantly increase the SOD value.[15] Further on the research of Subash et al. showed a significant increase in SOD activity in the cortex and hippocampus of rats with 4% date supplementation compared to Alzheimer's transgenic mice.[2]

The results showed that in the 21-day treatment, the ADE group had significant differences from the donepezil group. It showed that the administration of Ajwa date extract could increase SOD activity as an Alzheimer's prevention effort but did not have the same effectiveness as donepezil in increasing SOD activity. In contrast to the 28 days treatment, it was found that the ADE group with a dose of 800 mg/kgBW did not significantly differ from the donepezil group. Administration of ADE at this dose has the same effectiveness as the drug donepezil in increasing SOD activity. This aligns with the Research of Alqarni et al., which showed that administering Ajwa date polyphenol extract could increase SOD activity in rats.[21] Ajwa dates can increase the antioxidant defense system. Furthermore, Research by Al-Yahya et al.[9] and Taleb et al.[19] showed that dates significantly increased the activity of SOD and catalase enzymes. Dates contain various vitamins with solid antioxidant potential (e.g., vitamins E, A, and C) capable of inhibiting other radicals in non-enzymatic reactions. Dates also contain several macro and micronutrients (e.g., zinc, manganese, selenium, and copper) that play a role in many biological functions in the body. [22]

This study has limitations; for instance, the dose and duration of ADE could not be equivalent to normal treatment or healthy rats. So that further Research is recommended to increase the dose and duration of date extract. The relationship of Alzheimer's with other varieties or the level of maturity of dates can also be discussed in further study.

Based on these experimental studies and the active ingredient profiles, it can be concluded that giving Ajwa dates extract to rats is associated with elevated antioxidant activity (SOD) and reduced inflammation levels (IL-6). Administration of ADE 400 and 800 mg/kg BW for 21 days and ADE 200 and 400 mg/kg BW for 28 days can increase SOD activity in Alzheimer's but does not have the same effectiveness as donepezil. Administration of ADE 800 mg/kg BW for 28 days can increase SOD activity and has the same effectiveness as donepezil. It could be worthwhile to explore the clinical potential of Ajwa dates extract at another stage of maturity in the managing of Alzheimer's Disease.

References

1. Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimers Dement. 2016;12:459–509.
2. Subash S, Essa MM, Al-Asmi A, Al-Adawi S, Vaishnav R et al. Effect of dietary supplementation of dates in alzheimer's disease appsw/2576 transgenic mice on oxidative stress and antioxidant status. Nutr

Neurosci. 2015;18(6):281–8.
3. Alzheimer's Disease International. World Alzheimer Report 2015, The global impact of dementia, an analysis of prevalence, incidence, cost and trends. london: alzheimer's disease International (ADI); 2015.
4. Huang WJ, Zhang X, Chen WW. Role of oxidative stress in alzheimer disease. Biomed Rep. 2016;4(5):519–22.
5. Ataie A, Shadifar M, Ataee R. Polyphenolic antioxidants and neuronal regeneration. Basic Clinical Neurosci. 2016;7(2):81–90.
6. Essa MM, Akbar M, Khan MAS. Beneficial effects of date palm fruits on neurodegenerative Diseases. Neural Regen Res.2016;264:1071–2.
7. Gruol, Donna L. IL-6 regulation of synaptic function in the CNS. Neuropharmacology. 2014;96:42–54.
8. Xu PX, Shao-wei W, Xiao-lin Y, Ya-jing S, Teng W, et al. Improves spatial memory in alzheimer's disease transgenic mice by reducing Aβ oligomer level and attenuating oxidative stress and neuroinflammation. Behav Brain Res. 2014;264:173–80.
9. Al-Yahya M, Raish M, AlSaid MS, Ahmad A, Mothana RA, Al-Sohaibani M, et al. Ajwa dates (phoenix dactylifera l.) extract ameliorates isoproterenolinduced cardiomyopathy through downregulation of oxidative, inflammatory and apoptotic molecules in rodent model. Phytomedicine. 2016;23(11):1240–8.
10. Rodrigues GP, Cozzolino SMF, Marreiro DDN, Caldas DRC, da Silva KG, et al. Mineral status and superoxide dismutase enzyme activity in alzheimer's disease. J Trace Elem Med Biol. 2017;44:83–7.
11. Mahaman YAR, Huang F, Wu M, Wang Y, Wei Z, Bao J, et al. Moringa oleifera alleviates homocysteine induced alzheimer's disease-like pathology and cognitive impairments. J Alzheimers Dis. 2018;63(3):1141–59.
12. El-Far AH, Shaheen HM, Abdel-Daim MM. Date palm (Phoenix dactylifera): protection and remedy food. Curr Trends Nutraceuticals. 2016;1(2);1–10.
13. Khan F, Khan TJ, Kalamegam G, Pushparaj PN, Chaudhary A, et al. Anti-cancer effects of ajwa dates (Phoenix dactylifera L.) in diethylnitrosamine induced hepatocellular carcinoma in wistar rats. BMC Complement Altern Med. 2017;17(1):1–10.
14. El Abed H, Mouna C, Zaineb A, Noureddin D, Naziha M, Hafedh M, Bassem K. Antioxidant, anti-inflammatory, and antitumoral effects of aqueous ethanolic extract from phoenix dactylifera l. parthenocarpic dates. Biomed Res Int. 2018;2018:1–7
15. Pujari RR, Neeraj SV, Prasad A, Thakurdesai C. Protective effects of phoenix dactylifera against oxidative stress and neuronal damage induced by global cerebral ischemia in rats. Biomedicine Aging Pathol. 2013;3:75–81.
16. Hussain SM, Awwad IA, Taha M dan Khan O. A laboratory quest on use of date fruit (Phoenix dactylifera L) extract in prevention of chemically induced memory deficit in mice. Asian J Biomed Pharmaceutical Sci. 2015;5(49):5–11.
17. Winarsi H, Alice Y, Agus P. Aging detection in female based on antioxidant status. Majalah Kedokteran Bandung. 2013;45(3):141–6.
18. Winarsi H, Siwi PMW, Agus P. The activity of superoxide dismutase, catalase, and glutathione peroxidase enzymes in metabolic syndrome women. Majalah Kedokteran Bandung. 2012;44(1):7–12.
19. Taleb H, Maddocks SE, Morris RK, Kanekanian AD. Chemical Characterisation and The Anti-Inflammatory, Anti-Angiogenic and Antibacterial Properties of Date Fruit (Phoenix dactylifera L.). J Ethnopharmacol. 2016;94:457–68.
20. Zhang CR, Aldosari SA, Vidyasagar PS, Shukla P, Nair MG. Health-benefits of date fruits produced in Saudi Arabia based on in vitro antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory assays. J Saudi Society Agricultural Sci. 2017;16(3):287–93.
21. Alqarni MM, Magdi AO, Dalia SA, Mustafa AG, Abdulrahman SA, et al. Antioxidant and antihyperlipidemic effects of ajwa date (Phoenix dactylifera L.) extracts in rats fed a cholesterol-rich diet. J Food Biochem. 2018;43(8):1–12.
22. Ali Z, Jianke L, Yuhuan Z, Naureen N, Shoaib Y, Farah J. Dates (Phoenix Dactylifera) and date vinegar: preventive role against various diseases and related in vivo mechanisms. Food Rev Int. 2020;38(4):480–507.
23. Rizma, Amalina. Pengaruh Dosis dan Lama Pemberian Ekstrak Buah Kurma Ajwa (Phoenix Dactylifera L.) terhadap Memori, Kadar MDA dan SOD pada Tikus Model Alzheimer [thesis]. Surakarta: Universitas Sebelas Maret; 2022.