***Original Article***

# Ajwa Date (*Phoenix dactylifera L.*) Ethanolic Extract Ameliorates Oxidative Stress in Rats: Alzheimer's Disease Model

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**Abstract**

***Background:*** Alzheimer's is a degenerative disease that results in decreased memory and cognitive function. The pathogenesis of Alzheimer's disease is related to oxidative stress, which can promote damage to the brain. One of the natural ingredients that can be used as a treatment for Alzheimer's disease is Ajwa dates. Ajwa dates contain lots of phytochemical compounds that act as antioxidants, providing a repair effect against disease. ***Objective:*** This study aimed to analyze the effect of dose and duration of Ajwa date extract administration on MDA (malondialdehyde) and SOD (superoxide dismutase) levels in Alzheimer's model rats. ***Materials and Methods:*** Male Sprague Dawley rats, weighing 150-200 g, were orally given Ajwa date extract at dose of 200, 400 and 800 mg/kg body weight/day after the injection of homocysteine. MDA and SOD levels were measured before treatment, day seven and day 14. Data were then analyzed using the One-way Anova test. ***Results:*** The group of Alzheimer's rats that received Ajwa date extract for seven and 14 days experienced a decrease in MDA levels along with an increase in SOD levels compared to the control group. The group with highest doses had the same effect as the donepezil group on decreasing MDA levels and increasing SOD levels on day 14. ***Conclusion:*** Ajwa date fruit extract administration can reduce MDA levels and increase SOD levels in Alzheimer's model rats. Ajwa dates have the potential for the treatment of Alzheimer's disease.

**Keywords:** MDA, SOD, Alzheimer's disease, Rats.

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## Introduction

Alzheimer's is a degenerative disease of the brain that results in decreased memory and cognitive function. This disease is one of the most common types of dementia cases.[1] Alzheimer's Disease International estimated that 46.8 million people worldwide developed dementia in 2015. It was also estimated that cases would double every 20 years.[2]

Alzheimer's disease is characterized by the accumulation of amyloid-beta plaques and tau protein in the brain.[3] The deposition of these two proteins causes damage to the cortex, and over time, it will develop into deeper parts of the brain.[4] The accumulation of these two proteins is also associated with oxidative stress, which can play an essential role in the pathogenesis of Alzheimer's.[5] Oxidative stress can exacerbate amyloid-beta plaque accumulation and promote phosphorylation of tau protein in Alzheimer's disease.[6] The brain is an organ prone to oxidative stress because it contains many lipid cells susceptible to oxidation, very high oxygen consumption, and lower levels of antioxidant enzymes compared to other tissues.[7] Research conducted by Rani *et al.* on Alzheimer's disease biomarkers proved that the MDA (malondialdehyde) levels of Alzheimer's patients were higher than the control group.[8] The

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research results by Rodrigues *et al.* also showed that the severity of the disease in respondents with Alzheimer's was related to the activity of an antioxidant enzyme, namely SOD (superoxide dismutase).[9]

Many studies on experimental animals have proven that many natural ingredients can be used to treat Alzheimer's disease.[10] One of the natural ingredients that have many health benefits is dates.[11] Dates have been shown in several studies to have antioxidant, anti-inflammatory, anti-cancer, hepatoprotective, and nephroprotective properties.[12–17]

Ajwa dates are one of the best types of dates because they contain many phenolic compounds, flavonoids, melatonin, carotenoids, and vitamins. These compounds act as antioxidants that can provide a repair effect against disease.[18] A study by Ragab *et al.* revealed that the ethanol extract of Ajwa dates could increase levels of antioxidant enzymes along with a decrease in lipid hydroperoxide in rabbits experiencing toxicity.[19] Moreover, research by Al-Yahya *et al.* also confirmed that Ajwa date extract could prevent the depletion of vital antioxidants, such as glutathione peroxidase, superoxide dismutase, and carnitine acyltransferase.[20] Therefore, this study aimed to analyze the effect of dose and duration of Ajwa date extract administration on MDA and SOD levels in Alzheimer's rats.

## Materials and Methods

### Ajwa Date Fruit Extraction

The date fruit used in this study was the Ajwa variety, with *tamr* maturity obtained from Medina. Dates were washed, seeded, and mashed. Dates that had been refined were extracted employing the maceration method. Dates were soaked in 70% ethanol solvent, with a ratio of 1: 2 for 48 hours. The macerate was filtered using filter paper to separate the dregs and filtrate I. The dregs were soaked again with ethanol for 48 hours and filtered to obtain filtrate II. The filtrate I and II were mixed and evaporated with a rotary evaporator at a speed of 100 rpm and a temperature of 60$^0$C. The date fruit extract was then put in a tightly closed container and stored in the refrigerator.

### Homocysteine

Homocysteine (Hcy) was used to induce Alzheimer's disease in rats based on research by Mahaman et al. (2018), with the duration of modified Hcy administration.[21] The rats were given Hcy at a dose of 0.4 mg/kg body weight/day for 21 days to achieve the condition of the Alzheimer's animal model. Homocysteine was injected through the caudal vein.

### Experimental Animals and Treatment Groups

The experimental animals in this study were male rats of Sprague Dawley strain aged 8-12 weeks with a weight of 150-200 grams. The experimental animal control was started through an adaptation process for seven days. Rats were kept in a hygienic cage in a special room, with a temperature of 27-29$^0$C, 12 hours of bright lights, 12 hours of the dark cycle, and 70-90% humidity. Standard feed and drinking water were provided ad libitum.

Rats were divided into six treatment groups, and each group consisted of eight rats. The treatment group comprised (1) normal: healthy untreated rats, (2) control: Hcy + aquades, (3) donepezil: Hcy + donepezil 1 mg/kg body weight/day, (4)−(6) Ajwa date extract (ADE) group: Hcy + date fruit extract 200, 400 and 800 mg/kg body weight/day. Donepezil and date fruit extract were given orally for seven and 14 days.

### Measurement of MDA and SOD Levels

The rats' blood was used to check the MDA and SOD levels. The blood sample was taken three times: before the treatment given, seven days, and 14 days after treatment. Blood was drawn through the orbital sinus and collected in the blood tube. The collected blood was then centrifuged at 1000 rpm for ten minutes at 4$^0$C.

MDA level examination was carried out utilizing the TBARS (Thiobarbituric Acid Reactive Substance) method. The absorbance values were measured at a wavelength of 532 nm. SOD levels were measured using the WST (Water-Soluble Tetrazolium) method at a wavelength of 450 nm.

### Statistical Analysis

The data from the MDA and SOD levels were shown in mean ± standard deviation (SD). Data were analyzed then using the One-Way ANOVA test followed by post hock. Significant differences in MDA and SOD levels between groups were shown if the p-value was $<0.05$.

## Results

### The Effect of Ajwa Date Extract on MDA Levels

All groups of Alzheimer's rats had higher MDA

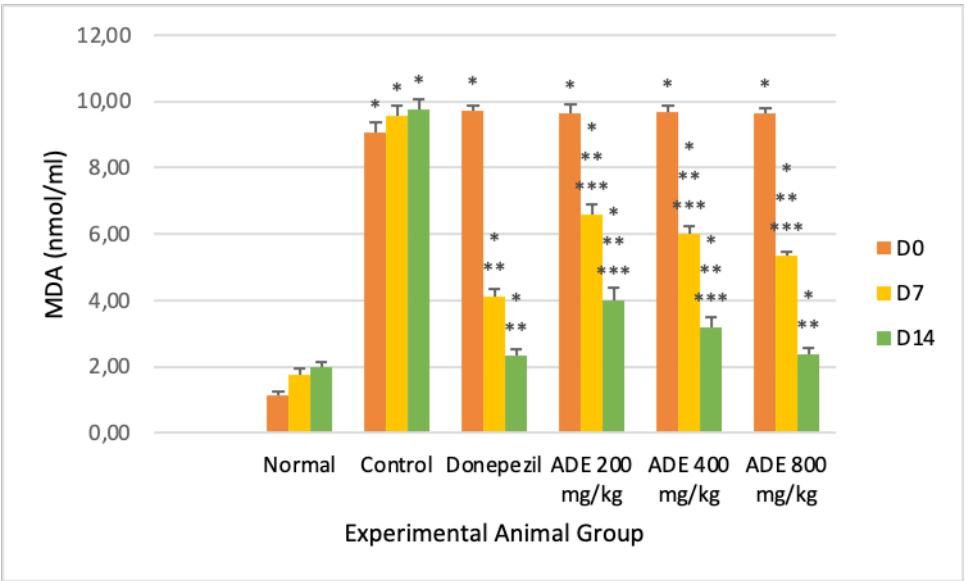


**Figure 1:** Effect of Ajwa date extract (ADE) on MDA level of Alzheimer rats induced by homocysteine. *p<0.01 compared to normal, **p<0.01 compared to control, ***p<0.01 compared to donepezil.

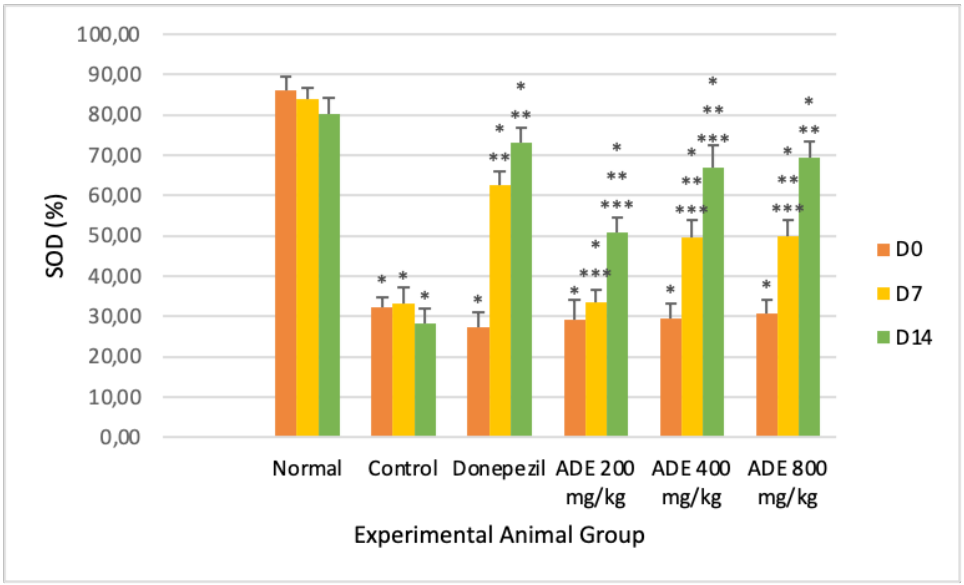


**Figure 2:** Effect of Ajwa date extract (ADE) on SOD level of Alzheimer rats induced by homocysteine. *p<0.01 compared to normal, **p<0.01 compared to control, ***p<0.01 compared to donepezil.

levels than the normal group on day 0. MDA levels in the control group experienced an increase in MDA levels on days 7 and 14. Alzheimer's rats given donepezil and Ajwa date fruit extract for 7 and 14 days experienced a significant decrease in MDA levels compared to the control group. The decrease in MDA levels in the group of rats that received the greatest dose of Ajwa date extract (ADE 800 mg/kg) was the same as the group of rats given donepezil on day 14 (Figure 1).

**The Effects of Ajwa Date Extract on SOD Levels**

The normal group had the highest SOD levels than the Alzheimer's rats group before being given the intervention. SOD levels in the control group increased on day seven but decreased on day 14. The donepezil and date fruit extract administration to the Alzheimer's animal model

group for 7 and 14 days showed a significant increase in SOD levels. On day 14, SOD levels in the group of Alzheimer's rats given the highest dose of Ajwa date extract (ADE 800 mg/kg) were not significantly different from the group of rats that received donepezil (Figure 2).

**Discussion**

This study analyzed the effect of Ajwa date fruit extract on MDA and SOD levels in Alzheimer's rats. The results of this study indicated an increase in MDA levels and decreased SOD levels in rats after being injected with homocysteine (Hcy). Our results are consistent with Kamat *et al.*'s research, which proved that intracerebral injection of Hcy significantly increased levels of malondialdehyde, nitrite, acetylcholinesterase activity, TNF-α, IL-1□, etc., and decreased glutathione levels compared to the control group.[22] This condition is caused by high homocysteine levels (hyperhomocysteinemia), triggering oxidative stress in brain tissue. Oxidative stress further activates amyloid-beta cleavage and hyperphosphorylation of tau to form plaque, which will develop into Alzheimer's.[23] Oxidative damage in Alzheimer's disease can be characterized by increased levels of lipid peroxidation (MDA) and oxidation of protein (carbonyl), accompanied by decreased levels of antioxidants (vitamins C and E) or antioxidant enzymes (SOD, catalase).[7]

The data obtained in this study indicated that giving Ajwa date fruit extract at a dose of 200, 400, and 800 mg/kg body weight/day for seven and 14 days could reduce MDA levels and increase SOD levels in rats. Besides, giving Ajwa date extract at 800 mg/kg body weight/day had the same effect as donepezil on reducing MDA levels and increasing SOD levels on day 14. This result aligns with Subash et al.'s (2015) research, showing that supplementation of dates in feed could attenuate oxidative damage in Alzheimer's rats, characterized by a decrease in MDA levels and protein carbonyl and restoration of antioxidant enzymes levels, such as SOD, catalase, GSH, and GPx.[24] It is because Ajwa dates contain many phytochemical compounds, such as phenolics and flavonoids, which are beneficial for health.[18] These compounds have antioxidant activity that can inhibit protein oxidation and clean up free radicals so that they can repair damaged cells.[25] Moreover, dates also contain the mineral selenium, which plays a vital role in activating many enzymes related to the detoxification of reactive oxygen species (ROS).[26]

Alzheimer's disease is a neurodegenerative disease characterized by memory loss, cognitive decline, and behavioral changes. This disease is often associated with the accumulation of amyloid-beta (A□) plaques and the aggregation of protein tau hyperphosphorylation (neurofibrillary tangles) in the brain.[27] This condition is initiated by oxidative stress, which occurs due to increased free radicals and a decrease in antioxidant defenses.[5] Oxidative stress causes inflammation of nerve cells by activating microglia and astrocytes, which results in increased production of pro-inflammatory mediators. Furthermore, the activation of glial cells will release toxic radicals that can worsen nerve damage.[28]

On the other hand, dates are a source of natural antioxidants that can be used as an alternative treatment for ailments associated with oxidative stress and infections.[29] Dates have an intense antioxidant activity because they are high in phenolics and flavonoids.[30,31] It has been evidenced by Kchaou *et al.*'s research, which states that antioxidant activity was positively correlated with total phenolic and flavonoid content.[32] The antioxidant activity and concentration of antioxidant compounds in dates can also be influenced by the stage of fruit maturity.[33] The highest total phenolic content, flavonoids, and antioxidant activity are the *besser* stage, then *rutab*, and the lowest is *tamr*.[34] It may be related to the administration of Ajwa date fruit extract for seven days, not comparable to the drug donepezil in decreasing MDA levels and increasing SOD levels. This result is supported by Awad *et al*.'s research that the antioxidant capacity and concentration of antioxidant compounds (phenols, tannins, and vitamin C) decreased along with the increasing level of fruit maturity. Besides, the activity of antioxidant enzymes, peroxidase, catalase, and polyphenol oxidase increased with an increasing level of maturity but decreased at the final stage of maturity.[35] Still, dates can contribute to improving health because they contain high amounts of polyphenols, especially in the early stages of fruit maturity, which is ready for consumption, namely *khalal*.[36]

## Conclusion

Based on our research in rat as an animal model of Alzheimer's disease, giving Ajwa date extract at doses of 200, 400, and 800 mg/kg body weight/ day for seven and 14 days could reduce MDA levels and increase SOD levels. Ajwa dates have the potential for the treatment of Alzheimer's disease. However, further research is needed regarding the effective dosage and duration of Ajwa date fruit extract administration at an earlier stage of maturity so that it is expected to provide better results.

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**Conflict of Interest:** Authors state no conflict of interest.

**Ethical Approval:** All research stages have referred to animal ethics in managing experimental animals. The Health Research Ethics Commission, Faculty of Medicine, Universitas Sebelas Maret declared this research's ethical clearance with Number 078 /UN27.06.6.1/KEPK/EC/2020.

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**Authors' contribution:** Concepts and material gathering of this study: Amalina Rizma, Brian Wasita; Study design: Amalina Rizma, Brian Wasita, Ari Probandari; Performed the experiment: Amalina Rizma; Data analysis and interpretation: Amalina Rizma, Brian Wasita, Ari Probandari; Written and submitted the manuscript: Amalina Rizma.

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