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Research Article

Plasma Malondialdehyde and Vitamin E Levels after Date Palm Seeds (*Phoenix dactylifera*) Steeping Administration

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Abstract

Background and Objective: Free radicals in the body increased along with aging. Free radical oxidation in fat tissue will produce malondialdehyde (MDA). Amount of antioxidant vitamins was required to reduce the oxidation rate. The objective of study was to determine the effect of date palm seed steeping to free radical products (MDA) and antioxidant vitamins (vitamin E) in alloxan induced rat model. **Materials and Method:** A quasi-experimental research with pre- and post-test design was used. Thirty rats were randomly distributed into 6 groups. From 1st-4th groups were given with 0.25, 0.5, 0.75 and 1 g kg⁻¹ BW of date seed steeping. The 5th group was a positive control (vitamin C 1 g kg⁻¹ BW) and the 6th group was a negative control. Before the date palm seed steeping was given, rats induced by alloxan. Blood was taken before and after treatment for MDA and vitamin E measurement. The data were analyzed by one-way ANOVA and continued by LSD *post hoc* test. **Results:** The study showed that date palm seed steeping can decrease MDA levels and increase vitamin E levels in all treatment groups significantly ($p < 0.05$), but did not occur in negative control group ($p > 0.05$). **Conclusion:** The results showed that date palms seed could reduce the levels of MDA and increase the vitamin E levels.

Key words: Malondialdehyde, palm seed steeping, free radicals, antioxidant, alloxan

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

INTRODUCTION

The high free radicals in the body could increase the degenerative diseases risk. Free radicals can be derived from food, exposure to pollution and microorganism. These may oxidize normal cells, causing a chain reaction. Free radical species oxidizes the surrounding molecules, resulting in disturb to lipids, cell organelles and proteins. Injury to these cells will have an impact on the occurrence of cell damage, cell death and apoptosis¹.

Currently, the strategy to decrease free radicals is done by consuming functional food or drink that contains antioxidants such as vitamin E, vitamin C, beta carotene and another source antioxidant as date palm seed. Date palm seed contains many antioxidants². However, it is not used much. Phenolic content in date palm seed called gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic, caffeic acid, p-coumaric acid, m-coumaric and o-coumaric³. Palm seeds have been proven to rise sperm count by decreasing lipid oxidation products (MDA) in the sperm cells of mice induced monosodium glutamate (MSG)⁴. Furthermore, palm seed steeping could increase sperm count by preventing cell membrane damage. Furthermore, it has been shown that it could rise SOD and GPX enzyme activity⁵. The current studies commonly used methanol extraction to antioxidant test capacity⁶⁻⁸. However, people use steeping for herbal consumption such functional food. Steeping process on date palm seed can move of active compound and soluble in the water. Phenolic compounds in the date palm seeds is expected to reduce levels of free radicals. Therefore, this study was investigated the effect of date palm seed steeping against free radical products (MDA) and antioxidant vitamins (vitamin E) in the alloxan induced rat model.

MATERIALS AND METHODS

Setting: This study was conducted in April, 2015-November, 2016.

Study design: An experimental study, pre- and post-test with control group design was used. Sample of the study were male rats age 2-3 months (wistar albino strain) with a body weight of 150-200 g, then randomly assigned into six groups. From 1st-4th groups were given with 0.25, 0.5, 0.75 and 1 g kg⁻¹ BW of date seed steeping. The 5th group was a positive control (vitamin C 1 g kg⁻¹ BW) and the 6th group was a negative control. After acclimatization for a week, the rats

were induced by alloxan to increase levels of free radicals. Principles of laboratory animal care were followed, as well as specific animal laws were applied. Blood samples were taken for examination of MDA and vitamin E before and after administration of date palm seed steeping for 7 days.

Preparation of the date palm seed steeping: The matured seeds of *Phoenix dactylifera* were purchased from local market. Date palm seed of Deglet Nour was selected and washed with tap water, left to dry under the sun for one day. The dried date palm seed was roasted and ground with blender. Filtered the flour to obtain a fine powder. A fine date seed powder was added with hot water and mix to make steeping. The date palm seed steeping was given a daily ingestion of dose as it groups.

Laboratory step: Five milliliters of blood from orbitals vein were drawn and stored in Ethylenediaminetetra acetic acid (EDTA) vacutainer tubes (BD vacutainer produced by Becton Dickinson Company, New Jersey, USA) to be used in the laboratory experiments. Examination of MDA level and Vitamin E was measured according to previous study^{2,4}. The MDA and vitamin E levels were expressed in $\mu\text{mol L}^{-1}$ and Ug mL^{-1} . This study followed the principles of laboratory established by Ethic Committee of Medical School, University of Jenderal Soedirman, Purwokerto, Indonesia.

Statistical analysis: Data was presented in a descriptive analysis, including mean and standard deviation. Comparison between treatment and control groups was performed by using one-way analysis of variance (ANOVA) and continued with *post hoc* test ($p < 0.05$).

RESULTS AND DISCUSSION

Malondialdehyde level: This was the first study to explore antioxidant and free radical product after date seed treatment with boiled water. The recent studies focused on date seed extraction while commonly human consumed by steeping like coffee or tea. The mean MDA levels before treatment were highest in 5th group ($1.78 \mu\text{mol L}^{-1}$) and the lowest in the 1st group ($1.57 \mu\text{mol L}^{-1}$). There were no significant difference in MDA levels among all groups ($p > 0.05$). The MDA levels before treatment showed a mean almost the same in the all groups (Fig. 1a). The initial condition before the treatment could be concluded homogeneous, so that research resulted can be an indicator that obtained after treatment only.

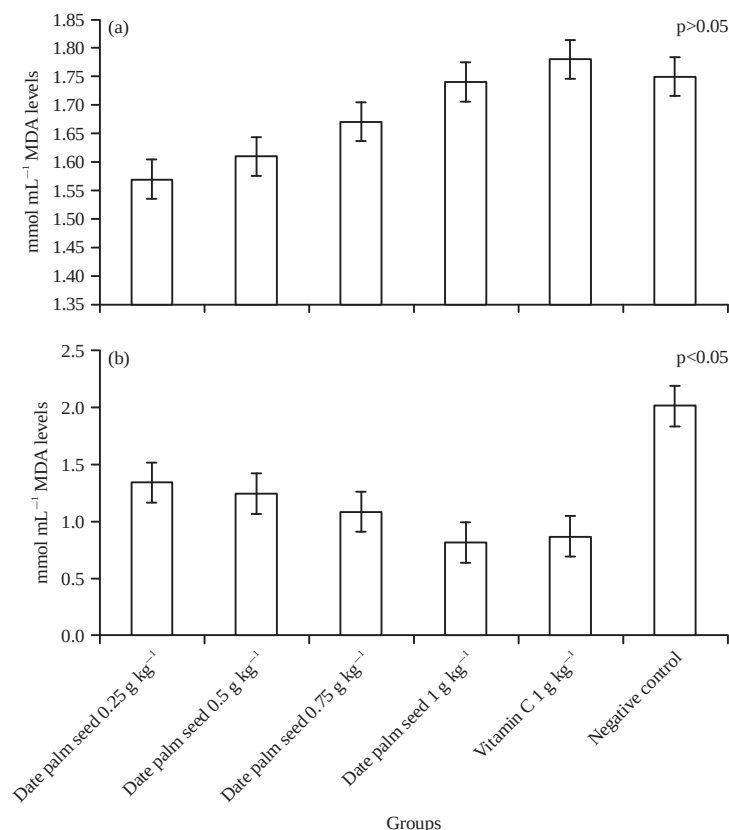


Fig. 1(a-b): MDA level, (a) Before treatment and (b) After treatment between groups

x = Groups of date palm seed, y = Mean $\pm$ SD

Table 1: LSD post hoc test of MDA after treatment between groups

Groups	Date palm seed (0.25 g kg ⁻¹)	Date palm seed (0.5 g kg ⁻¹)	Date palm seed (0.75 g kg ⁻¹)	Date palm seed (1 g kg ⁻¹)	Vitamin C (1 g kg ⁻¹)	Negative control
Date palm seed 0.25 g kg ⁻¹ (1st group)	-	0.179	0.002*	0.000*	0.000*	0.000*
Date palm seed 0.5 g kg ⁻¹ (2nd group)	0.179	-	0.032*	0.000*	0.000*	0.000*
Date palm seed 0.75 g kg ⁻¹ (3rd group)	0.002*	0.032*	-	0.001*	0.007*	0.000*
Date palm seed 1 g kg ⁻¹ (4th group)	0.000*	0.001*	0.001*	-	0.401	0.000*
Vitamin C 1 g kg ⁻¹ (5th group)	0.000*	0.007*	0.007*	0.401	-	0.000*
Negative control (6th group)	0.000*	0.000*	0.000*	0.000*	0.000*	-

*Significant (p<0.05)

The MDA levels in each group showed changes after treatment for a week. The average of MDA levels was highest in the 6th group (2.01 $\mu\text{mol L}^{-1}$) and the lowest in 4th group (0.81 $\mu\text{mol L}^{-1}$) (Fig. 1b). On the negative control group that only given an aquadestilata has the highest levels of MDA, whereas in the group by date palm seed steeping with a dose of 1 g kg⁻¹ had a mean lowest MDA.

One-way ANOVA test showed F-value of 78.147 (p<0.05), there were differences in MDA levels between groups after treatment significantly. Award date palm seeds steeping shown to reduce levels of MDA in all treatment groups, but no decline in the negative control group. The LSD's *post hoc* test

showed that there were different between groups of 1, 3, 5 and 6, while groups 1 and 2 did not different significantly (Table 1). The results also showed that the 4th and 5th groups were not significantly different.

Level of Vitamin E: The mean level of vitamin E before treatment of date seed steeping highest in 4th group (group with date palm seed steeping 1 g kg⁻¹), while lowest was in the 2nd group. In the entire group before treatment, the mean levels of vitamin E were relatively similar (Fig. 2a). There was no significant difference of vitamin E levels between the groups before treatment date palm seed steeping.

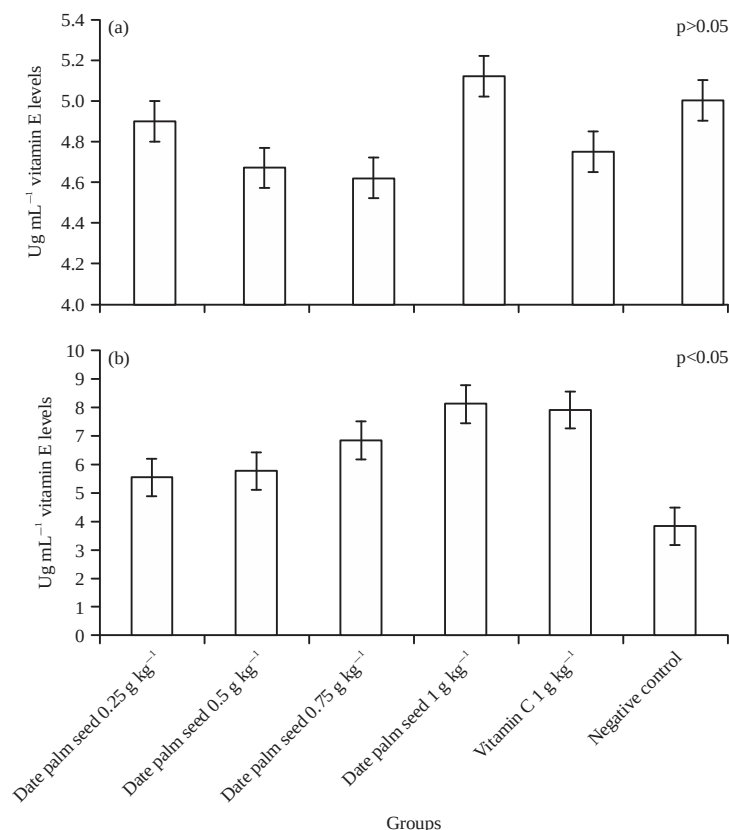


Fig. 2(a-b): Vitamin E levels, (a) Before treatment and (b) After treatment between groups

x = Groups of date palm seed, y = Mean $\pm$ SD

Table 2: LSD post hoc test of vitamin E after treatment between groups

Groups	Date palm seed (0.25 g kg ⁻¹)	Date palm seed (0.5 g kg ⁻¹)	Date palm seed (0.75 g kg ⁻¹)	Date palm seed (1 g kg ⁻¹)	Vitamin C (1 g kg ⁻¹)	Negative control
Date palm seed 0.25 g kg ⁻¹ (1st group)	-	0.574	0.004*	0.000*	0.000*	0.000*
Date palm seed 0.5 g kg ⁻¹ (2nd group)	0.574	-	0.000*	0.000*	0.000*	0.000*
Date palm seed 0.75 g kg ⁻¹ (3rd group)	0.004*	0.014*	-	0.004*	0.014*	0.000*
Date palm seed 1 g kg ⁻¹ (4th group)	0.000*	0.000*	0.004*	-	0.617	0.000*
Vitamin C 1 g kg ⁻¹ (5th group)	0.000*	0.000*	0.014*	0.617	-	0.000*
Negative control (6th group)	0.000*	0.000*	0.000*	0.000*	0.000*	-

*Significant (p<0.05)

The average levels of vitamin E after date palm seeds steeping administration was the highest in 4th group (8.12 Ug mL⁻¹), while lowest in the 6th group (3.85 Ug mL⁻¹) (Fig. 2b). There was a significant difference in level of vitamin E between the groups after treatment (p<0.05). The highest levels of vitamin E were in a 4th and 5th groups (groups with date palm seeds steeping dose of 1 g kg⁻¹ and positive control group that given vitamin C dose of 1 g kg⁻¹) and fall within the subset. This study showed that the quality of date palm seed steeping a dose of 1 g kg⁻¹ was comparable with vitamin C dose of 1 g kg⁻¹.

The LSD's *post hoc* test showed that there were significant differences between groups of 1st, 3rd, 5th and 6th,

but there was no significant differences between 1st and 2nd groups and between 4th and 5th groups. This study result suggested that 1 g kg⁻¹ date palm seeds administration have same effect with Vitamin C dose of 1 g kg⁻¹ administration (Table 2).

Malondialdehyde level: The results showed that average levels of MDA was highest in group 6 and the lowest in group 4. The negative control group that were only given a drink aquabidest have the highest levels of MDA, whereas in the group by date palm seed steeping with a dose of 1 g kg⁻¹ had a mean lowest MDA. The malondialdehyde is a product of lipid oxidation. Along with the addition of antioxidants from the

seeds of the palm, MDA levels in rats was decreased. Date seed that used are dried dates seeds, which have phenolic and flavonoid content higher than the wet and semi-wet palm⁸. From the research descriptively indicated that the higher dosage of date seed steeping, the lower the MDA levels in the blood.

There was a significant difference in MDA levels between groups after treatment. Date seed steeping administration shown to reduce levels of MDA in all treatment groups, but not in the negative control group. Date seeds contain many active compounds such as antioxidants⁹, which will donate electrons to form the outer track pairs on the skin, the compound becomes unstable and can reduce the oxidation of fat in the body. Phenolic content in date palm seeds is powerful to attack free radical⁷. Phenolic content in the date seeds is 48.64 mg/100 g¹⁰. However, date palm seeds have capacity as radical scavenging activity and reducing antioxidant power^{11,12}.

Peroxidation on the lipid with free radical would release MDA. Free radical was a compound which had unpaired electron in the outer trajectory. To achieve its stability, a free radical compound will be oxidizing other compounds. Due to compound electron around is taken, thus will damage the stability so that free radical chain reaction will occur. Lipids are easily oxidized molecules and easily break up so that MDA could be produced. The accumulation of MDA in the body will cause cell damage.

Level of vitamin E: The mean levels of vitamin E after treatment of date palm seed steeping administration were highest in 4th group (group with a dose of 1 g kg⁻¹), while the lowest in the 6th group. There were significant differences in the levels of vitamin E between the groups after treatment of date palm seed steeping administration. The highest levels of vitamin E were in 4th and 5th groups (group by date seed steeping with a dose of 1 g kg⁻¹ and positive control group that given vitamin C dose of 1 g kg⁻¹). Despite being in one subset, but the highest increased of vitamin E levels contained in the group that given date palm seed steeping dose of 1 g kg⁻¹. Vitamin E as an antioxidant works on the cell membrane because it is hydrophobic, comparable with date palm seed which may also working on cell membranes¹³. This study also found the antioxidant effect of palm seed and vitamin E with the same doses has comparable strength. It showed the power of date seed steeping administration a dose of 1 g kg⁻¹ comparable with vitamin C dose of 1 g kg⁻¹.

Lipophilic antioxidants are important in preventing lipid oxidation. This role may arise due to date palm seeds contain

a lot of oleic acid, vitamin E and others antioxidant compound¹⁴⁻¹⁶. Consumption of date palm seed increase the antioxidant capacity in the body. Therefore, this reserve of antioxidant will be to rise vitamin E. Roasted date seeds have highest content of flavonoids and antioxidant capacity than raw date seed¹⁷.

Furthermore, date seeds contain flavonoid, phenolic compound, vitamin E and other antioxidants. Flavonoid and phenolic compound would deliver electron so that free radical could stable and chain oxidation process will stop, whereas, vitamin E will produce more antioxidants in the body. Vitamin E will be oxidized by vitamin C so that the number of vitamin E will be more. Then, vitamin E will work as antioxidant by theses processing.

The limitation of this study are steeping product has difficulties to determine accurate doses. Each of date palm seed has differences active substance level, thus in the daily living should be selective to keep healthy¹⁸.

CONCLUSION AND RECOMMENDATIONS

Date palm seed steeping shown to reduce levels of MDA and increase vitamin E in all treatment groups, but does not occur in the negative control group. There were significant difference in the levels of MDA and vitamin E among the groups after treatment date palmseed steeping administration. The power of date palm seed steeping a dose of 1 g kg⁻¹ is comparable with vitamin C dose of 1 g kg⁻¹ administration.

Need further studies on the effects of long-term administration on organs of the body. Thus, it is safe for consumption. The findings were useful to develop herbal therapy for pre-elderly to inhibit aging process by to decrease free radical level in the human body. Further study needed for safety test and toxicity effect before human application.

SIGNIFICANCE STATEMENTS

This study discovered the date palm seed steeping that could be considered a promising source of new natural antioxidant agent to decrease MDA levels. This steeping (not extract) can be beneficial for common people. This study will help researcher to explore date palm seed steeping as antioxidant functional drinking or for use various pharmaceutical products and formulation. Thus, a new theory of steeping on date palm seed was possible for daily use to increase antioxidant level.

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