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Research Article

Effect of Antioxidant Status and Oxidative Stress Products in Pre-menopausal Women after Treatment with Date Seed Powder (*Phoenix dactylifera* L.): A Study on Women in Indonesia

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Abstract

Background: Degenerative diseases have the highest death rates in the elderly and these rates begin to rise starting in the pre-menopausal period. One degenerative disease is caused by an increase in free radicals, which induce oxidative stress. **Objective:** This study aimed to examine the effects of date seed powder on the antioxidant status and oxidative damage in pre-menopausal women in Gununglurah Village, Banyumas, Indonesia. **Methodology:** Deglet noor dates were cleaned, sun-dried, roasted and mashed. The date powder was poured into boiling water (250 cc). The antioxidant status (superoxide dismutase (SOD), glutathione peroxidase (GPX) and vitamin E) and oxidative damage (malondialdehyde (MDA)) were measured in women pre and post-treatment with date seed powder (2.5 g) for 2 weeks. The sample size of this study was 31 pre-menopausal women. The data were analyzed using a paired t-test. **Results:** The MDA level was significantly decreased after date seed consumption ($p < 0.05$), whereas the mean SOD and GPX enzyme activities and the vitamin E were increased after consuming the date seed powder ($p < 0.05$). **Conclusion:** Date seed powder increased the antioxidant status and decreased oxidative damage in pre-menopausal women.

Key words: Superoxide dismutase, glutathione peroxidase, vitamin E, oxidative damage, date seed powder, antioxidant, malondialdehyde

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

INTRODUCTION

Degenerative diseases have the highest death rates in both adolescents and the elderly. The incidence of degenerative diseases continues to grow as economic welfare and aging increase¹. The most common degenerative diseases are stroke, diabetes mellitus, hypertension, cancer and joint diseases². A previous study showed that free radicals were deleterious to the aging process³ and potentially worked through signal transduction mechanisms⁴. The signal transduction systems are very essential for homeostasis; thus, any deviation from a normal condition may lead to disease. The influence of free radicals and oxidative stress on vascular dysfunction disrupts the endothelial layers and causes cardiovascular disease⁵. Disruption of blood vessels may cause atherosclerosis, potentially leading to hypertension, stroke, coronary heart disease and myocardial infarction.

One way to improve body antioxidants is via the consumption of exogenous antioxidants obtained from plants⁶, such as date seeds. Date seeds are usually left unconsumed after being removed from the flesh, despite their high antioxidant content⁷. Previous studies have largely focused on the use of date seed extracts as a treatment⁸⁻¹⁰. Conversely, this study focuses on the initial product and tests the date seeds in powder form, which can be consumed like coffee. Therefore, the effects of treatment with date seed powder on the antioxidant status and oxidative damage were examined in pre-menopausal women.

MATERIALS AND METHODS

Setting: This study was conducted from July, 2015 to October, 2016 in Gununglurah Village, Banyumas, Indonesia.

Preparation of the deglet noor date powder: Deglet noor date seeds were separated from the fruit, washed with running water and laid out to dry for 1 day under the sun. The dried date seeds were roasted using medium heat, mashed with a blender and filtered with flour filters to obtain a fine powder. The date seed powder was weighed and aliquoted into approximately 2.5 g per pack. The participants consumed a pack of date seed powder mixed with 250 cc boiling water every day for 2 weeks.

Study design: The quasi-experimental study included pre and post-test designs. Thirty one pre-menopausal women aged 45-58 years were randomly recruited from Gununglurah Village, Banyumas, Indonesia. The oxidant status (superoxide

dismutase (SOD), glutathione peroxidase (GPX) and vitamin E) and oxidative damage (malondialdehyde (MDA)) were measured by pre and post-test treatment with 2.5 g of date seed powder for 2 weeks. Blood samples (5 cc) were collected pre and post-treatment. The SOD, GPX, vitamin E and MDA analysis were performed as described in previous studies^{11,12}. The SOD and GPX activity were expressed in U mL⁻¹ and mmol L⁻¹ and vitamin E and MDA were expressed by μmol L⁻¹. Informed consent was obtained from each participant using the standard informed consent procedure. The principles of respect for the autonomy, anonymity, confidentiality and privacy of the participants were practiced. The participants were treated with respect and were well informed concerning their right to freely decide whether to participate in the study and to withdraw anytime without penalty.

Statistical analysis: The data were analyzed using t-tests with a 5% significance level. This study was approved by the research ethics committee at the School of Nursing, Jenderal Soedirman University, Indonesia (Approval No. 091/KEPK/VI/2015).

RESULTS AND DISCUSSION

This study is the first to focus on the consumption of date seed powder by pre-menopausal women. Steeping date seed powder resulted in a significant decrease in the free radical product level (MDA), which was tested as an indicator of oxidative damage (Table 1, Fig. 1).

The second week of treatment with date seed powder resulted in a significant increase in the body's antioxidant capacity (vitamin E, SOD and GPX) (Table 2, Fig. 1).

Steeping the date seed powder did not disturb glomerular filtration, based on the creatinine levels before and after treatment (Table 3).

This study showed that the pre-treatment MDA level was higher than the level observed post-treatment ($p < 0.05$).

Table 1: Free radical oxidation products in lipids

| Variable | Pre-test | | Post-test | | t | p-value |
|----------|----------|------|-----------|------|-------|---------|
| | Mean | SD | Mean | SD | | |
| MDA | 2.21 | 0.39 | 1.52 | 0.40 | 10.75 | 0.00 |

Table 2: Differences in antioxidant levels pre and post-treatment

| Variables | Pre-test | | Post-test | | t | p-value |
|-----------|----------|-------|-----------|-------|--------|---------|
| | Mean | SD | Mean | SD | | |
| Vitamin E | 16.26 | 4.89 | 18.71 | 5.22 | -8.59 | 0.00 |
| SOD | 129.29 | 17.19 | 146.45 | 19.08 | -13.79 | 0.00 |
| GPX | 82.85 | 11.38 | 94.48 | 12.93 | -11.15 | 0.00 |

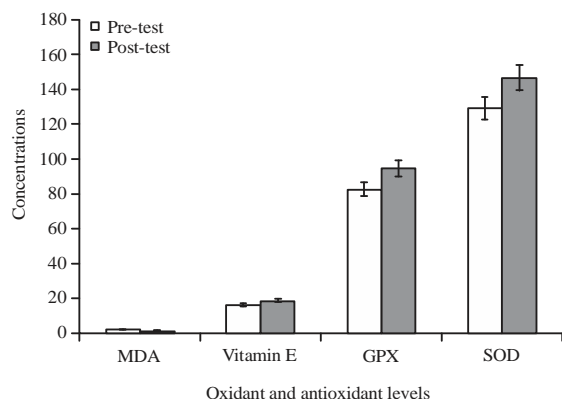


Fig. 1: Effect of date seed powder on increases in the vitamin E, GPX and SOD levels and decreases in the MDA level

Table 3: Safety of date seed powder on the kidney

| Variable | Pre-test | | Post-test | | t | p-value |
|-----------------------------------|----------|------|-----------|------|------|---------|
| | Mean | SD | Mean | SD | | |
| Creatinine (mg dL ⁻¹) | 1.14 | 0.30 | 1.03 | 0.19 | 2.02 | 0.05 |

Furthermore, this study indicated that free radicals caused oxidative damage. Elderly individuals are commonly accepted to have higher free radical production levels, which oxidize nearby molecules. The oxidized normal molecule becomes unstable when it loses its electron and forms new free radicals. Oxidation by free radicals on lipid membranes produces malondialdehyde products. The damage due to the oxidative process causes cell function degradation, which leading to cell death.

The results were consistent with a previous study in experimental animals¹¹, which showed that the MDA levels decreased after date seed treatment. Date seeds act as antioxidants to inhibit the oxidation of free radicals in lipids within the cell membrane. Therefore, the MDA level was decreased.

Treatment with date seed powder can significantly enhance vitamin E oxidant (tocopheryl) content ($p < 0.05$). Vitamin E is a fat-soluble oxidant that functions at the cell membrane to facilitate chain initiation or decompose chain propagation reactions. This vitamin also prevents oxidant formation. Date seeds contain large amounts of vitamin E as oxidants. Nehdi *et al.*¹³ showed that the tocopheryl alpha content in Tunisian date oil reached 66%. Tocopheryl is commonly recognized as the strongest reductor and thus is able to inhibit free radical oxidation in pre-menopausal women.

The results of this study also indicate that pre-menopausal women have lower enzymatic antioxidant

(SOD and GPX) activity on average with date seed powder. High oxidant levels in foods, pollution and an unhealthy life style can cause a high free radical contents in the body, which results in a decrease in enzymatic antioxidants. Enzymatic oxidants function to prevent free radical formation by the body. Glutathione peroxide (GPX) and catalase reduce hydrogen peroxide to water¹⁴. The SOD and GPX levels increased significantly ($p < 0.05$) following treatment with date seed powder. Furthermore, date seeds generally contain high total phenolic levels, which function as antioxidants.

Phenolics and flavonoids are closely related to antioxidant capacities and function as the chelating agent and reducing power, respectively. Various types of phenolic acid are found in date seeds¹⁵. Korkobbian stage dates have the highest phenolic acid content (0.5466 g kg^{-1}), this content increases significantly during the drying process as a result of tannin degradation and the maturation of enzymes at high temperatures¹⁶. The flavonoids, particularly phenolic acid, function as metal and free radical chelating agents that can break chain reactions due to free radicals.

These findings were consistent with a previous study in rabbits, which showed that treatment with Ajwa date seed extract increased the SOD and GPX activities and decreased lipid hydrogen peroxide production¹⁷. Date seeds exert functions as a chelating agent of Reactive Oxygen Species (ROS) by functioning as antioxidants. Based on the aging theory, there is an increase in free radical ROS in the elderly that are obtained from foods, pollutants and endogenous products of energy metabolism. The ROS cause mitochondrial and cytoplasmic membrane damage, which can lead to damage in other tissues. Consequently, ROS produce lipid hydrogen-peroxide. The components of the antioxidant compounds in date seed powder are undoubtedly able to fight oxidative stress, which decreases free radicals and improves the endogenous antioxidant enzymes.

The antioxidant contents vary in different date seed types. The antioxidant content of deglet noor dates is higher than the contents of other dates, which indicates better oxidative stability. Another study found a significant amount of gallic acid in date seeds³. The polyphenol content and antioxidant activity of date seeds are 10 times higher than the contents of date flesh, which is similar to other products such as grape seeds¹⁸. These facts all support the findings of this study that date seeds powder increase the antioxidant status of pre-menopausal women.

Date seed steeping had no effect on kidney glomerular filtration based on the stable blood creatinine levels after date seed consumption. Because date seed powder does not affect the blood filtration ability of the kidneys, creatinine excretion remains normal. Conversely, increasing creatinine levels

indicate the presence of glomerular damage, resulting in a decrease in blood creatinine excretion. The results of this study showed that steeped date seeds are safe for consumption.

The implication of these findings is that date seed powder can replace habitual coffee consumption. Date seed powder is healthier than coffee, particularly for pre-menopausal women and will decrease the free radical levels in the body. Further studies are needed to obtain a deeper understanding of the decreases in free radicals induced by different date seed varieties. The limitation of the current study is that the researchers only chose the best seeds of the deglet noor date, however, the use of deglet noor seeds should be selective, because the various deglet noor seeds have differences in their active substances.

CONCLUSION AND FUTURE RECOMMENDATION

Date seeds can decrease the MDA level, which serves as an oxidative stress product in pre-menopausal women. Date seed powder increases the antioxidant status in terms of the activity of enzymes such as SOD, GPX and the vitamin E level. The product obtained by steeping date seeds is safe for consumption. A further study analyzing the stability of the product of date seed packages using a complete indicator is strongly suggested.

SIGNIFICANCE STATEMENTS

This study reveals a possible synergistic effect of date seed powder, which can be beneficial and increase the SOD and GPX enzyme activities and the vitamin E level in pre-menopausal women. This study will help researchers uncover critical areas needed to increase the antioxidant status and decrease oxidative damage in pre-menopausal women. Thus, a new theory on the action of date seed powder has been presented.

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