

Research Article

Antioxidant enzyme status on rat after date seeds (*Phoenix dactylifera*) steeping treatment

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ABSTRACT

Background: Free radicals can damage normal cells thereby accelerating aging. Endogen antioxidant enzymes play an important role in inhibiting the oxidation rate of free radicals. This study aimed to assess the effect of date seed steeping on the indigenous antioxidant enzymes status in rats induced by alloxan.

Methods: This research used pre and post-test with control group design. A total of 30 rats were randomized to six groups. There were the treatment groups with different dosage of date seeds steeping (0.25; 0.5; 0.75; 1) g/kg, positive control group with vitamin C 1 g/kg per day and negative control group. Deglet Noor date seeds variant were cleaned and dried and was roasted and mashed. Examination of the glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity were conducted in accordance of the research of Castenmiller et al., (1999). Data were analyzed by Anova and continued by post-hoc test.

Results: The results showed that GPx and SOD levels increased significantly. There were differences in SOD and GPx activity between the groups after treatment of date seed steeping. Increasing of SOD activity in the group that was given date seed steeping dose of 0.75 g/kg and vitamin C as a positive control group was comparable. The GPx activity between treatment group of date seed steeping a dose of 1 g/kg is not significantly different from positive control group.

Conclusions: Date Seeds (*Phoenix dactylifera*) steeping have potential to increase antioxidant enzyme states.

Keywords: Free radical, GPx, SOD, Date seed steeping

INTRODUCTION

Aging is a physiological process that can be inhibited. One of the factors that can accelerate aging is increased levels of free radicals in the body. The higher levels of free radicals will increase endogenous antioxidants requirement to stop the chain reaction due to free radical oxidation. Endogenous antioxidants such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) are limited in number.

Date seed contains many polyphenolic compounds that can act as antioxidants.¹⁻³

Utilization of palm seed is still very limited. Polyphenols in date seeds have shown to maintain the strength of the sperm cell membrane thus increasing the number of sperm cells in mice exposed to monosodium glutamate.⁴ Polyphenol compounds in the date seed allegedly acted as a source of antioxidants to enhance the activity of endogenous antioxidant enzymes in the body, but the exact mechanism have not been known so that further research is needed. This study aimed to assess the effect of date seed steeping on the activity of the enzyme glutathione peroxidase and superoxide dismutase in rat models of induced alloxan.

METHODS

The ethical of this study was approved by the institutional review board in Faculty of medicine, Jenderal Soedirman University. This research used pre and post-test with control group design. A total of 30 rats were randomized to six treatment groups. Rats were randomized to six groups, there are negative control group, the treatment group with different dosage of date seed steeping (0.25; 0.5; 0.75; 1) g/kg and positive control group with antioxidants vitamin C 1 g/kg. Deglet Noor date seed variant were cleaned and dried. After the date seeds were dried, it were roasted and mashed. Blood samples were taken before and after treatment for 7 days. The examination of glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzyme activity conducted in accordance research of Castenmiller et al (1999).⁵ Levels of SOD and GPx activity were expressed in U/ml and mmol/l respectively. Data were analyzed by ANOVA test and post-hoc test continued.

RESULTS

SOD enzyme activity

The mean of SOD activity among groups before treatment showed normal distribution. The highest mean value is in the 6th group (87.75 U/ml), while the lowest is in the 2nd group (70.25 U/ml). Nevertheless, based on ANOVA test, there is no difference of SOD activity between groups before ($p > 0.05$). This indicates that SOD activity among groups before treatment is relatively balanced to protect the rats against oxidants.

After treatment for 7 days, the highest SOD enzyme activity found in the positive control group (who were given vitamin C), while the lowest activity is in the negative control group (Figure 1).

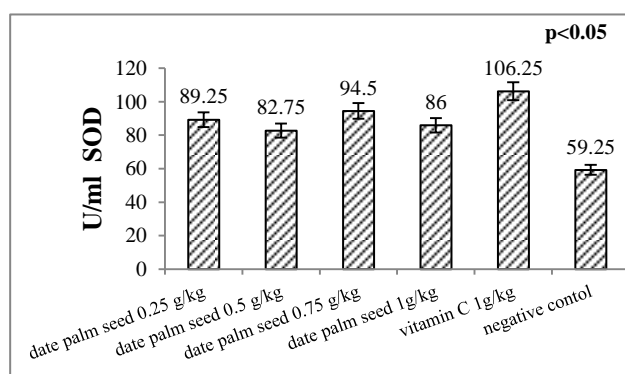


Figure 1: SOD level after treatment of date palm seed steeping.

The results of data analysis using ANOVA test obtained F-value of 15.44 with a p-value of 0.000 ($p < 0.05$). It is indicating that there is a difference in SOD activity among the groups after treatment of date seed steeping administration. SOD activity with vitamin C treatment as

a positive control showed the highest activity comparable to the provision of date seed steeping dose of 0.75 g/kg. Based on the Duncan post-hoc test, the group that given of date seed steeping did not show any difference, whereas the group treated with vitamin C is different from 1st, 2nd, 3rd and 4th groups. The negative control group is different from other groups.

GPx enzyme activity

The mean of GPx enzymes activity before treatment is highest in 5th group, a group with a dose of vitamin C, 1 g/kg as a positive control, while the lowest activity is in the 3rd group. Based on ANOVA test, there is difference of GPx enzyme activity among the groups before treatment of dates seed steeping ($p < 0.05$). The initial condition before treatment showed different GPx activity thus needs to be seen is there any difference in the increased activity of GPx which is the difference in GPx activity changes before and after treatment.

The highest mean of GPx enzyme activity after treatment of date seeds steeping is found in 4th group that given 1 g/kg of dates seed steeping, while the lowest activity present in 6th group of the negative control group. The ANOVA test showed that there are differences in the GPx enzyme activity among treatment groups after administration of date seeds steeping ($p < 0.05$). GPx activity is highest in the group with the 1 g/kg dose of date seed steeping administration and positive controls were given vitamin C 1 g/kg, but there is no significant different.

Based on different between average, oneway ANOVA test showed that that the GPx enzyme activity significantly different among groups ($p < 0.05$). The largest changes occurred in the group of date seed steeping dose of 1 g/kg and the control group who were given vitamin C 1 g/kg (Figure 2). Duncan's post hoc test result indicated that there is a difference among group 1st, 3rd and 5th, but no difference between 4th and 5th groups as well as 1st and 2nd groups. This indicates that administration of date seed steeping a dose of 1 g/kg is not different with vitamin C dose of 1 g/kg.

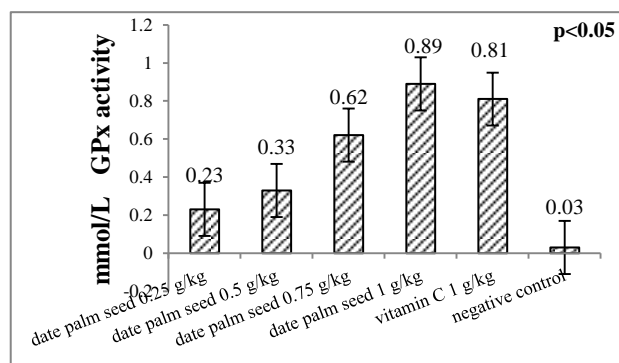


Figure 2: GPx activity changes after date palm seed steeping administration.

DISCUSSION

Enzyme activity of superoxide dismutase (SOD)

The highest mean of SOD enzyme activity after treatment was found in the positive control group (who were given vitamin C), while the lowest activity was found in the negative control group. Superoxide dismutase is endogenous antioxidant enzyme that works in the cytoplasm. Vitamin C is also an antioxidant that works in the cytoplasm that using to reduce re-tocopherol is oxidized. The results showed that the positive control group were given vitamin C is more potent in improving SOD enzyme activity compared to the group given the dates seeds steeping. Allegedly, the date seeds steeping worked in the cell membrane to protect the lipids from oxidation, whereas vitamin C and SOD works in the cytoplasm so that the effect of vitamin C is more significant in improving SOD activity. Several enzymes contribute in oxidant-antioxidant balance in cells.⁶

There are differences in SOD activity among the groups after treatment administration of steeping seeds of dates (Figure 1). SOD activity in the treatment of vitamin C as a positive control showed the highest activity comparable to the provision of date seeds steeping dose of 0.75 g/kg, but different to other groups. From Duncan's post-hoc test results, vitamin C group were significantly different from the group that was given date seed steeping or the negative control in improving SOD activity. This difference in activity indicates that date seed more work in the cell membrane, not in the cytoplasm, which is in line with the nature of the seeds of palm fatty acids. Fatty acids in date seed consists of saturated and unsaturated fats. Date seeds contain 14 types of fatty acids, such as oleic acid, lauric, linoleic, palmitic, myristic acid, caproate, myristoleic, palmitoleic, stearic, and linolenic acid.⁸ This observations confirm the finding of several studies, which reported alterations in antioxidant enzyme activity after date seeds treatment.⁷

Glutathione peroxidase activity

GPx enzyme activity was significantly different among groups as given in Figure 2. The increasing of GPx activity was greatest in the group with the administration of date seed steeping dose of 1 g/kgbw followed with a control group who were given vitamin C 1 g/kgbw. Glutathione peroxidase is needed to change the $H_2O_2 + H_2O$ to reduce glutathione and glutathione becomes oxidized. Glutathione peroxidation require mineral elements Selenium (Se). In present study showed that the date seed treatment significantly increases GPx enzyme activity. The study about date seed effect to increase antioxidant level was still limited. This research supports previous finding that date seed can increase of antioxidant level.⁷

Date seeds also contain quersetin that compounds flavonoids with really potential antioxidant

characteristic.³ Quersetin have antioxidant characteristic equal as alpha tocopherol at inhibiting lipid peroxidation by free radicals. Free radicals are actually formed naturally as part of the body's metabolic processes. However, free radicals can also be influenced by environmental factors, including smoking, toxic substances in food, pollution and radiation. When free radicals attack the molecule, which is initially neutral molecule eventually transformed into a radical, causing a chain reaction that can lead to destruction of cells.

There are differences in SOD activity among the groups after administration of dates steeping. Increasing of SOD activity in the group that given of date seed steeping dose of 0.75 g/kg is equal with the group that given vitamin C as a positive control. The group that given of date seed steeping did not show any differences, whereas the group treated with vitamin C is different from group 1, 2, 3 and 4. The negative control group was different from other groups.

There are significantly differences in the GPx enzyme activity among groups. The change has been occurred in the group that given of date seed steeping dose of 1 g/kg. Treatment of date seed steeping dose of 1 g/kg is not significantly different from vitamin C dose of 1 g/kg.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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