

EFFECT OF JUJUBE LEAVES BREWED TO MALONDIALDEHIDE (MDA) LEVEL OF WISTAR RATS EXPOSED PSYCHOLOGICAL STRESS

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ABSTRACT

Background: Psychological stress is often experienced without realized. One of the causes is the inverted sleep-guard pattern. Psychological stress causes oxidative stress resulting in increased reactive oxygen species (ROS). ROS as a trigger of high free radicals exceeds the amount of antioxidants in the body causing accumulation of oxidative damage and increase level of MDA (*Malondialdehyde*) as one of psychological stress markers. High MDA may be prevented by high antioxidant food that found in plants. One of plants that use to prevent this, called Jujube leaves. Jujube leaves contain flavonoid can lower MDA. The aim of this study is to analyze the influence of Jujube Leaves brewed to MDA levels in white rat strains wistar exposed to psychological stress by compared the difference of MDA levels in each group.

Materials and Methods: The type of research that used in this study is true experimental, by post-test control group design. Psychological stress condition of wistar rats was made by changing the sleep-patterns reverse. The doses of jujube leaves are given for 3,9 gr, 7,9 gr, and 15,8 gr. Statistical analysis that used is ANOVA.

Result: Based on statistical analysis, showed that there are differences of MDA levels in stress control group, first treatment, second treatment, and third treatment, significant ($p. < 0.05$). Levels of MDA in the normal group compared with the high MDA group (stress control group). The third treatment showed a significant difference ($p. < 0.05$).

Conclusion: Jujube leaves brewed can decreased MDA levels after treatment, those can be due to the flavonoid and polyphenol of Jujube leaves brewed.

Keywords: *Malondialdehyde (MDA), Jujube leaves, flavonoid, psychological stress*

1.0 Introduction

Psychological stress is often experienced without realized. One of the causes is the inverted sleep-guard pattern. Psychological stress is a condition where a person feels the excessive environmental demands on his ability to adapt (Cohen *et al*, 2007). Psychological stress causes oxidative stress. Oxidative stress is a condition where the amount of anti-oxidants is lower than free radicals (Granger *et al.*, 2001). The body's reaction to stressors is marked by changes in the endocrine system, the secretion of norepinephrine hormone and the high cortisol hormone that can cause other physical diseases including increased blood glucose (Sengupta, 2012). The body's reaction to stress conditions stimulates the HPA (Hypothalamic-Pituitary-Adrenal) axis so that the hormones cortisol and adrenal increase. Increased cortisol is associated with glucocorticoids that increase gluconeogenesis and glycogenolysis resulting in high blood glucose. Some hormones such as cortisol and adrenal due to these reactions trigger the occurrence of vasoconstriction and increased frequency of heartbeat that causes blood pressure to rise. As a result the amount of circulating blood and oxygen circulating in the blood decreases (Guyton and Hall, 2016) resulting in turbulence that triggers free radical production (Segestrom; Miller, 2004). Psychological stress is also able to disrupt the work of the pancreas resulting in decreased insulin and result in increased amounts of glucose in the blood. the production of excessive free radicals in the body occurs as a result of elevated blood glucose levels.

Free radical production increases in pathological conditions due to physical and psychological stress (Lei *et al*, 2007). Free radicals are often associated with the cause of a number of degenerative diseases, both coronary heart disease and atherosclerosis and cancer (Eibond *et al.*, 2004). Free radicals cause lipid peroxide, the process which free radicals take electrons in the lipid cell membranes resulting in cell damage. One of the products produced is MDA (Catala, 2006). Free radicals in the body can be reviewed from the levels of Malondialdehyde (MDA) with the molecular formula $C_7H_{16}O_4$ in plasma. MDA is the most commonly studied product in oxidative stress with peroxidase of polyunsaturated fatty acids (Del Rio *et al*, 2005).

MDA is formed from the reaction of free radical ROS with the fatty acid component of the cell membrane. The high concentration of MDA indicates the presence of oxidation processes in cell membranes. MDA has a longer half-life than free radicals whose half-life is very short (Halliwell, Gutteridge, 2007). MDA is formed from the reaction of free radical ROS with the fatty acid component of the cell membrane. The high concentration of MDA indicates the presence of oxidation processes in cell membranes. MDA is the main secondary product of lipid peroxidation because it is more mutagenic than other aldehydes. Psychological stress increases the production of free radicals that indicated by increased MDA.

Exposure of psychological stress on the occurrence of oxidative stress involves a multi-lane mechanism. The provision of eksogen antioxidants such as proteins, vitamins, minerals, and natural materials can be the solution. High MDA may be prevented by high antioxidant food that found in plants. One of plants that use to prevent this, called Jujube leaves (*Ziziphus mauritiana*). Jujube is a bush or spiny tree with height up to 15 m, stem diameter 40 cm or more. The bark of the trunk is dark gray or black, cracked irregularly. The leaves are single and crisscrossed, 4-6 cm long and 2.5-4.5 cm wide. Hairy petiole and on the leaf edge there are very fine teeth. One-seeded fruit, round to ovoid, size of 6x4 cm, smooth or rough, glossy, yellowish to blackish or blackish skin, white flesh, crunchy, slightly sour to sweet (Tangkelangi., 2017).

In previous research (Niamat et al, 2012), Jujube leaves used to decrease blood glucose level. Jujube leaves contain antioxidants that inhibit a free radical reaction by forming reactive free radical reactions and relatively stable. One of the compounds in plants that have activity as an antioxidant is flavonoids (Neldawati, 2013). Flavonoids are reducing compounds that can inhibit many oxidation reactions and have the ability as an antioxidant because it can transfer an electron to free radical compounds (Haeria et al, 2016). *Ziziphus mauritiana* which contains flavonoid can reduce MDA level (Batool et al., 2017). The aim of this study is to measure MDA levels of normal wistar rats (normal control group) and wistar rats exposed psychological stress without (stress control group) and with giving jujube leaves brewed.

2.0 Materials and Methods

The type of research that used in this study is true experimental, with post-test control group design. This design enables the researcher to measure the effect of treatment / intervention with a simple randomly-determined experimental group by comparing between the control group and the experimental group. This study was conducted in Medical Faculty, University of Airlangga, Surabaya. The subject of this research is rats strain wistar and meeting the sample acceptance criteria. Sample acceptance criteria were white wistar rat, age \pm 2-3 months, body weight 150-200 grams. Criteria for rejection of the sample include rat whose psychological stress is characterized by increased aggressiveness, rats that do not want to eat and drink, and rats that die before being treated. The number of samples that used are five per treatment group (normal group; stress group; stress and jujube laves brewed with three doses). Rats are stressed by changing their sleep-guard patterns by utilizing light.

Rats are acclimatized for 7 days. The weight of the rats ware weighed to see the homogeneity of body weight from the rats sampled. During the acclimatization process rats were fed standard rodensia and drank ad lithium. Stress exposure is given for 24 hour after acclimatization and the doses are given in the next day. Giving each dose in a rat is divided for 4 times in the same day. The doses of jujube leaves are 3,9 gr, 7,9 gr, and 15,8 gr which is brewed in 100 ml warm water (70°C) for 5 minutes. The temperature used to active flavonoid in jujube leaves. The optimal resistance to flavonoid compounds has a temperature range of 0°C - 100°C (Putri et al., 2014). Research of Dewata et al (2017) about effect of temperature and old time to antioksidan activity in herbal tea of avocado leaves mention that the lower temperature and the duration of brewing cause the total amount of flavonoids in herbal tea is lower.

The last of the treatment (9th days), the rats are drawn the blood to measure MDA levels after treatment. MDA was measured by Enzym-linked immunosorbent assay (ELISA) technique, then data was processed with SPSS version 20.0, descriptive analysis, normality test, and homogeneity variant, analyzed statistically (Anova one way), then presented in tables and graphs. Statistical analysis is using ANOVA to notice the difference of MDA levels in each group. This study was conducted to prove the hypothesis that there are the influence of jujube leaves brewed to MDA levels in white rat strains wistar exposed to psychological stress by compared the difference of MDA levels in each group. The difference is considered meaningful when the value of $p < 0.05$.

3.0 Result

3.1 MDA levels bivariate analysis

Effect of giving jujube leaves on MDA levels in rat exposed to psychological stress seen from the difference in average MDA levels after being given jujube leaves. The difference of mean and standar deviation of MDA in control group and treatment group of wistar rats showed in the following table 1.

Table 1. The Mean and Standar Deviation Difference of MDA in Control Group and Treatment Group of Wistar Rats

Group	n	MDA (nmol/ml)
		Mean \pm SD
Normal Control (NC)	5	3.35 \pm 0.214
Stress Control (SC)	5	4.50 \pm 0.226
First Treatment (FT) (3.9 gr)	5	3.34 \pm 0.042
Second Treatment (ST) (7.9 gr)	5	3.26 \pm 0.052
Third Treatment (TT) (15.8 gr)	5	3.19 \pm 0.032

Levels of MDA are measured once at sixth after first administration The average calculations and standard deviations of MDA from each group show different results. MDA level in the normal control group (NC) is 3.35 nmol/ml and the highest level is in the stress control group (PC), 4.50 nmol/ml. MDA level in first treatment group (FT) decreased to approximately the mean of normal control group MDA level, that is 3,34 nmol/ml. Then in treatment group (ST) is 3,26 nmol/ml, and MDA level in treatment group (TT) is 3.19 nmol/ml.

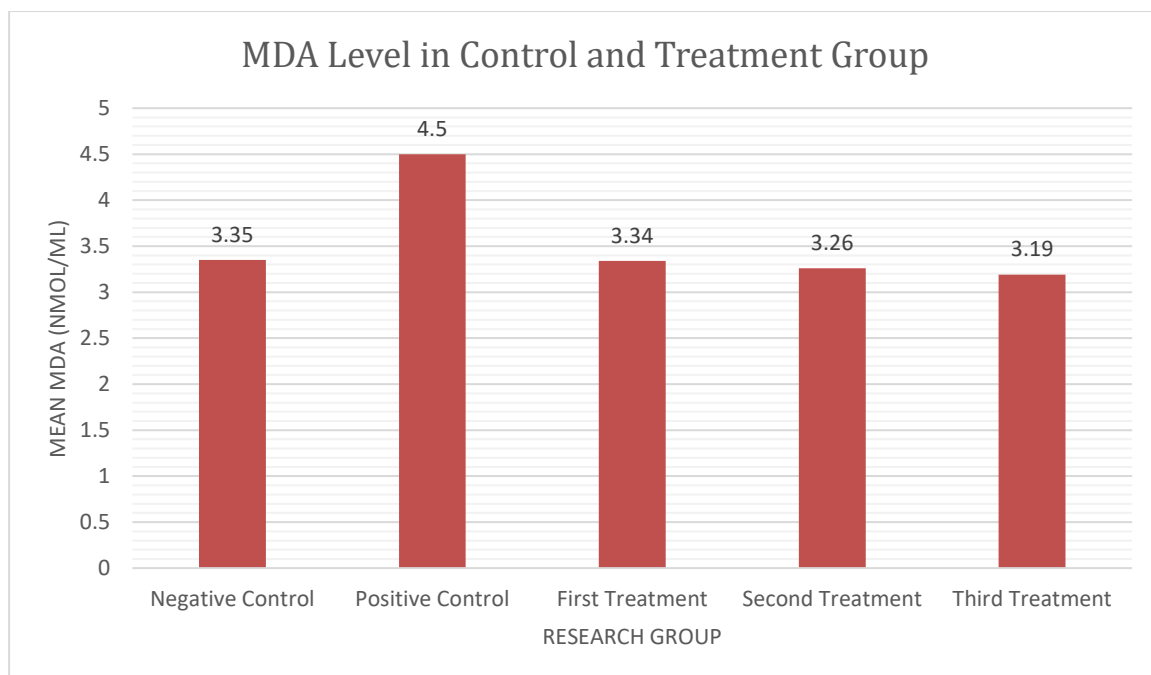


Figure 1. Graph of MDA Levels in Each Group

Descriptive statistic of this research exhibit that there are differences of MDA levels in the normal control group and stress control group, first treatment, second treatment, and third treatment, significant ($p. < 0.05$). MDA level in stress control group is higher than others. While MDA level in treatment group, that is first treatment, second treatment, or third treatment showed no much different from normal control group. The comparison of MDA level in every group showed in figure I.

3.2 MDA levels changes analysis

Based on the ANOVA test, the significance value obtained is 0.000 ($p. < 0.05$). Consequently, there are differences in MDA levels in among groups (normal control, stress control, first treatment, second treatment, and third treatment). Then, to find out the different groups significantly based on p . value can be seen in table 2.

Table 2. The Comparison of MDA Levels in Each Group

Group	NC	PC	FT	ST	TT
NC	-	0,000	1,000	0,841	0,401
PC	0,000	-	0,001	0,001	0,001
FT (3,9 gr)	1,000	0,001	-	0,148	0,002
ST (7,9 gr)	0,868	0,001	0,148	-	0,203
TT (15,8 gr)	0,526	0,001	0,002	0,203	-

Levels of MDA in the normal control group compared with the stress control group showed a significant difference ($p. < 0.05$). Levels of MDA in the stress control group compared with first treatment, second treatment, and third treatment group showed a significant difference ($p. < 0.05$). Levels of MDA in first treatment group compared with third treatment group exhibit a significant difference ($p. < 0.05$). Level of MDA in first treatment and second treatment didn't show a significant difference ($p. > 0.05$). Level of MDA in second treatment and third treatment also didn't show a significant difference ($p. > 0.05$). While level of MDA in first treatment and third treatment show a significant difference ($p. < 0.05$).

4.0 Discussion

Stress is the reaction or the body's response to stressors (mental pressure /load of life). Diseases of the blood circulation and ulcers in the gastrointestinal tract are closely related to the incidence of stress. In this study the rats to be exposed to stress by being awakened using shocks and utilizing lighting lamps. The lights are off at night and turned on during the day. The treatment was administered for 24 hours which can cause circadian rhythm disturbance in *Supra Ciasmaticnucleus* (SCN) in the hypothalamus (Martinez et al., 2008). Based on the results of research that has been done on the five treatment groups, it was found that the average value of MDA levels in the normal group was 3.35 nmol / ml. While MDA levels in the group who were given only exposure to psychological stress showed a higher average of 4.50 nmol / ml. That

showed that the provision of exposure to psychological stress can increase MDA levels significantly.

Psychological stress in rats causes high blood glucose levels that trigger oxidative stress, a condition in which the amount of free radicals in the body is higher than the number of antioxidants. The high free radical effect on high levels of MDA toxic enzymes as oxidative stress markers and is the final product of high lipid peroxidation. Peroxidant compounds that generally play a role in the reaction of damage in the body such as hydroxyl radicals (OH), superoxide radicals (O), radicals nitric oxide (NO), and peroxy fatty radicals (LOO). Free radicals are intermediate which can be converted into other substances quickly and occur in a chain and continuously. High free radicals lead to further peroxidation with MDA higher end products.

Based on statistical analysis, the effect of MDA levels based on jujube leaves brewed is significant ($p < 0.05$). It means that there is influence of giving jujube leaves brewed to MDA level of rats. The means of the stress control group is higher than the normal control group. The means of first treatment is lower than the stress control group and the lowest is in third treatment. Higher levels of MDA are caused by the influence of psychological stress resulting in oxidative stress due to increased amount of free radicals than antioxidants in the body. Whereas in the treatment group previously exposed to stress showed lower MDA levels due to the giving of jujube leaves.

MDA level is lower in treatment with jujube leaves brewed because it contains phenolic compounds, and flavonoid that are valued as a group of potent natural antioxidants (Duthie & Morrice, 2012). These compounds are helpful in reducing the chances of occurrence of oxidative stress (Huang et al., 2012; Pandey & Rizvi, 2009). Flavonoid treatment reduced MDA levels by alter oxidation reactions by connecting an electron to unpaired electrons (OH^-) in free radicals (Fidzaro, 2010). Decreased MDA levels are caused by the ability of flavonoids to prevent elevated levels of MDA by preventing the formation of lipid peroxidation that acts as a vasoconstrictor and reduces the formation of peroxynitrite radicals (Grossman, 2004).

This study is supported by previous studies in which the antioxidant activity test of flavonoids can inhibit MDA (Rizki and Hariandja, 2015). In another study also mentioned that the administration of flavonoids can shrink the increase in MDA in rats (Olayinka et al, 2015). While research conducted by Dewi et al, (2014) about antioxidant activity of flavonoids extract of ethanol extract of eggplant seeds of solo (*Solanum betaceum*, syn) in inhibiting fat peroxidation reaction in blood plasma of wistar rat showed that peberian extract of ethyl acetate seeds of eggplant of dutch with dose 200 mg / kg BW equivalent to vitamin E (100 mg / kgBB) can inhibit the fat peroxidation reaction characterized by decreased plasma blood plasma MDA levels of Wistar rats. The results of this study are also in accordance with previous research studies on chemical composition and biological activity leaf *Ziziphus mauritiana* leaves native to Pakistan. The study concluded that *Ziziphus mauritiana* leaf extracts with efficient biological activity could be explored for potential use as antioxidant agents for the pharmaceutical industry (Ashraf et al., 2015). Another research before concluded that leaves of *Z. mauritiana* could may serve as alternative medicine oxidative stress in Wistar albino rats (Dutta and Patil, 2017).

5.0 Conclusion and recommendation

In this study, Jujube leaves brewed could decrease MDA levels after treatment. These effects can be due to the active compound in that plants that is flavonoid and polyphenol, which alter oxidation reactions by connecting an electron to unpaired electrons (OH^-) in free radicals. Decrease in MDA levels due to the effect of dosage on the distressed rat. Consumption of jujube leaves in daily life must be along with physical activity or exercise to prevent the increase of MDA and require further research about mechanism of flavonoid in jujube leaves to decrease MDA levels.

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Declaration

Author(s) declare that there's no conflict of interest in this research.

Author's contribution

Author 1: Concept, idea, data collection, statistical analysis, and manuscript writing

Author 2: Concept, idea, literature review and manuscript writing

Author 3: Concept, idea, literature review, and manuscript writing

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