**International Journal of Healthcare Sciences** ISSN 2348-5728 (Online)
Vol. 7, Issue 2, pp: (454-458), Month: October 2019 - March 2020, Available at: www.researchpublish.com

# The Effect of Ethanol Extract from Red Dragon Fruit (*Hylocereus polyrhizus*) Peels on Malondialdehyde Levels in Menopause Model Rat

Nur Aliyyah Binti Harun[1], I Wayan Sugiritama[2], IG Nyoman Sri Wiryawan[3]

[1]Medical Student of Udayana University, Bali, Indonesia

[23]Department of Histology, Faculty of Medicine, Udayana University, Bali, Indonesia

***Abstract:*** **The ovaries stops producing estrogen during menopause which turns out to have an effect on various organ functions and the body's metabolism, including lipid oxidation. Previous studies have shown that when examining the levels of Malondialdehyde (MDA) in women who have reached the age of menopause, an increase in MDA levels can usually be observed. One of the food ingredients that is rich in antioxidant is the skin of red dragon fruit (*Hylocereus polyrhizus*), so it is hoped that the skin of red dragon fruit can be a source of phytoestrogens that can play a role in improving MDA levels in menopausal women. This study aims to determine the effect of ethanol extract of red dragon fruit peels (*Hylocereus polyrhizus*) on the levels of Malondialdehyde in menopausal model rats. This study is a purely experimental study. A randomized post-test only control group design research was conducted on 30 female Wistar rats. The study sample was divided into the control group (P0), the ethanol extract of red dragon fruit peels at a dose of 60mg / 200grBB group (P1), and the ethanol extract of red dragon fruit peels dose 90mg / 200grBB group (P2). The highest MDA Plasma / mm level was found in group (P1), 2,789 µmol / L, followed by P2, 2,157 µmol / L and (P0) with a mean of 1,944 µmol / L. The results showed that there is no significant effect of ethanol extract from red dragon fruit peels on Malondialdehyde Levels in menopause model rats.**

***Keywords:*** **Menopause, Malondialdehyde, Antioxidant, Red Dragon Fruit Peels, *Hylocereus polyrhizus***

## 1. INTRODUCTION

Menopause is a physiological process that every women will go through. During menopause, some changes can be observed such as loss of traction, lack of excitement and many others.[1] Menopause can be divided into a few stages namely being: perimenopause, menopause, and postmenopause. These stages vary from the hormone levels involved as well as the symptoms experienced. Various mechanism is involved in this process. One the mechanisms involved is a decrease in the estrogen level thus causing the antioxidant level in the body to also decrease. This in return, causes the imbalance between the free radicals and antioxidant known as oxidative stress.[2] Oxidative stress occurs naturally in the body and can be seen from several different signs, namely through direct measurement of oxidative agents such as ROS production in peripheral blood cells, the effect of oxidative stress on molecular targets (oxidized lipid products and oxidized proteins), or through the response of antioxidant capacity in plasma.[3] This condition can be prevented by reducing exposure to free radicals or adding antioxidant intake. Additional antioxidant intake is important to ensure prevention of cell damage caused by oxidative stress.

Antioxidants have been widely studied and is found to reduce the total effects of menopausal symptoms, as well as body symptoms and psychological symptoms when compared with low antioxidant capacity.[4] Antioxidants are very important to protect the body from free radicals because they are substances that can prevent or slow down cell damage caused by free radicals, unstable molecules produced by the body in reaction to environmental stresses and others. If the amount of

free radicals in the body exceeds the amount of antioxidants, this may lead to oxidative stress. The activity of antioxidants can be assessed from the levels of Malondialdehyde (MDA) in the plasma of rats given free radicals and compared with plasma from groups of mice that were not given free radicals. The lower the MDA level, the better the antioxidant activity. Parts of some plants such as skin, fruit, roots, fruit, stems, leaves and seeds can be used as antioxidants. One of the plants that have the potential to become antioxidants is the peel of the red dragon fruit.

Red dragon fruit or its scientific name *Hylocereus polyrhizus* comes from the pitahaya tribe and is mostly found in the Southeast Asia due to its tropical nature.[5] Red dragon fruit contains antioxidants, phenols, flavonoids, phytoalbumins, and betalains such as betacyanins and betaxanthins which functions as antioxidants.[6] It is high with antioxidants such as polyphenol due to its red skin.[7] Studies have shown that the skin of a red dragon fruit is rich in antioxidants and can even be considered as an inhibitor of growth of cancer cells. This study aims to examine the effect of ethanol extract of red dragon fruit skin on the levels of Malondialdehyde in menopause model rats.

## 2. METHODOLOGY

This study is a purely experimental study with a post-test only control group research design that uses animals as test material. This study uses a menopausal mouse model done by ovariectomy of rats. Based on Fredere's minimum sample formula, this study used 9 Wistar rat samples in each treatment group. An additional rat is added in each group to anticipate death or any other exclusion criteria. Female Wistar strain rats were chosen as research subjects because they have characteristics and physiological aspects that are relevant to humans, as well as being easy to obtain, easy to care for and do not require large spaces. To avoid bias in the study, only healthy rats were selected to participate in the study, and to maintain the health condition of the rats, the researchers provided adequate pens, as well as adequate food and drinks during the study. The rats were first acclimated in the lab for a duration of one week were then divided into a control group where the rats only received ovariectomy procedures without any treatment (P0), group 1 (P1) where an ovariectomy mouse is given red dragon fruit extract at a dose of 60mg / 200gramBB, while in group (P2) were rats that had undergone ovariectomy along with the administration of red dragon fruit extract at a dose of 90mg / 200grBB. This treatment was carried out for 30 days until the plasma were taken to measure the MDA levels using the thiobarbiturate acid-reactive substances (TBARS) method. Red dragon fruit (*Hylocereus polyrhizus*) was obtained from a supermarket located in Denpasar. The dragon fruit skin used is the skin of dragon fruit (*Hylocereus polyrhizus*) that is ripe and red in colour. Extraction is done by separating the skin of the dragon fruit with its flesh, washing the skin of the fruit with clean and flowing water, then chopped finely, and dried by air-drying the chopped pieces in the oven for better drying. It is then blended and sieved to make a smooth simplicial and is then soaked in 96% ethanol solution with a ratio of 1: 7 for 3 days while stirring 2 times a day. After maceration is complete, the macerate is separated using filter paper. The results of maceration were evaporated using a rotary evaporator until a thick extract of red dragon fruit peel is obtained. Ovariectomy was carried out according to the modified Ingle DJ and Grith JQ method, namely the rats were anesthetized using ketamine at a dose of 40 mg / kg, im. The abdominal hair of the rat was shaved, sterilized using savlon - betadine, then covered with sterile cloth. A transabdominal incision is made over the uterus 1.5 to 2 cm layer by layer until it penetrates the peritoneal wall. The uterus is then searched, and followed by the left uterine-oviduct-ovary cornu. The ovary is released from the surrounding fat and connective tissue, then the distal and ovarian oviduct is ligated and then removed. The same procedure is done in the right section. Exploration did not result in bleeding. The incision wound was sutured layer by layer, the operation was complete. Postoperative therapy is given gentamicin injection at a dose of 60-80 mg / kgBB / day for 3 days. However, one of the sample died postoperative due to infection and thus making the sample count to: P0 (9 rats), P1 (10 rats) and P2 (10 rats). P1 and P2 was then given doses of red dragon fruit extract according to the measurement stated above for 30 days while P0, as the control group, is given no doses of the extract. As for the plasma sample, blood is first taken as much as 3ml and placed into the Eppendorf tube which is then centrifuged at a speed of 14,000rpm for 5 minutes. Blood plasma fluid that is separated from the solid portion is transferred to an empty tube to measure MDA levels. Examination of MDA plasma levels was done using the thiobarbiturate acid-reactive substances (TBARS) method and was carried out in the Biochemistry laboratory of the Faculty of Medicine, Udayana University, Bali, Indonesia. Descriptive analysis is conducted to obtain a central tendency value and standard deviation (SD) of the dependent variable. Statistical analysis is also conducted to test the normality of the data using the Shapiro-Wilk test, and homogeneity test is also done using the Levene Statistic. It is then proceeded with parametric statistical test, one-way ANOVA. A post-hoc test using Least Significant Difference (LSD) is also done to conclude the data.

## 3. RESULTS AND DISCUSSION

### *3.1 Descriptive Test*

Table 1 shows descriptive test results of mean, standard deviation, lower and upper limits of 95% confidence interval and the maximum and minimum values of MDA plasma levels in ovariectomy Wistar rats. It is found that the highest average MDA Plasma/mm level is found in group 1 (P1), which is 2,789 µmol / L, followed by P2, which is 2,157 µmol / L. The lowest average plasma MDA level was found in the treatment group 0 (P0).

**TABLE 1: Descriptive Test of MDA Plasma Levels in Ovariectomy Wistar Rats**

| | | | | 95% Confidence Interval | | | |
|---|---|---|---|---|---|---|---|
| | N | Average | Standard Deviation | Lower Limit | Upper Limit | Min | Max |
| P0 | 9 | 1.9443 | 1.25144 | 0.9824 | 2.9063 | 0.62 | 4.13 |
| P1 | 10 | 2.7884 | 1.31012 | 1.8512 | 3.7256 | 1.08 | 5.47 |
| P2 | 10 | 2.1571 | 0.75447 | 1.6174 | 2.6968 | 1.08 | 3.38 |
| Total | 30 | 2.3088 | 1.14682 | 1.8725 | 2.7450 | 0.62 | 5.47 |

### *3.2 Normality & Homogeneity Test*

Normality and homogeneity tests are useful for determining the type of mean comparative test that will be continued thereafter. In this study, the normality test was performed using the Shapiro-Wilk test for studies with a small total sample. It was found that all treatment groups had a mean value of MDA Plasma levels normally distributed with a p value greater than 0.05. While based on the Levene normality test it was found that the average MDA Plasma data in this study was homogeneous (p> 0.05). Table 2 shows normality and homogeneity results.

**TABLE 2: Normality and Homogeneity Test Results for MDA Plasma Levels in Ovariectomy Wistar Rats**

| **Normality Test** *Saphiro Wilk* | | **Homogeneity Test** *Levene Test* | | |
|---|---|---|---|---|
| **Group** | **Significant (p)** | | **Statistic** | **Significant (p)** |
| P0 (Control) | 0.118 | **Based on Mean** | 1.325 | 0.283 |
| P1 (60 mg / 200grBB) | 0.474 | | | |
| P2 (90 mg / 200grBB) | 0.315 | | | |

### *3.3 Comparability Test*

The mean MDA plasma levels in rats in each treatment group that have been proven to be normally distributed are then compared with the One Way ANOVA test where significant results will be symbolized by p values < 0.05. In this study it was found that the mean MDA Plasma levels in each treatment group had a statistically insignificant difference (p = 0.250). Table 3 shows the complete data.

**TABLE 3: ANOVA Analysis of MDA Plasma Levels in Wistar Rats that Have Been Ovariectomized**

| | Sum of Squares | Mean of Square | Significant (p). |
|---|---|---|---|
| Between Groups | 3.726 | 1.863 | 0.250* |
| Within Group | 33.100 | 1.273 | |
| Total | 36.825 | | |

### *3.4 Post-Hoc Test*

Comparison of mean values between groups was done by Post-Hoc test using Least Significant Difference (LSD). In this study it was found that there were no statistically significant differences in the mean MDA plasma levels between the treatment groups in this study (p <0.05). Table 4 shows the complete data.

**TABLE 4: LSD Post-Hoc Test Results on MDA Plasma Levels in Wistar Rats that Have Been Ovariectomized**

| | P0 | P1 | P2 |
|---|---|---|---|
| P0 | | 0.116 | 0.685 |
| P1 | 0.116 | | 0.222 |
| P2 | 0.685 | 0.222 | |

### *3.5 Discussion*

MDA is the result of lipid peroxidation from polyunsaturated fatty acids, where polyunsaturated fatty acids will oxidize if there is an increase in ROS levels under stress oxidation conditions. This causes MDA to be a marker in indicating the presence of oxidative stress in the body.[8] This study looks at MDA levels with the assumption that there are oxidative stress conditions in the menopause state. When the body begins to age, antioxidant levels decrease plus a decrease in estrogen levels in the female reproductive system causes oxidative stress. Estrogen has an inhibitory effect on 8-hydroxylation guanine in DNA bases which can reduce the production of free radicals.[9]

Red dragon fruit is known to have a lot of antioxidant content, where a study found that red dragon fruit powder has a total flavonoid of 171.79 mg / 100gr, anthocyanin 47.7mg / 100gr, carotene 0.25 mg / 100gr, 157.34 mg / 100 gr phenolic acid, 35.92 mg / alkaloid / 100 gr, and vitamin C 88.17 mg / 100gr as a source of antioxidants.[10] Another study states that red dragon fruit extracts contain beta carotene alkaloids and trepenoids which are antioxidants that break free chain chains. This causes beta carotene to react with peroxyl radicals to produce stable radicals called hydroxyperoxicals (HOO) that react directly with free radicals causing a decrease in free radical levels and oxidative stress.[11] Other antioxidants found in red dragon fruit are anthocyanin pigments derived from the purplish color of red dragon fruit. Anthocyanins work by giving hydrogen atoms to free radicals to convert them into stable forms.[12] In this study no further analysis was carried out to determine the antioxidant content in the red dragon fruit so it cannot be known of the antioxidant content in the red dragon fruit extract.

Based on the descriptive test data in this study, it can be seen that the level of MDA plasma in P0 where the rats did not receive any extract has the lowest mean MDA plasma level compared to other groups. Whereas the highest mean MDA level was found in the group of rats given red dragon fruit ethanol extracts with 60mg / 200gram BB (P1), followed by group of rats given red dragon fruit ethanol extracts with 90mg / 200gram BB (P2). Based on ANOVA analysis test it was found that the mean difference between group was statically insignificant (p=0.250), after multivariate analysis was done using Post-Hoc LSD it was found that it is not statistically significant.

Although various studies found a significant decrease in MDA levels after administration of the red dragon fruit extract, this study did not find any significant difference between the average plasma MDA levels in each treatment group, where in this study the maximum dose given was 90 mg / 200grBB for 30 days.

## 4. CONCLUSION

Based on the results of studies that have been described, it can be concluded that the extract of the red dragon fruit does not reduce MDA levels but does increase levels of Plasma MDA in rats that have undergone ovariectomy. This average increase is not statistically significant. The difference in effectiveness of extracts at different dosage levels cannot be concluded since there is no difference in mean levels of plasma MDA between treatment groups. Further studies with a larger sample size and a longer duration might be possible to show the different in effectiveness at each dose of red dragon fruit ethanol extract.

## REFERENCES

[1] Hoffman B, Schorge J, Halvorson L, Bradshaw K, Cunningham F. 2nd ed. New York City: The McGraw Hill Companies; 2012. William's Gynecology; pp. 1–1399.

[2] Yoshikawa, T. and Naito, Y. (2002). Oxidative Stress. [online] Med.or.jp. Available at: http://www.med.or.jp/english/pdf/2002_07/271_276.pdf [Accessed 15 Jan. 2019].

[3] Yanbaeva D.G., Dentener M.A., Creutzberg E.C., Wesseling G., and Wouters E.F. 2007. Systemic effect of Smoking. Chest 135(5): 1557-1566

[4] Dabelstein W, Reglitzky A, Schütze A, Reders K (2007). "Automotive Fuels". Ullmann's Encyclopedia of Industrial Chemistry. doi:10.1002/14356007.a16_719.pub2. ISBN 978-3-527-30673-2.

[5] Hardjadinata, S. 2010. Budi Daya Buah Naga Super Red Secera Organik. Bogor : Penebar Swadaya

[6] Harahap, N., Simatupang, N., Suprayitno. 2020. Potential of The Red Dragon Fruit (Hylocereus polyrhizus) as an Antioxidant in Strenuous Exercise. Biotechnology; 19(1): 18-22.

[7] Li, Q., Geng, X., Zheng, W., Tang, J., Xu, B. and Shi, Q. (2012). Current understanding of ovarian aging. Science China Life Sciences, [online] 55(8), pp.659-669. Available at: https://www.ncbi.nlm.nih.gov/pubmed/22932881 [Accessed 15 Jan. 2019].

[8] Sari, W., Wahdaningsih. S., Untari, E. 2014. Efek Fraksi n-Heksana Kulit Hylocereus polyhizusTerhadap Kadar Malondialdehida Tikus Stres Oksidatif. Pharm Sci Res; 1(3): 155- 165.

[9] Doshi, S., Agarwal, A. 2013. The role of oxidative stress in menopause. Journal of Mid Life Health; 4(3): 140-146.

[10] Maigoda, T., Sulaeman, A., Setiawan, B., Wibawan, W. 2016. Effects of Red Dragon Fruits (Hylocereus polyrhizus) Powder and Swimming Exercise on Inflammation, Oxidative Stress Markers, and Physical Fitness in Male Obesity Rats (Sprague dawley). International Journal of Sciences: Basic and Applied Research; 25(1): 123-141.

[11] Sani, H., Baharoom, A., Ahmad, M. 2009. Effectiveness of Hylocereus polyrhizus extract in decreasing serum lipids and liver MDA-TBAR level in hypercholesterolemic rats. Sains Malaysiana; 38(2): 271-279.

[12] Mahdi, C., Hendrawan, V., Viestaria, K. 2019. The Effect of Red Pitaya Peel (Hylocereus polyrhizus Extract) on Malondialdehida Levels and Histopathology Profile in Diazinon Induced Rat (Rattus norvegicus). Indones J Cancer Chemoprevent; 10(2): 88-93.