The Potential of Papaya Leaf Extract to Reduce Malondialdehyde Levels in the Gastric Organ of Wistar Rats Given to Used Cooking Oil

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Abstract. Indonesian people use cooking oil extensively in food processing. Free radicals created when cooking oil is repeatedly heated to high temperatures. Oxidative stress is a result of free radical formation in tissues or cells. By Malondialdehyde (MDA), the degree of oxidative stress can be determined. Antioxidants are necessary in order to minimize oxidative damage. Papaya leaves (Carrica papaya L.), containing tannin, alkaloid, terpenoid, saponin, and flavonoid compounds, are one natural ingredient that may reduce free radicals. The purpose of this study is to compare the amounts of MDA in the gastric organs of Wistar rats that were treated with papaya leaf extract and exposed with used cooking oil. The method of this study is using a visible spectrophotometer to measure MDA levels. The initial MDA levels in the gastric organs of Wistar rats were measured at 16.5987 mg/kg before giving them of papaya leaf extract in the control group. After papaya leaf extract treatment, it was determined that the extract decrease MDA levels in the gastric organs of Wistar rats. The rats that received the treatment showed the greatest reduction in MDA levels following the consumption of 110 mg/200 g body weight of papaya leaf extract, which could lower MDA levels by as much as 71%. This result suggest that papaya leaf extract have the ability to reduce MDA levels in the gastric organs of rats.

Keywords: Malondialdehyde, Used Cooking Oil, Papaya Leaf, Wistar Rats, Gastric organ.

1. Introduction

Hemoglobin electrophoresis is the gold standard in screening thalassemia carriers. One culinary item that Indonesians consume on a regular basis is cooking oil. Cooking oil is typically used by the community to prepare food. This is due to the fact that fried food has a higher flavour [1]. Cooking oil that has been used repeatedly at high temperatures during the frying process is referred to as used cooking oil. This reduces the food's calorific value

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and nutritional value, as well as its quality [2]. When cooking oil exposed to high temperatures while utilize it frequently, unsaturated fatty acid chains undergo oxidation, hydrolysis, and polymerization processes that result in the formation of free radicals [3]. If free radical levels are too high, they may damage the normal tissue by dealing with the formation of MDA, lipid coatings in cell walls, blood vessels, prostaglandin production, cell damage, and a decrease in the capacity of cells to adapt to their surroundings.

Excessive exposure to free radicals has the potential to harm DNA and cells in internal organs, including the gastric organ [4]. As one of the digestive system's organs, the gastric organs support the human system break down food and absorb various food essences [5]. Food is going to be crushed in the gastric organ [6]. Before food is transferred to the duodenum, it is stored and processed in the gastric organ. As a result, the gastric organ is constantly exposed to various substances that may cause damage to the gastric organ [7].

Used cooking oil is one thing that may cause damage the cells or tissues in the gastric organ. The presence of free radicals will lead to oxidative stress in the cells, which may affect and harm various cells in the body. MDA can be used to determine the oxidative stress that free radicals produce to cells or tissues [8].

The end product of lipid peroxidation in the body, either by enzymatic or non-enzymatic processes, is malondialdehyde (MDA), a dialdehyde compound [9]. MDA is a sign that free radicals have oxidized unsaturated fatty acids. Long-term oxidative stress triggers cell or tissue damage, which is the main cause of inflammation, atherosclerosis, ischemia, malignancy (cancer), and aging [10].

Antioxidants is needed to reduce the damage caused by oxidative stress. Antioxidants can be put into two main groups: synthetic and natural, based on their chemical type. The synthetic type of antioxidants includes Butyl Hydroxy Anisole (BHA), Butyl Hydroxy Toluene (BHT), and synthetic Zeolite ZSM-5. While the natural antioxidants are derived from many plants like aloe vera, coconut shells, mustard leaves, papaya, and others [11].

The substances that have activity as antibacterial, antifungal, antiseptic, and anti-inflammatory can be extracted from papaya leaves (Carica papaya L.). Papaya leaves contain many different substances such as tannins, alkaloids, terpenoids, saponins, and flavonoids. These compounds have the ability to stimulate the gastric organ's production of progstaglandins and inhibit the production of free radicals [12].

The average MDA level in rats before and after receiving mango juice was decreasing, according to research by [13] on the impact of giving mango juice on the lipid profile and MDA in rats given used cooking oil. This indicates that there was a highly significant decrease in MDA levels in experimental rats from before receiving mango juice. Wening [14] was used to measure the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) after treating papaya leaf juice jelly. The use of papaya leaf juice jelly had a significant impact on reducing MDA.

Based on the background information provided above, studies on MDA levels in the gastric organ organs of Wistar rats given used cooking oil utilizing papaya leaf therapy are required.

2. Materials And Methods

The following materials and equipment were used in this study such as: used cooking oil, 96% ethanol, papaya leaves, wistar rats, distilled water, trichloroacetic acid (TCA), hydrochloric acid, thiobarbituric acid (TBA), chloroform. Also, jars, blenders, measuring glasses, pipettes, enlementers, test tubes, rotary evaporators, sterile gauze, UV spectrophotometer, cuvette, centrifuge, water bath, vortex, filter, stirring rod, and distilled water were utilized.

2.1. Papaya Leaf Extractions

After papaya leaves are separated into leaves and stems, they are cleaned, cut into small pieces, and either dried in the sun or at a temperature below 50ºC in a cabinet dyer. The dried papaya leaves are then ground into a powder by blending them until they are smooth. The powder is prepared for the maceration process by filtering it through a 60 mesh sieve.

The resultant fine powder was soaked for three times for twenty-four hours, stirring periodically, and macerated with 96% ethanol using a 1:2 ratio (1 part papaya leaf powder and two parts 96% ethanol). Following filtering, the filtrate is gathered in a glass beaker.

2.2. Optimizing Wave Length to Determine MDA Level

There are four microtubes prepared. There was 1.4 mL of distilled water in Microtube 1 (which was used as a blank). 1 mL of distilled water was added to each of the microtubes 2-4, and 400 µL of MDA standard at concentrations of 12.5 µg/mL, 25.0 µg/mL, and 50.0 µg/mL was added.

One milliliter distilled water, two hundred microliters each of 10% TCA, 1% TBA, and 1N HCl were added to each of the four microtubes. Afterwards, it was homogenized for ten minutes in a water bath and a vortex formed. Next, let it to reach room temperature until it becomes cold, and then transfer it into a cuvette. At wavelengths of 524-536, absorbance was measured. The ideal wavelength is the outcome of the maximum absorbance attained.

2.3. MDA Standard Curve

Prepare 11 microtubes. There was 1.4 mL of distilled water in Microtube 1 (which was used as a blank). Each of the microtubes 2 through 11 received 400 µL of MDA standard, that range in concentration from 2.5 to 25.0 ppm.

All microtubes were added with 1 mL distilled water, 200 µL 10% TCA, 200 µL 1% TBA and 200 µL 1N HCl. After that, it was homogenized for ten minutes in a water bath at 95 oC and then again using a vortex at 3000 rpm. Next, allow it to reach room temperature until it becomes cold and then transfer it into a cuvette. When measuring absorbance, the ideal wavelength is used. A line equation was then created using the absorbance results.

2.4. Preparation of Experimental Animals

Total of 25 rats were acclimatized before being used for research, with the aim of providing adaptations to the rats to minimize stress. Acclimatization was carried out for 7 days. Rats are given standard food and drink, and their husks are regularly replaced and their cages cleaned.

2.5. Treatment of Rats Samples

A total of 25 Wistar white rats (which had been adapted for 7 days) were divided into 5 groups with 5 rats in each group. The first group (K1) was a negative control, specifically rats that received nothing but food and liquids (without treatment). The second group (K2) served as a positive control, consisted of rats that were given food and drink and exposed to 1 mL of used cooking oil per day for a period of 14 days. This

The treatment groups are the third through fifth groups. The rats in the third, fourth, and fifth groups (P1, P2, and P3) received food and drink, were exposed to used cooking oil, and received treatment with papaya leaf extract. For a period of 14 days, a total of 15 rats received food and drink and were exposed to 1 mL of used cooking oil daily. The rats were given therapy with papaya leaf extract on days 15 to 21 (7 days). Two milliliters (mL) of a solution containing 27.5 mg (P1), 55 mg (P1), and 110 mg (P3) of papaya leaf extract was given to the rats in groups P1, P2, and P3.

2.6. Assessment of MDA Levels in the Rats Stomatch

The rats were given ketamine and xylazine anesthesia on the 22nd day of treatment, and their gastric organs were surgically removed. Rats from each treatment group had their gastric organ organs cleaned with physiological NaCL. Following the grinding and 400 mg weight of the clean organs, 2 mL of physiological NaCl was added, and the centrifuge was run for 10 minutes at 300 rpm. The supernatant was taken.

The supernatant from the gastric organ was then pipetted at 400 µL and added with 1 mL of distilled water, 200 µL of 10% TCA, 200 µL of 1% TBA and 200 µL of 1N HCL and homogenized using a centrifuge at 3000 rpm for 10 minutes, then heated using a water bath at 950C for 10 minutes. After heating, it was cooled to room temperature, and after that the mixture was filtered using filter paper and transferred to a cuvette and then the absorbance was read using a UV-Vis spectrophotometer at the optimum wavelength.

3. Results And Discussion

3.1. Papaya Leaf Extraction

Extraction is made by washing clean papaya leaves, cutting them, then drying them in the oven/sunlight, then grinding them to get papaya leaf powder. The resulting fine powder is macerated with 96% ethanol in a ratio of 1: 2, soaked for 3x24 hours; the filtrate is filtered and then collected in a glass beaker.

3.2. MDA Levels in Rat Gastric Organ

MDA levels of rat's gastric organ were determined by measuring absorbance with a UV-Vis spectrophotometer. The following are the outcomes of the MDA level measurement:

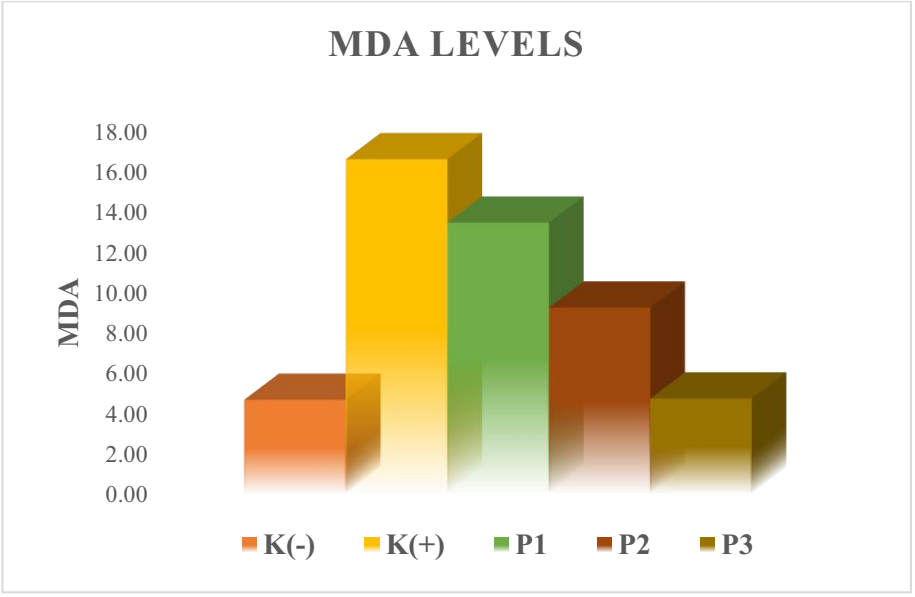

Fig. 1. MDA Levels in Rat's Gastric Organ (mg/kg).

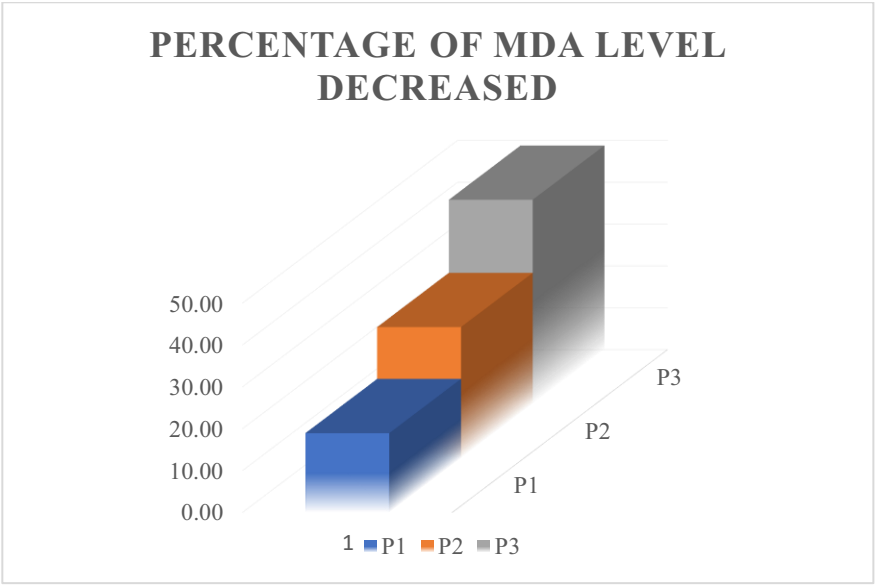

Fig. 2. Percentage of MDA Level Decreased

From Fig 1 and 2 above, it can be seen that the MDA levels in negative control, positive control, treatment 1, 2 and 3 rats were respectively 4.6725, 16.5987, 13.4545, 9.2416 and 4.7347 mg/kg. The highest percentage reduction in MDA levels was obtained in treatment 3 with a reduction percentage of 71.48%.

3.3. Differential Data Analysis

A one-way ANOVA statistical test, which had previously been conducted with a data normality test (Shapiro Wilk), was conducted based on the research findings obtained. The test produced a p-value >0.05, indicating that the data was normally distributed. The data was not homogeneous, as indicated by the homogeneity test result of <0.05, which indicates that the observation data for the treatments 1, 2, and 3 were not identical to those of the negative control and positive control. Subsequently, one way ANOVA was used to test it, and the results showed a significant decrease in MDA levels in the gastric organs of rats treated with different concentrations of papaya leaf extract (sig value < 0.005).

4. Discussion

The purpose of this study was to investigate the effects of giving papaya leaf extract at doses of 27.5, 55, and 110 mg/200g rats body weight on MDA levels in the gastric organs of

Wistar rats that were given 1 mL of used cooking oil per day for 14 days.

The first step in this research was to make papaya leaf extract using the maceration method with a solvent ratio 1:2 (1 part papaya leaf powder and 2 parts 96% ethanol) and soaking for three times for twenty-four hours,. The filtrate was then filtered and collected in a glass beaker. After the solvent had not leaked, the maserate's results were gathered and concentrated using a rotary evaporator, and then a water bath was used to create a thick extract. The maceration method offers several benefits, including the use of a very simple solvent, an easy-to-follow working technique, low operating costs, the ability to extract thermo labile compounds due to the lack of heating, and the extraction itself, which is made with 96% ethanol as the thick solvent (pure), which facilitates identification [15].

To help the rats adjust to their new surroundings, a total of twenty-five Wistar rats were acclimated for seven days. Following this period, the negative control group received nothing but food and drink. Rats that received food and drink and were exposed to 1 mL of spent cooking oil every day for 14 days served as the positive control. The Treatments 1, 2, and 3 groups received food and drink, 1 mL of used cooking oil per day for 14 days, and 7 days of papaya leaf extract therapy. Papaya leaf extract was administered to rats in treatment groups 1, 2, and 3 at concentrations of 27.5, 55, and 110 mg/g body weight. After that, the rats were dissected on the thirtieth day to take gastric organ in order to measure the MDA levels and remove the gastric organs.

MDA levels were measured using spectrophotometry. MDA levels in the gastric organs of the negative control rats were 4.6725 mg/kg. The positive control group of rats was then exposed to 1 mL of used cooking oil per day for 14 days. The positive control had an increase in MDA levels to 16.5987 mg/kg. This study demonstrates that MDA levels rise when exposed to cooking oil. Under typical conditions, the body produces free radicals very slowly. Oxidative instability (stress) is caused by an imbalance between the amount of free radicals and endogenous antioxidants when free radical production exceeds the capacity of endogenous defences. Excessive lipid peroxidation is a result of oxidative stress. MDA is the end product of lipid peroxidation, thus elevated lipid peroxidation may raise the body's MDA levels [3].

Increasing of peroxide levels can be triggered on by oil content damaged by the oxidation process of using oil at high temperatures. This may cause oxidative stress, which in turn raises MDA levels. The end result of lipid peroxidation in the body, either by enzymatic or non-enzymatic processes, is MDA, a dialdehyde compound [9]. MDA is a sign that free radicals are producing unsaturated fatty acids. Used cooking oil has a high concentration of free radicals that can damage cell organs like the gastric structurally by attacking unsaturated fatty acids found in cell membranes or organelles.

For seven days, rats were given 1 mL/day of papaya leaf extract at three different concentrations—27.5, 55, and 110 mg/BW—to prevent oxidative stress and elevated MDA

levels. The MDA level in control + was 16.5987 mg/kg. Following treatment with varying concentrations of papaya leaf extract, the MDA level in treatments 1, 2, and 3 was 13.4545, 9.2416, and 4.7347 mg/kg, respectively.

Based on the results of research carried out using the one way anova statistical test which had previously been carried out with a data normality test (Shapiro Wilk) wirh p-value >0.05, indicating that the data are normally distributed. The data was not homogeneous, as indicated by the homogeneity test result of 0.000, which indicates that the observation data for the treatments 1, 2, and 3 were not identical to those of the negative control and positive control. Subsequently, one way ANOVA was used to test it, and the results showed a significant decrease in MDA levels in the gastric organs of rats treated with different concentrations of papaya leaf extract (< 0.005).

Papaya leaf extract can reduce MDA levels in the gastric organs of rats because papaya leaves contain active ingredients such as flavonoids, tannins, terponoids and saponins. Flavanoids are active ingredients that have anti-inflammatory effects. Flavonoids have the ability to inhibit the enzyme that generates superoxide anion radicals, thereby stopping the reaction and eliminating the radicals [15]. Tannin is a polyphenolic compound with a relatively high molecular weight that possesses antimicrobial, anticancer, antiradical, and antimutagen properties as active ingredients. While saponins are known to have antimicrobial effects, inhibit fungi and can lower cholesterol, have antioxidant, antiviral, and anticarcinogenic properties, and are known to manipulate rumen fermentation, terpenoid compounds exhibit pharmacological activity as antiviral, antibacterial, anti-inflammatory, and as an inhibitor of cholesterol synthesis and anticancer [3][16].

5. Conclusions And Suggestion

5.1. Conclusion

Papaya leaf extract can reduce MDA levels in the gastric organs of rats. The most effective concentration of papaya leaf extract in reducing MDA levels in the gastric organs of Wistar rats is a concentration of 110g/weight of rats which can reduce MDA levels by 71.48%.

5.2. Suggestion

Further research is needed regarding the use of varying concentrations of papaya leaf extract as a therapeutic effect and the length of time between using used cooking oil and papaya leaf extract.

References

1. Sutanto, U. 2016. Antioksidan Pangan. Gadjah Mada University Press. Yogyakarta
2. Nita, N., Dewi, E., Analekta, T. P., Ninditasya, W., & Widhi, W. 2013. Analisis Penggunaan dan Syarat Mutu Minyak Goeng pada Penjaja Makanan di Food Court UAI. Jurnal Al-azhar Indonesia Sari Sains dan Teknologi, 1 (3), 147-150.
3. Erni,W. 2016. Efek Ekstrak Kulit Buah Rambutan Terhadap Kadar MDA dan SOD Tikus Yang Dipapar Asap Rokok. Skripsi. Fakultas Matematikan Dan Ilmu Pengetahuan Universitas Negeri Semarang. Semarang
4. Natalia, E. S., & Wasi, S. W. P. 2018. Pengolahan Minyak Goreng Bekas (Jelantah) Sebagai Pengganti Bahan Bakar Minyak Tanah (Biofuel) Bagi Pedagang Gorengan di Sekitar Fmipaunnes. Jurnal Ilmu dasar, 9(3), 90-96.
5. Noviriolla, M., I Ketut, B., I made, K., & Samsuri. 2017. Studi Histopatologi Lambung Tikus Putih Yang Diberi Parasetamol dan Suplemen Propolis. Jurnal Buletin Vateriner Udayana, 9(1), 95-100.
6. Sarini, V.M., & Malita, I., 2019. Rancangan Sistem Pakar Mendiagnosa nPenyakit Lambung Menggunakan Metode Forward Chsining. Jurnal of Informatics and Computer Science, 5(1), 11-15.
7. Yoakim, W.S., A.Agung, G.J., I Made, K., & Luh Made, S., 2017. Perbandingan Gambaran Histopatologi Lambung Tikus Putih (Rattus norvegicus) Jantan Yang Diberi Amoxicillin Dikombinasikan Dengan Asam Mefenamat dan Deksametason. Jurnal Kesehatan, 6(3), 33-39.
8. Andesty, N. P. & Hendri, B. 2020. Potensi Antioksidan Kedelai (Glycine Max L) Terhadap Penangkapan Radikal Bebas. Jurnal Ilmu Kesehatan Sandi Husada, 11(1), 498-504.
9. Novera, H., & Endah, B. P. P. 2018. Pengaruh Ekstrak Buah Naga Merah Terhadap Kadar MDA Tikus yang Diberi Paparan Asap Rokok. Jurnal Kesehatan, 2(1). 56-63..
10. Novrina, S., & Zulham. 2020. Malondialdehyde (MDA). Jurnal Keperawatan dan Fisioterapi, 2(2), 118-226.
11. Ketaren, S. (1986). Pengantar Teknologi Minyak dan lemak Pangan. UI Press. Jakarta
12. Monalisa, M., & Dwi, R.F. 2019. Uji Aktifitas Antibakteri Ekstrak Biji Pepaya (Carica papaya L) Terhadap Pertumbuhan Escherichia coli. Jurnal Ilmiah, 2(2), 95-102.
13. Zaki, I., & Johan, A. 2015. Pengaruh Pemberian Jus Mangga Terhadap Profil Lipid dan Malondialdehyde Pada Tikus Yang Diberi Minyak Jelantah. Jurnal Gizi Indonesia (The Indonesian Journal Of Nutrition), 3(2), 108-115.
14. Wening, Dyah K., Maryanto, S., Purbowati. 2023. The Effect of Papaya Leaf Juice Jelly (Carica papaya L.) on the Levels of SOD (superoxide sismutase) and MDA (malondialdehyde). The 6th International Conference on Energy, Environment, Epidemiology and Information System (ICENIS) 2021: Topic of Energy,

Environment, Epidemiology, and Information System, SemarangMarjoni, R. 2016. Dasar-dasar Fitokimia untuk Diploma III Farmasi. Jakarta : Trans Info Media.

15. Nisa, K. 2017. Pengaruh pemberian Ekstrak Etanol 70% Biji Rambutan (Nephelium lappaceum L) terhadap enaikan kadar SOD dan penurunan kadar MDA Pankreas Mencit (Mus musculus) yang diinduksi Streptozotocin. Universitas Islam Negeri Maulana Malik Ibrahim.
16. Rustiani, E., Fitriani, A., & Wardatun, S. 2021. Analysis of Flavonids and Terpenoids in Ethanol Extract of Colocasia esculenta L. (Schoot) Stalk and Leaves. Journal of Tropical Pharmacy and Chemistry, 5(4), 359–364. https://doi.org/10.25026/jtpc.v5i4.349.