Analysis of Malondialdehyde Levels in The Heart, Small Intestine, and Testes in White Rats Induced MSG with Treatment of Kesum Ethanol Extract (*Polygonum minus Huds.*)

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Abstract. Monosodium glutamate (MSG) is a food additive used as a food flavoring. Excessive use of MSG can increase Reactive Oxygen Species which has an impact on rat organ damage, characterized by increased levels of malondialdehyde (MDA). Kesum leaves (*Polygonum minus Huds*.) contain antioxidants which can reduce MDA levels. Research Purpose to determine the effect of ethanol extract of kesum leaves on reducing MDA levels in the organs of white rats induced MSG. Method is experiment with post-test control group design. This study used 35 male Wistar rats divided into 5 groups, a Control (-) group without treatment, Control (+), P1, P2 and P3 were induced MSG 35 mg/Kg BW for 14 days and P1, P2 and P3 were given ethanol extract of kesum leaves for 7 days with 27.5; 55.0; 110.0 mg/200g rat body weight/day. MDA levels were measured using spectrophotometer. Data were analyzed using One Way ANOVA and Tukey's Post Hoc test. Results MDA levels in the heart, small intestine and testes of mice induced MSG were 6.6020; 15.966; 5.58867 mg/kg. MDA levels in the three organs of mice induced by MSG, then given ethanol extract of kesum leaves (P1, P2, and P3) decreased. The level of kesum leaf ethanol extract which can reduce MDA levels is a maximum of 60%; 92%; 83% is a concentration of 110.0 mg/200gbw rat. Post Hoc Tukey test results show that K(+) is different from P1 (p=0.007), P2 (p=0.000), and P3 (p=0.000). There was an effect of variations in the concentration of kesum leaf ethanol extract on reducing MDA levels. The final conclusion is ethanol extract of kesum leaves (*Polygonum minus Huds*.) can reduce MDA levels in the organs of white rats were induced MSG.

Keywords: Kesum leaves, Malondialdehyde, Monosodium glutamate, Organs.

1. Introduction

Monosodium Glutamate (MSG) is a sodium salt derived from the amino acid glutamate

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with a content of 78% glutamate, 12% sodium and 10% water [1]. According to the Information Handling Services (IHS) report, in 2014 Asia ranked first in both MSG production, exports and consumption [2]. Indonesia is ranked second as the MSG exporting country in the world after China with total exports of 16%. Of the population in Indonesia, around 77.6% consume MSG more than once per day, with the amount of consumption increasing by an average of around 24.1% per year [3]. WHO sets the ADI (Acceptable Daily Intake) for humans at 120 mg/kg BW. If MSG is consumed by someone more than 3gram/day it will cause the effects of Chinese Restaurant Syndrome, problems with the heart, reproductive system and small intestine [1], which will disrupt health. MSG is a combination of monosodium salt components and glutamic acid-L. The glutamate in MSG is not bound to protein molecules, so it can form free radicals [4].

Free radicals cause an increase in sodium salt levels in the blood and make it a toxic substance due to high levels of Reactive Oxygen Species (ROS) which exceed the capacity of endogenous antioxidant enzymes in the body [1]. An increase in ROS will reduce cellular antioxidant capacity so that it can damage organs and cause oxidative stress [5][6]. Oxidative stress causes cell swelling and cellular damage [7], which is characterized by increased levels of malondialdehyde (MDA) [8]. MDA is the result of a lipid peroxidation process between free radicals that attack lipids in rat organs with double carbon bonds.

One way to reduce the level of oxidative stress in the heart, small intestine and testicles is by consuming foods that contain natural antioxidant compounds such as kesum leaves [9]. The kesum leaf plant (*Polygonum minus Huds*.) is one of the endemic plants in the West Kalimantan region, containing natural antioxidant compounds, namely phenolic compounds, steroids, flavonoids (quercetin, myricetin, flavonols), alkaloids, tannins, saponins and beta-carotene which can absorb free radicals [10].

The aim of the research was to prove that the MDA levels in the heart, small intestine, and testes of rats induced by 35 mg/kg MSG were higher than in rats without treatment. To prove that the MDA levels of rats induced by MSG and given a dose of 27.5 doses of kesum leaf ethanol extract; 55.0; and 110.0 mg/kg BW of mice experienced a decrease. Analyzed the relationship between variations in the concentration of ethanol extract of kesum leaves on MDA levels in the organs of white mice induced by MSG.

2. Methods

This type of research is experimental, posttest only with control group design. The minimum sample size is 25 mice divided into 5 treatment groups and an additional 2 mice per treatment. Examination of MDA levels was carried out using the spectrophotometric method.

2.1. Material and Equipment

The equipment used in this research were rat cages, erlenmayer flask, 60 mesh and 100 mesh sieve, measuring flask, water bath, thermometer, analytical balance, oven, mortar, test tube, rat organ container pot, 2mL microtube, dropper pipette, funnel, filter paper, cuvette, UV-Vis spectrophotometer, vortex, yellow tip, blue tip.

The materials used for this research were MSG (Monosodium glutamate), white rat, kesum leaves, rat feed, MDA Raw, HCl 1N (SMART-LAB), NaCl (Merck), TCA 10% (Supelco), TBA 1% (Merck), Aquadest, Ethanol Absolut (Supelco), PBS 1x (Supelco).

2.2. Making Ethanol Extract of Kesum Leaves

Kesum leaves (*Polygonum minus Huds*.) 3kg of clean were dried using a cabinet dryer at a temperature of 35-38°C. After that, it is blended, sifted with a 60 mesh and 100 mesh sieve. Making ethanol extract of kesum leaves using the meseration method (Ubaidah, 2023), then making it with varying concentrations of 27.5; 55.0; 110.0 mg/2mL.

2.3. The Phytochemical Screening Test

The phytochemical screening test for kesum leaf extract is presented in Table 1.

Table 1. The phytochemical screening test

Examination	Reagent	Result
Alkaloids	Larutan Mayer Larutan	Yellowish white precipitate
	Dragen-droff	Orange precipitate
Flavonoids	HCl 2N	Orange precipitate
Steroid / Triterpenoid	Kloroform, CH_3COOH, H_2SO_4	Brownish ring
Saponin	hot water	Constant foam
Tanin	+ $FeCl_3$ 1%	Dark blue solution
	+ Gelatin 1%	White precipitate
Fenol	$FeCl_3$ 10%	Blackish blue solution

2.4. Experimental Animal Treatment

Thirty-five mice were acclimatized for 7 days [11] by being given food and drink, then divided into 5 treatment groups. Negative control (K-) was given standard food and drink, Positive control (K+) and treatment groups (P1, P2, P3). given food and drink and MSG 35 mg/2mL/kg BW for 14 days. Next, groups P1, P2, P3 were given ethanol extract of kesum leaves at successive doses of 27.5; 55.0; 110.0 mg/2mL/200g BW of mice for 7 days. After that, the mice were terminated to have their heart, small intestine and testicles removed.

2.5. Preparation of Mouse Organ Samples

Each mouse organ was washed with physiological NaCl, then placed in 1x PBS solution. Next, the rat organs were weighed at 400mg, add 2mL of PBS once and centrifuged at a speed of 3000rpm for 10 minutes. The resulting supernatant was used to examine MDA levels.

2.6. Check MDA Levels

The stages of examining MDA levels are determining wavelength optimization, creating an MDA standard curve, and examining MDA levels in mouse organ samples. MDA levels were examined using the TBARS test, pipetting 400µl of supernatant from each rat organ and adding 1mL of distilled water, 200µl of 10% TCA, 200µl of 1% TBA and 200µl of 1N HCl and homogenized with Fortex, then heated using a water bath at 95ºC for 10 minutes. After that, it was cooled to room temperature, filtered, and poured into a cuvette to read the absorbance of the sample using a UV-Vis spectrophotometer at a wavelength of 532nm.

2.7. Data Analysis

Data analysis for each organ used the Statistical Package for the Social Sciences (SPSS) with normality test (Shapiro-Wilk), homogeneity test, One way ANOVA test, and Post Hoc test.

3. Result and Discussion

The yield results of the ethanol extract of kesum leaves (*Polygonum minus Huds*.) are presented in Table 2.

Table 2. Results of leaves kesum extract yield.

Examination	Reagent	Result	Information
Alkaloids	Larutan Mayer	Yellowish white precipitate	+
	Larutan Dragen-droff	Orange precipitate	+
Flavonoids	HCl 2N	Orange precipitate	+
Steroid/ Triterpenoid	Kloroform, CH_3COOH, H_2SO_4	brownish ring	+
Saponin	hot water	constant foam	+
Tanin	+ $FeCl_3$ 1%	dark blue solution	+
	+ Gelatin 1%	white precipitate	+
Fenol	+ $FeCl_3$ 10%	Blackish blue solution	+

Kesum leaf ethanol extract contains alkaloids, flavonoids, triterpenoids, saponins, tannins and phenols (Table 2). Wavelength optimization using 2.5 ppm; 5.0 ppm and 10.0 ppm MDA standard solution were read with a spectrophotometer at wavelengths 524, 526, 528, 530, 532, 534, 536nm. The absorbance versus wavelength curve is shown in Figure 1.

The standard absorbance of MDA at a wavelength of 524-532 nm increased, while at a wavelength of 534-536 nm the absorbance decreased, so it can be seen that the maximum wavelength of MDA is 532 nm. The MDA standard of 0.5-5.0 mg/L was intrapolated with the absorbance to obtain the standard curve presented in Figure 2.

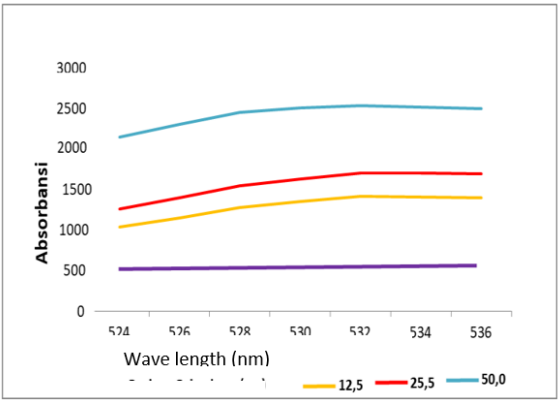

Fig. 1. Wavelength Optimization.

The MDA standard curve produces the line equation y=0.5605x + 0.1272 with R2 = 0.9771. The MDA levels in the organs of white mice were calculated using the line equation formula. The MDA levels obtained in the heart, small intestine and testicular organ samples are presented in Table 4.

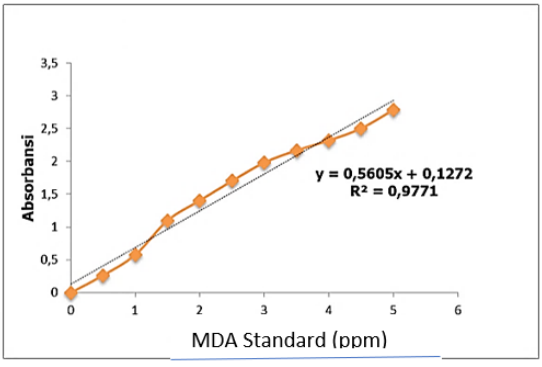

Fig. 2. MDA Standard Curve.

Table 4. Wistar Rat Organ MDA levels in the heart, small intestine and testes.

Treatment Group	Mean ± SD (mg/kg)			Information
	Heart	Small Intestine	Testes	
K(-)	0.5980 ±0.2105	0.5805 ±0.2429	1.4064 ±0.1899	given Feed+Drink
K(+)	6.6020 ±1.1284	15.966 ±2.1543	5.8867 ±0.8604	MSG 35mg/KgBB
P1	4.2701 ±0.3385	9.4524 ±1.0192	3.2884 ±0.3817	MSG+EDK 27.5mg/200gBB
P2	3.0151 ±0.9088	4.0987 ±1.3761	2.2449 ±0.3125	MSG+EDK 55 mg/200gBB
P3	2.6532 ±0.8612	1.2680 ±0.4629	0.9980 ±0.1430	MSG+EDK 110 mg/200gBB

Note = EDK: Kesum Leaf Extract, MSG: Monosodium glutamate, SD: Standard Deviation

Rat organ MDA levels in K(+) heart, small intestine, testes have an average value of 6.6020; 15,966; 5.58867 mg/kg which is higher than the levels in K(-). This is because exposure to MSG in mice produces free radical compounds due to MSG metabolism reacting with Poly Unsaturated Fatty Acid (PUFA) which is the main target of free radicals in cell membranes and produces toxic and reactive aldehyde metabolites. The formation of lipid radicals reacts with oxygen atoms (O2) to form peroxyl radicals (O=O) and produces MDA [12]. MDA is one of the main aldehydes that is formed and is toxic to body cells and causes oxidative damage. An excessive increase in Reactive Oxygen Species (ROS) can reduce endogenous antioxidants in the body, causing damage to rat organs, characterized by an increase in MDA levels. This is in accordance with the results of Maulina's research that MSG consumed continuously will accumulate in the body, thereby causing damage to several organs, namely the heart, neurological, respiratory, gastrointestinal tract, muscles, genital and urinary tract, skin, kidneys, liver. and vision [13].

In the treatment group, mice were induced by MSG and given ethanol extract of kesum leaves with a concentration of 27.5; 55.0 and 110.0 mg/200g BW of rats/day, MDA levels in rat organs decreased compared to MDA K(+) levels. This is because the ethanol extract of kesum leaves contains antioxidant alkaloids, flavonoids, triterpenoids, saponins, tannins and phenols which can provide electrons to free radicals that lack electrons, so that oxidative stress is reduced and MDA levels decrease. This is in line with Purwaningsih's research that kesum leaves contain phenol, steroid, flavonoid, alkaloid, tannin, saponin, terpenoid and beta-carotene compounds and have been reported to have very strong antioxidants because they have an IC50 < 50 ppm, namely 20,632 ppm [14].

Kesum leaf antioxidants are able to neutralize free radicals in the body by providing hydrogen atoms to free radical compounds so that they become stable and prevent chain

reactions. The flavonoid compound content works by donating hydrogen atoms and increasing the expression of endogenous antioxidant genes through activation of Nuclear Factor Erythroid 2 Relates Factor 2 (Nrf2) so that the expression of the SOD (superoxide dismutase) gene increases. Tannin compounds have OH groups that can reduce free radicals and act as scavengers of hydrogen peroxide (H2O2), so that H2O2 does not react further to form hydroxyl radicals (OH-) and lipid peroxidation [15]. The percentage reduction in MDA levels in treatment groups 1, 2, and 3 can be seen in Figure 3.

The percentage reduction in MDA levels increased from P1, P2, and P3 compared to K+. The higher the concentration of kesum leaf extract, the greater the antioxidant content, so the ability to reduce MDA levels in the organs of Wistar rats induced by MSG is also greater. The highest percentage reduction in MDA levels was obtained when giving ethanol extract of kesum leaves at a concentration of 110mg/200g BW to rats. The higher the concentration of kesum leaf extract means the amount of antioxidant substances increases, so that oxidative stress in rat organs decreases, as indicated by a decrease in MDA levels in rat organs [16]. The results of this study are in line with research by Tobing (2017) that administering extract therapy with a dose of 1.28 mg/day of aloe vera extract can reduce MDA levels in the stomach organs of rats induced by Indomethacin. So the higher the dose of extract given in the study, the lower the MDA levels.

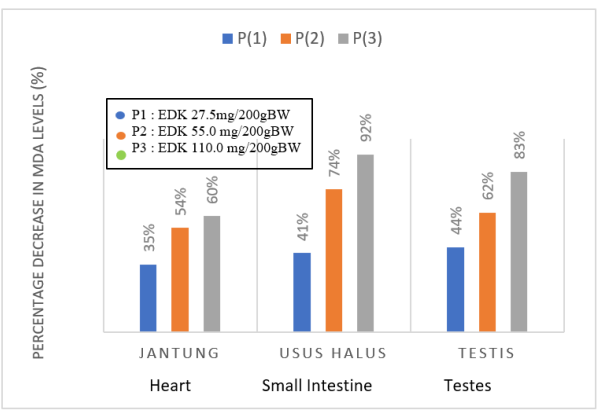

Fig. 3. Percentage Decrease in MDA in Rat Organs.

Data analysis was continued using bivariate analysis including normality test, homogeneity test, One way ANOVA test and Post Hoc test using the Statistical Package for the Social Sciences (SPSS) method. The following results were obtained: Normality test results using the Shapiro-Wilk test for all groups were obtained. p-value ≥0.05 means that the data is normally distributed. Data analysis continued by calculating the similarity of data variations (homogeneity) using the Levene's Test.

This research obtained data on MDA levels which were analyzed with SPSS using the analysis of variance test. Before analyzing the ANOVA test, a normality test and data homogeneity test were first carried out. The results of the ANOVA analysis showed that the p-value for examining the MDA of the heart, small intestine and testes of mice was 0.000 (p□0.05) so that it meant there was a difference. The results of the ANOVA test followed by the Post Hoc Tukey test to see significant differences between treatment groups showed that K(-) was significantly different from K(+) with the respective values for the heart, small intestine, and testes of mice, namely (p = 0.000). This is because in K(-) the experimental animals were only given food and drink, while the K(+) experimental animals were induced by MSG which can damage the mice's organs thereby increasing MDA levels.

The K(+) of the heart, small intestine and testes of mice is different from the P1 of the heart, small intestine and testes of mice, namely (0.001; 0.000; 0.000), P2 (0.000; 0.000; 0.000), and P3 (0.000; 0.000; 0.000). Varying concentrations of kesum leaf extract have an effect on reducing MDA levels. The most effective concentration of kesum leaves was in the treatment group (P3) 110.0mg/200g BW of mice, which reduced MDA levels in the heart, small intestine and testes of mice by 60%; 92%; 83%.

4. Conclusion

There was an effect of variations in the concentration of kesum leaf ethanol extract on reducing MDA levels. The effective level of ethanol extract of kesum leaves is 110.0 mg/200gBW in mice. It can reduce MDA levels in the heart, small intestine and testes of mice by the highest, respectively 60%; 92%; 83%.

Authors' Contributions. All authors contributed equally to this work.

References

1. Yonata A dan Indah I. Efek Toksik Konsumsi Monosodium Glutamate, Semarang. FK Unila. 2016
2. Theodorus E, Muhartono M, Putri G. T. Pengaruh Pemberian Ekstrak Etanol Rimpang Lengkuas (Alpinia Galanga) Terhadap Gambaran Histopatologi Otak Mencit (Mus Musculus L) Yang Diinduksi Monosodium Glutamate. Semarang. FK Unila. 2019
3. Kurtanty D, Daeng M. F, Upa, N. P. Monosodium Glutamat How To Understand It Properly, Jakarta: Primer Koperasi Ikatan Dokter Indonesia, 11, p. 1. 2018.
4. Simon H, Muhartomo H, Pudjonarko D. Pengaruh Pemberian Monosodium Glutamat peroral terhadap Degenerasi Neuron Piramidal CA1 Hipokampus pada Tikus Wistar. Medica Hospitalia. Universitas Diponegoro. 2013. 1(3):175–181. DOI: https://doi.org/10.36408/mhjcm.v1i3.67

5. Pratama M. R. Dampak Mengkonsumsi Alkohol terhadap Kesehatan Lambung. Lampung. FK Unila. 2019
6. Chen D, Huang C, & Chen Z. A review for the pharmacological effect of lycopene in central nervous system disorders. Biomedicine & Pharmacotherapy. Nantong University Cina. 2019. 111, 791-801. DOI: https://doi.org/10.1016/j.biopha.2018.12.151
7. Larasati S, Rahman H, Wigati S. Kajian Ilmiah Problema Kesehatan Gambaran Histologis Jantung pada Pemberian Monosodium Glutamate (MSG). Jurnal Endurance : Kajian Ilmiah Problema Kesehatan. Universitas Jambi. 2020. 5(2): 259–270. DOI: http://doi.org/10.22216/jen.v5i2.4698
8. Situmorang N, Zulham Z. Malondialdehyde (MDA) (Zat Oksidan Yang Mempercepat Proses Penuaan). Jurnal Keperawatan Dan Fisioterapi (JKF). Universitas Sumatera Utara. 2020. 2 (2): 117–123. DOI: https://doi.org/10.35451/jkf.v2i2.338
9. Togatorop D, Pasiak T. F, Wongkar D, Kaseke, M. M. Gambaran histologik ginjal tikus Wistar yang diberikan jus tomat setelah diinduksi dengan monosodium glutamat. Jurnal e-Biomedik (eBm). Universitas Sam Ratulangi. 2016. 4(2). DOI: https://doi.org/10.35790/ebm.v4i2.14664
10. Christapher P, Parasuraman S, Christina J, Asmawi M. Z, Vikneswaran M. Review on *polygonum minus. Huds*, a commonly used food additive in Southeast Asia. Pharmacognosy Research. Medknow Publications. 2015. 7(1): 1–6. DOI: 10.4103/0974-8490.147125
11. Dalimunthe T. A, Hariaji I. Pengaruh Pemberian Jus Nanas (Ananas comosus l.merr) terhadap Penurunan Kadar Trigliserida pada Tikus Putih Jantan (Rattus novergicus l.) yang Diinduksi Monosodium Glutamate. Medan. FK Umsu. 2017
12. Mayangsari E, Kalsum U, Pragiwaksana R. G. Efek Ekstrak Daun Kencana Ungu (Ruellia Tuberosa) Terhadap Kadar Malondialdehida (MDA) Usus Tikus Yang Diinduksi Indometasin. Majalah Kesehatan. Universitas Brawijaya. 2020. 7(2). DOI: https://doi.org/10.21776/ub.majalahkesehatan.2020.07.02.3
13. Maulina M. Zat-Zat yang Mempengaruhi Histopatologi Hepar, Unimal Press, 49, p. 1. 2018.
14. Purwaningsih I, Sapriani R, Indrawati R. Aktivitas Antioksidan Ekstrak Metanol Daun Kesum (*Polygo-num minus Huds*.) Metode DPPH. Jurnal Laboratorium Katulistiwa. Analis Poltekes Pontianak. 2018. 2(2): 161-165. DOI:10.30602/jlk.v1i2.156
15. Firdaus A. N, Dewi A. R, Purnomo Y. Efek Dekokta Daun Pulutan (Urena Lobata) Terhadap Kadar Superoxide Dismutase (SOD) Jaringan Otak Dan Motilitas Ikan Zebra (Danio Rerio) Fase Juvenil Yang Dipapar Malathion Secara Kronis. Malang. FK Unisma. 2015
16. Oktaviananda T. Z. Pengaruh Pemberian Ekstrak Biji Anggur (Vitis vinifera) Terhadap Kadar MDA (Malondialdehyde) dan Gambaran Histopatologi Bronkus Pada Tikus

Putih Model (Rattus norvegicus) Strain Wistar Yang Diberi Paparan Asap Rokok. Malang. FKH Universitas Brawijaya. 2017.