PROTECTIVE EFFECT OF JAVA WOOD BARK EXTRACT (*LANNEA COROMANDELICA*) ON LDH LEVEL, CKMB LEVEL AND HEART HISTOPATHOLOGYIN MALE WISTAR RATS INDUCED BY MSG

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Abstract

Monosodium glutamate (MSG) is used as a flavor enhancer with the aim of increasing sweetness and saltiness, and reducing sourness and bitterness. L-glutamate binds to taste cell receptors in the taste organ and gives rise to the umami taste, which is the characteristic flavor imparted by MSG. There is an effect of Monosodium Glutamate (MSG) on organ damage, increased cardiovascular risk factors and resulting oxidative stress due to ROS. To prevent the undesirable effects of free radicals, antioxidants are needed. One of the plants that can be used as a natural medicine and rich in antioxidants is the Java Wood plant (*Lannea coromandelica*). It is known that the stems and leaves contain saponins, flavonoids, and tannins. Flavonoids can function as antioxidants that prevent free radicals. This study aims to determine the protective effect of Lannea Coromandelica extract on the levels of LDH enzymes, CK-MB, and MSG-induced heart histology. Rats were divided into five treatment groups (KS = 0mg, KN = 3 ml/gBB, KPA = 250mg/KgBB, KPB = 500mg/KgBB, and KPC 750mg/KgBB), each group consisting of 6 rats. MSG administration was carried out for 28 days, then examined LDH enzymes, CKMB and heart histology. The results of LDH enzyme levels, CKMB were analyzed using one-way ANOVA test while the percentage of histological damage was analyzed using Kruskal-Wallis test. The results showed that there was a significant difference in blood CKMB levels ($p<0.05$), while blood LDH levels did not show a significant difference ($p>0.05$). It was concluded that dosing MSG and Lannea Coromandelica Extract for 28 days can cause mild damage to the histological structure of the rat heart and no visible effect on LDH levels.

Keywords: Monosodium Gluatamate, Histology, LDH, CKMB

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1. Introduction

Food flavoring ingredients are used to preserve or add delicious flavor to food. One widely used food flavoring is *Monosodium Glutamate* (MSG), a sodium salt of glutamic acid. Glutamic acid is one of the amino acids that is also contained in food, usually around 5%-20% of the total amino acid content, either in free form or bound to peptides or proteins. Glutamic acid levels in human blood begin to increase significantly at consumption of 150 mg/kgBB/day, this effect will be strong if consumption inthe short term and large or in high doses (3gr or more in one meal). MSG is easier to cause effects when served in the form of soupy food (Larasati, 2020). Excessive consumption of MSG can cause damage to several parts of the body such as the heart, skin, respiratory system, digestion, vision, and nervous system. There is an effect of *Monosodium Glutamate* (MSG) on organ damage, increasing cardiovascular risk factors and causing oxidative stress. Increased levels of glutamate in the blood willcause increased expression of glutamate receptors in the central nervous system (CNS) resulting in increased formation of *reactive oxygen species* (ROS), as a trigger for oxidative stress that can lead to cell damage. Oxidative stress is a state of imbalance between prooxidants and anti-oxidants and excess production of free radicals. This is in line with research conducted by (Diana, 2018) that giving MSG to rats will interfere with lipid metabolism, causing the risk of hypertension and heart disease. In addition, the administration of MSG as much as 4 mg / gr body weight can cause oxidative stress so that it can act as a predisposing factor that initiates the occurrence of atherosclerosis. The heart muscle can be damaged due to atherosclerosis of the heart blood vessels and can result in ischemia to infarctionof the heart muscle. Glutamic acid metabolism in tissues occurs through the process of oxidative deamination or through transamination with pyruvate which produces *oxaloacetic acid* with the help of α-ketoglutarate. Glutamate metabolism is important to the process of decarboxylation into *gamma- aminobutyrate (*GABA) and amidation of glutamine. (Faqih, 2018). Glutamate in MSG plays a role inactivating glutamate receptors that help in the entry of Ca^{2+} (N.Taufik, 2012).

To prevent the undesirable effects of free radicals, antioxidants are needed. The use of antioxidants has begun to be widely used along with the increasing understanding of the role of antioxidants in the inhibition of degenerative diseases such as heart disease, arteriosclerosis, cancer and symptoms of aging. Plants contain various free radical inhibiting molecules, such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and several other endogenous metabolites rich in antioxidant activity. One of the plants that can be used as a natural medicinal material and is rich in antioxidants is the Kayu Jawa plant (*Lannea coromandelica*) from the Anacardiaceae family. Phytochemical analysis of *Lannea coromandelica* leaf extracts revealed the presence of phenolic and flavonoid compounds that act as antioxidants, as well as ß-sitosterol, ellagic acid, quercetin, quercetin-3-arabinoside, leucocyanidin and leucodelphinidin. Based on research, it is knownthat the stems and leaves contain saponins, flavonoids, and tannins. Flavonoids can function as antioxidants that prevent free radicals. Research conducted at Thailand Universitay Khan Kaen Facultyof Pharmaceutical Sciences, showed that ethanol extract of Java wood can inhibit free radicals (Febriansyah 2019).

It is known that the excessive effects of MSG can trigger an increase in several compounds in cellular activity which causes an increase in ROS compounds in cells and initiates oxidative stress. Oxidative stress will cause cell swelling and lead to cellular damage and result in various cardiovascular diseases so that the medicinal plant *Lannea coromandelica is* needed which contains various free

radical inhibiting molecules but this research is still very limited, so on this basis the researchers are interested in conducting research on the effect of *Lannea coromandelica* administration on cardiac histopathologyin MSG-induced male Wistar rats.

2. Materials and Methods

Preparation of Monosodium Glutamate (MSG)

Monosodium glutamate used was obtained from the free market with the trademark Ajinomoto produced by PT Ajinomoto Indonesia, MSG powder in the form of white crystals containing pure monosodium glutamate. The daily dose given to rats is 3 mg/gBB rat.

Preparation of *Lannea coromandelica* stem bark extraction

Lannea coromandelica bark was obtained from Pinrang Regency, South Sulawesi Province, Indonesia. 100 g samples were weighed and put into a reflux device. Then 96% ethanol was added until the sample was submerged. The reflux process was carried out for 3-4 hours. After that, filtering was carried out, and the filter results were stored to evaporate the solvent. The dregs of the filter were then refluxed again for 3-4 hours.

Preparation of Animal

Twenty-five male Wistar strain rats weighing between 180 to 300 grams and ± 3 months of age were adapted for 7 days in the animal laboratory before the start of the experiment. During this stage, all rats were given standard feed and water ad libitum, rats were housed individually in their respective cages.

Experimental Protocol

Twenty-five male Wistar rats were divided into five groups, the healthy group was given standard food, the negative group was given MSG 3 mg/grBW, treatment group 1 was given MSG and *Lannea coromandelica* extract at 250 mg/kgBW, treatment group 2 was given MSG and *Lannea coromandelica* extract at 500 mg/kgBW while treatment group 3 was given MSG and Lannea *coromandelica extract* at 750 mg/kgBW for 28 days. *Lannea coromandelica* bark extract was administered 1 hour before MSG administration. In this study, MSG and *Lannea coromandelica* bark extract were given to mice through the gavage method, namely by oral administration using a round. Mouse care was carried out in accordance with laboratory animal care standards, and all procedures involving animals were approved by the Animal Ethics Committee of the Faculty of Medicine, Hasanuddin University.

Enzym Level Analysis

Serum collection was done using a hematocrit tube through the eye. Blood samples were inserted into EDTA tubes. The tube containing blood was then centrifuged at 3000 rpm for 10 minutes. Serum obtained was measured for LDH and CKMB levels using the LDH and CKMB regen kit. Then examined Measurement of LDH levels with IFCC using ABX Pentra with LDH IFCC CP regen and CKMB levels with Immunology method. Using the ABX Pentra tool with CKMB RTU regen.

Histopathological examination

The rat heart was washed with saline solution, followed by 10% formalin fixation. The fixed tissue was placed in pa|rafin and cut into several sections (each section had a thickness of 5 µm). Each section was stained with hematoxylin and eosin (H&E). Microscopic observations

were made using a microscope. The microscope observed the presence of heart damage based on the damage degree score by looking for the presence of necrosis cells.

Statistical Analysis

Statistical analyses were performed using SPSS IBM 22 software. Data distribution was examined using the Saphiro Wilk test to determine whether the data were normally distributed or not. If the data were normally distributed, the analysis continued with ANOVA, while if the data were not normally distributed, the Mann-Whitney test was used. Differences were considered significant if the p value was <0.05.

3. Results and Discussion

Lactate Dehydrogenase (LDH) Enzyme Level Result

Comparison of LDH values in each group can be seen in the graph in Figure 1.

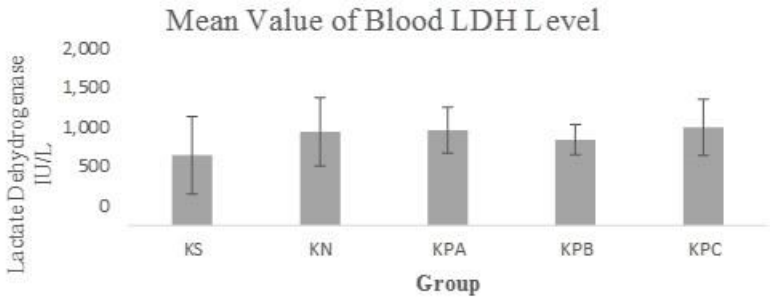

Figure 1. The graph above shows the mean value of blood LDH in each treatment group. Data presented as mean± SD (n=5)

The graph above shows that the lowest LDH value is owned by the healthy control group that is not given any treatment. Unlike the negative control group which has an LDH level value of 1178 ± 429 IU/L. In treatment group A (MSG + Lannea Extract 250 mg/gBB) LDH enzyme levels obtained an average value of 1199 ± 287.06 IU/L. Then there was a decrease in enzyme levels in treatment group B (MSG + Lannea Extract 500 mg/gBB) which had LDH levels of 1079 ± 188.92 IU/L. Treatment group C (MSG + Lannea Extract 750 mg/gBB) obtained a value of 1234 ± 400 IU/L. From the graph above, it can be seen that the administration of MSG as much as 3mg / kgBB can increase LDH levels,while the treatment group given Lannea Extract decreased LDH levels.

Creatine Kinase Myocardial Band (CKMB) Level Results

Comparison of CKMB values in each group can be seen in Figure 2.

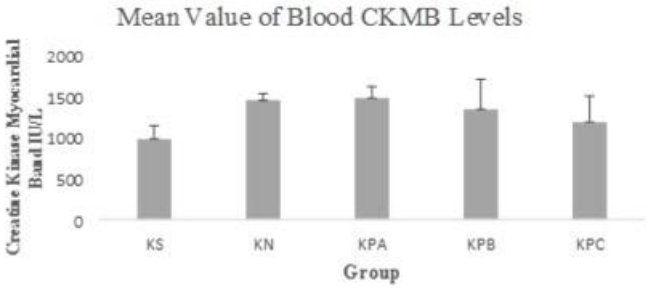

Figure 2. The graph above shows the mean value of blood CKMB in each treatment group. Data presented as mean± SD (n=5)

The figure above shows the value of CKMB enzyme levels in the healthy control group of 975.4 ±

161.17 IU/L. The enzyme levels in the negative control group were 1444 ± 79.75 IU/L. In treatment group A, the enzyme level obtained was 1470 ± 140.00 IU/L and then there was a decrease in enzyme levels in treatment group B of 1337 ± 363.73 IU/L. followed by treatment group C enzyme levels decreased significantly, namely 1181 ± 312.67 IU/L. Based on the graph above, it can be seen that the lowest CKMB enzyme levels in the treatment group were in treatment group C which was given MSG + Lannea Extract 750mg/gBB. While the negative control group and treatment group A have almost the same CKMB levels.

Heart Histopathology

The results of histopathological examination showed that in the healthy control group there was no tissue damage with a degree score of 0. While in the negative control group, treatment group A (MSG + Lannea Extract 250 mg / kgBB) and treatment group B (500 mg / kgBB) had the same average value of 0.33 ± 0.577. While the highest average value was obtained by treatment group C (MSG + Lannea Extract 750mg / kgBB).

The following are images of heart histopathology based on damage parameters in each group

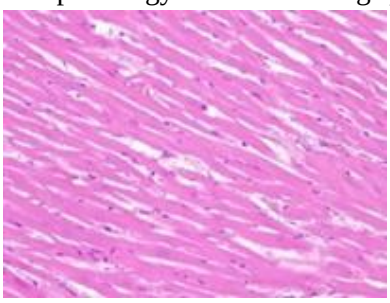

Figure 3. histopathology of the heart in the healthy control group. Showing no occurrence of damage

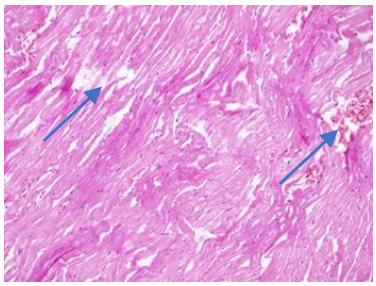

Figure 4. Histopathology picture of the heart in the negative control group. The picture showsthe presence of necrotic cells with a mild degree of damage: necrotic cells < 25%.

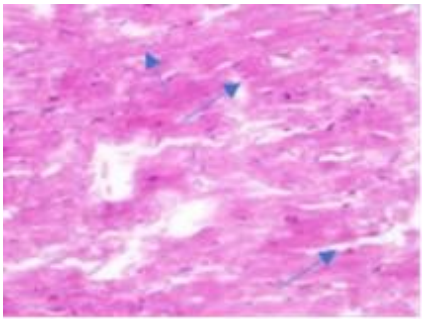

Figure 5. Histopathological picture of the heart in treatment group A. The picture shows thepresence of nectrotic cells with a mild degree of damage: necrotic cells < 25%.

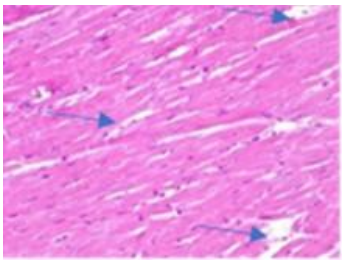

Figure 6. Histopathological picture of the heart of treatment group B. The picture shows thepresence of necrotic cells with a mild degree of damage: necrotic cells < 25%.

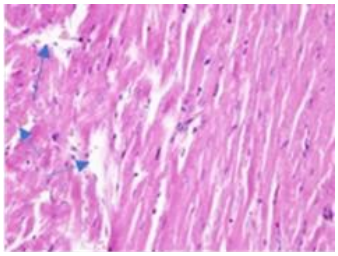

Figure 7. Histopathological picture of the Heart of Treatment Group C. The picture shows the presence of necrotic cells with a moderate degree of damage: necrotic cells 25-50%

Effect of *Lannea coromandelica* extract on LDH enzyme in MSG-induced rats

LDH catalyzes the reduction of pyruvate to lactate and produces NAD+. The product, lactate, is a by-product of this reaction. LDH affects the process of lactic acid formation, and LDH and lactic acid levels generally increase if there is cell damage (Novara, T., Harini, I, 2019). The National Cancer Institute in 2007 stated that the LDH value can describe the degree of damage that occurs in the tissue.(Hanifa, I, N, R. Andriani, and Handoko, 2017).. The results of statistical analysis showed a normality value of P = 0.251 (P>0.05), meaning that the data had a normal distribution. Then proceed with the homogeneity test and the results obtained are P = 0.251 (P>0.05) which means the data is homogeneous. After conducting normality and homogeneity tests, it is continued with the Anova test to see the average difference between groups. The Anova test results show P = 0.581 (P>0.05) which means there is no significant difference in value.

Increased LDH levels as an indicator of heart muscle cell disorders caused by MSG induction. An increase in free radicals can lead to lipid peroxidation in the cell membrane which inhibits the supply of oxygen to the mitochondria for energy formation. Thus, a decrease in oxygen supply to cells results in decreased metabolism of heart muscle cells in producing ATP. Therefore, to meet the needs of ATP cell metabolism from aerobic (using oxygen) will switch to anaerobic metabolism (Nonoxygen). This metabolism is catalyzed by the enzyme lactate dehydrogenase (Khoiriyah, S. I., As, N. A., & Zayadi, 2019).. Thus, if free radicals in the body increase followed by cell damage, the levels of lactate dehydrogenase (LDH) enzyme released from cells in the bloodstream are higher. The results of the average value of LDH enzyme levels show that there is a difference between the negative control group and the treatment group where the average value of the negative group is higher than the average value of the treatment group. This shows that the Java Wood stem is useful as an antioxidant that contains saponins, flavonoids, and tannins. (Galanki, V.; Venkatesham and K, 2014).. Flavonoids function as antioxidants that can prevent oxidation of substrates in chain reactions, so that antioxidants are able to provide protection to heart muscle cells from free radicals, by transferring their electrons to free radical molecules, so that free radicals can be stabilized and chain reactions are stopped.(Science and Health, no date) This has an impact on reducing the levels of the enzyme lactate dehydrogenase (LDH). Other phenolic compounds found in Java wood, namely tannins, are complex compounds that also combine with flavanoids to play a role in counteracting free radicals in the body that interfere with the function of heart muscle cells. (Risfianty and Sanuriza, 2021)..

Effect of *Lannea coromandelica* extract on CKMB enzyme in MSG-induced rats

One of the biomarkers to detect cardiac muscle cell death is CKMB (Creatine Kinase Muscle Brain). CKMB is found in the heart muscle, so total serum creatine kinase and CKMB concentrations increase when there is injury to the myocardium. Based on research conducted by Djabir YY, Arsyad MA, Sartini (2017)The normal value of CKMB ranges from 55.0 to 179.0 UI/L, these results are different from those obtained due to differences in measurement methods and differences in reagents used. From the results of CKMB measurements after 28 days of being given MSG and Lannea Extract, it was found that there was a significant increase in CKMB in the group of rats that were only given MSG (negative control group). The increase in CKMB in the negative control group indicates the occurrence of myocardial infarction or death in heart muscle cells which causes the release of CKMB from the heart muscle cells into the blood circulation. While CKMB levels began to decrease in treatment group A (MSG and Lannea Extract 250mg/KgBB) followed by treatment group B (Lannea Extract 500mg/KgBB) and treatment group C (Lannea Extract 750mg/KgBB). This indicates that Java wood stem which has antioxidant content can prevent the increase in CKMB enzyme levels due to MSG induction. The decrease in CK-MB enzyme levels in rat blood serum can result from increased relaxation and NO synthesis in smooth muscle and endothelial cells that stimulate guanylate cyclase to form cGMP so that vasodilation occurs. Ca2+ canal blockage occurs so that there is no increase in intracellular Ca2+ and MLC dephospholiration finally inhibits muscle contraction which is characterized by lower levels of CK-MB enzymes in the treatment than the negative control. (Qurroti A'yun et al., 2019)

Effect of *Lannea coromandelica* extract on Heart Histopatology enzyme in MSG-induced rats

The heart organ can be damaged by various types of chemical compounds. One compound that can damage heart function is free radicals or reactive oxygen species (ROS). Free radicals through lipid peroxides damage the mitochondria of the heart where ATP is formed which plays a role in blood circulation in the body(Sukalingan, 2016). Heart damage caused by free radicals can be inhibited by a chemical compound, namely antioxidants. Research that has been done Rahayu et al. (2021) found that there was a significant increase in heart mass in rats given MSG. In this study, the results obtained in the negative control treatment with an average value of 0.33 ± 0.577. These results are the same as treatment group A (MSG + 250mg / kgBB) and treatment group B (MSG + 500 mg / kgBB). While in treatment group C, different results were obtained with an average value of 1.00 ± 1.00. Based on the comparative analysis of the negative control group which was only given MSG on the histopathology of the heart, the value of P = 0.502 (P>0.05) meant that there was no significant difference between the groups. Histopathological examination of the heart in healthy control group animals found no histological changes with a degree of damage 0. While in the negative control group, treatment group A (MSG + Lannea Extract 500mg / KgBB) and treatment group B (MSG + 500mg/ kgBB) obtained histopathological analysis showing mild damage with a score of 1 where there are necrotic cells among normal cells or necrotic cells < 25% of the entire field of view. In treatment group C (MSG + 750mg/KgBB) the results obtained were that there was moderate damage to the tissue with a degree of damage 2: necrotic cells 25-50%. Based on the results of the analysis of the average histopathology score of heart damage where the average score of each group is not much different so that the damage that occurs in each group is also not much different. This shows that the administration of Lannea extract is not able to prevent heart damage due to MSG induction. Changes in the histology structure of the heart of rats given MSG for 28 days are caused by the active substance monosodium glutamate itself. According to (N, 2012) Glutamate in MSG (Monosodium Glutamate) will activate glutamate receptors that help in the entry of $Ca2+$. With excessive levels of intracellular $CC2+$, it triggers the activation of NO synthase and protein kinase which will stimulate the formation of free radicals that cause cell damage. The increase of several compounds in cellular activity causes an increase in ROS compounds in the cell and initiates oxidative stress in the cell which causes cell swelling and cellular damage.

Lannea coromandelica (Houtt.) Merr stem bark contains secondary metabolites: terpenoids, steroids, saponins, flavonoids, tannins and cardiac glycosides. The plant can be utilized by its leaves and stem bark by pounding or boiling to treat external wounds, internal wounds, and postpartum care. Stem bark can be used as a treatment for stomach pain, leprosy, ulcer and heart disease. (N, 2012).

Flavonoid content of phenolic compounds has strong antioxidant properties. The role of flavonoids as antioxidants is to capture unstable free radicals by donating one electron.

4. Conclusion

Based on the research that has been conducted, Lannea coromandelica extract has an effect that can reduce the increase in LDH enzyme and CKMB enzyme levels in MSG-induced heart damage and has no potential to repair damage to the heart histopathology of male Wistar rats due to MSG induction.

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