PROTECTIVE EFFECT OF LANNEA COROMANDELICA EXTRACT ON MDA LEVELS, SGOT/SGPT LEVELS AND LIVER HISTOPATHOLOGY IN MSG INDUCED MALE WISTAR RATS

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Abstract

Monosodium glutamate (MSG) is the sodium salt of glutamic acid which has been consumed widely throughout the world as a food flavor enhancer. Monosodium glutamate (MSG) is classified as a safe substance, but long-term consumption of MSG causes a number of toxic effects and is considered an excitotoxin, causing harmful effects on organs such as liver toxicity. MSG accumulation can cause hepatocyte damage due to the effects of free radicals, so important substances such as antioxidants are needed to reduce the negative effects of free radicals. The content of Java wood (*Lannea coromandelica)* was obtained, namely flavonoid compounds, terpenoids/steroids and tannins which are antioxidants so that they can overcome and prevent free radicals. The purpose of this study was to determine the protective effect of *Lannea coromandelica* extract on MDA, SGOT/SGPT levels and histopathology of rat liver induced by MSG. There were 5 treatment groups including healthy control (KS) without treatment, negative control (KN = MSG 3mg/BB, treatment control (KPA= extract 250 mg/kgBB + MSG), (KPB = extract 500 mg/kgBB + MSG) and (KPC = extract 750 mg/kgBB + MSG) orally. After 28 days blood and liver samples were taken. Data were analyzed using the ANOVA test on MDA, SGOT/SGPT levels in blood and Kruskal-Wallis test on liver damage level analysis The results showed that there were significant differences in blood levels of MDA, SGOT and SGPT ($p<0.05$), whereas there were no significant differences in the histopathological structure of the liver ($p>0.05$).The conclusion of this study was that MSG administration and *Lannea coromandelica* extract for 28 days can cause mild damage to liver histopathology and affect liver MDA, SGOT and SGPT blood levels.

Keywords: Lannea coromandelica ; histopathology; MDAs; SGOT/SGPT

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1. Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid. MSG contains 78% glutamic acid, 22% sodium and water (Tawfik and Nawal, 2012). Glutamate as a natural nutrient in many foods. To this day it is increasingly being used (in the form of salt, namely MSG (monosodium glutamate) in food processing and home cooking worldwide which aims to increase delicious food for humans. It delivers a certain taste called umami which is now famous as the fifth basic taste (Diab and Reham, 2016). Monosodium glutamate (MSG) was classified as a safe substance by the Food and Drug Administration (FDA) in 1959 (Jinap and Hajeb, 2010). However, empirical studies have revealed that long-term consumption of MSG causes a number of toxic effects on organs such as brain damage, retinal degeneration, and liver toxicity (Al-Ghamdi, 2021) .

The liver is an organ that functions to remove toxic substances that enter the body. Excessive glutamate metabolism in the liver such as chronic MSG intake can be a source of free radicals such as reactive oxygen species (ROS). Glutamate deamination produces ammonium ions ($NH4^+$). Excess amounts of $NH4^+$ can damage hepatocyte mitochondria through activation of Ca^+ independent intrinsic apoptotic pathways. Increased free radicals can cause oxidative stress which is characterized by lipid peroxidation and increased glutathione-s-transferase activity. Therefore, the accumulation of MSG can cause damage to hepatocytes due to the effects of free radicals (Aji, Ruri, and Ahmad, 2019).

The human body needs important substances such as antioxidants to reduce the negative effects of free radicals (Hardiningtyas et al, 2014). Sha Li et al (2015) stated that antioxidants have the ability to remove free radicals and protect the liver from oxidative stress. Based on research from Azzahrah, Abdul and Yuko (2019) the content of Java wood (*Lannea coromandelica)* was obtained, namely flavonoids, terpenoids / steroids and tannins. Flavonoids are compounds that are antioxidants that can overcome and prevent free radicals. The antioxidant effect of flavonoids is that they can increase the regeneration process (Hardiningtyas et al, 2014).

Based on the description above, excess MSG is known to cause damage to hepatocytes influence integrity cell, damage permeability membrane, And influence homeostasis volume cells due to the effects of free radicals. However, this can be prevented by administering antioxidants that may be found in *Lannea coromandelica* which contains several substances such as flavonoids as antioxidants so that they can overcome and prevent free radicals. This study aims to determine the protective effect *of Lannea coromandelica* on the livers of male Wistar rats induced by MSG.

2. Materials and Methods

The materials used in this study were Monosodium Glutamate (MSG), Male Wistar Rats (*Rattus Novergicus*) and reagents SGOT and SGPT . The Monosodium Glutamate (MSG) used is the Aji-no-motto brand produced by Aji-no-motto co. Inc. In the MDA examination, the materials used were 10% TCA and 1% TBA, each of 1000 µl using a UV-Vis spectrophotometer. The SGOT reagents used were AST and CP and the SGPT reagents used ALT and CP with the IFCC method using the ABX Pentra tool . The materials used in the manufacture of histological preparations include paraffin, hematoxylin eosin, methylene blue, xylol solution, and aquadest . The test animals used were male wistar rats (Rattus Novergicus) with a body weight of 180-300 grams as many as 25 rats aged 2-3 months. The rats used were healthy rats and showed normal behavior. The test animals were acclimatized for one week by *ad libitum giving standard food and drink*.

The treatment given to the test animals is using 3 doses of Laneea extract and MSG which will then be given orally to the experimental animals for 28 days of treatment. The treatments to be given were as follows: (Healthy controls that only received standard feed), (Negative controls given MSG 3 ml/gBB), (Treatment group A: Lannea extract 250 mg/KgBB + MSG 3 ml/gBB), (Treatment group B: Lannea extract 500 mg/KgBB + MSG 3 ml/gBB), (Group C: Lannea extract 750 mg/KgBB + MSG 3 ml/gBB). How to give Lannea Extract and MSG is done orally using a sonde. MSG is given by first dissolving it with distilled water. MSG administration is carried out with a frequency of 1x administration with a span of 1 hour. After being treated for 28 days, the rats were weighed and killed by decapitation. Furthermore, surgery was performed to take liver samples and cleaned with 0.9 % NaCl. Liver samples were taken on the left side to be tested for MDA levels using a UV-Vis

spectrophotometer. Serum collection is carried out using a hematocrit tube through the eye. The blood sample is put into the EDTA tube. The tube containing blood was then centrifuged at 3000 rpm for 10 minutes. The serum obtained was measured for levels SGOT/SGPT. To determine the level of liver histological damage, the score and percentage criteria for the degree of liver damage were used as presented in Table 1 regarding changes in the histopathological structure of the liver.

Table 1. Criteria for assessing the degree of liver damage based on histopathology

Criteria	Information	Score
Normal	There are no histological changes	0
Light	If there is parenchymatous degeneration <25% among normal cells	1
Currently	If there is hydrophilic degeneration 25-50% of the entire visual field	2
Heavy	If there is necrosis > 50% of the entire visual field and other damage that is more severe	3

(Cahya et al, 2022)

MDA, SGOT/SGPT levels were analyzed by *one-way* ANOVA analysis of variance. Score data on the degree of liver histological damage were analyzed using the non-parametric *Kruskal-Wallis test.*

3. Results

Analysis of MDA Levels

liver MDA levels in the treatment group can be seen in Figure 1. Liver MDA levels in rats increased in the negative control (HKN) induced by MSG for 28 days which showed an average value of liver MDA levels in KN was slightly higher, namely 6.31 ± 1.48 µg/ml compared to HKS, namely 4, 65 ± 1.76 µg/ml. In addition, there were differences in the treatment group that was given Lannea coromandelica extract (HKPA, HKPB and HKPC), namely a decrease in MDA levels starting from the HKPA group of 4.87 ± 1.42 µg/ml, but there was a slight increase in the HKPB group of 4. .92 ± 0.77 µg/ml and decreased in the HKPC group of 3.27 ± 0.95 µg/ml. Based on the results of the analysis of the homogeneity test data, it shows a significance of 0.837 (p>0.05), which means that the data is homogeneous. Then a normality test was carried out with a significance value of 639 (p>0.05) which showed that the data was normally distributed so that it could be continued with the One Way Anova test. The results of the ANOVA test obtained p = 0.037 (p> 0.05) where there was a significant difference between administration of the extract and induction of MSG for 28 days on liver MDA levels. In further analysis with Duncan's test showed that there were significant differences in each group given Java bark extract (Lannea coromandelica) both doses of 250 mg/kg, 500 mg/kg, and doses of 750 mg/kg, in rats induced with MSG for 28 days.

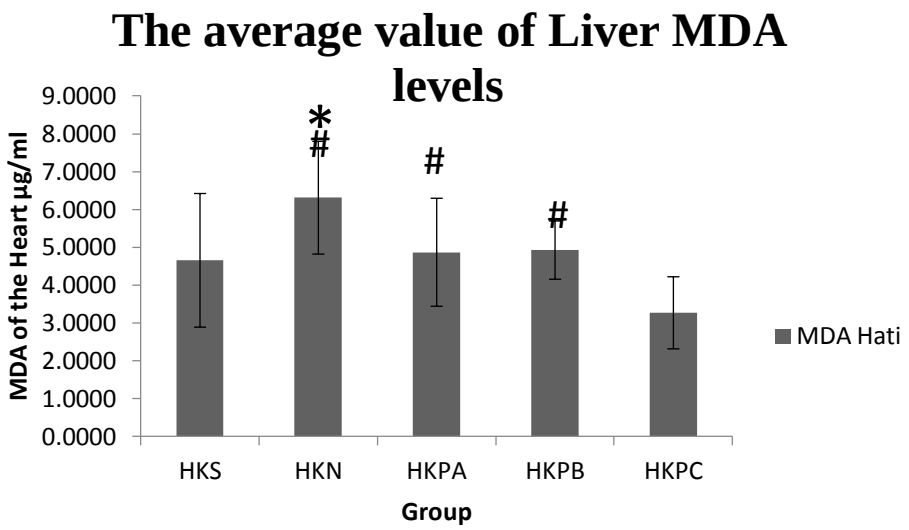

Figure 1. Graph showing the average value of blood SGPT levels in each group. Data are presented as mean ± SD (n = 5) and symbols * where p <0.05 is significantly different from healthy controls (KS). The symbol # where the value of p <0.05 is significantly different from the negative control

Liver SGOT levels

Comparison of SGOT levels for each group can be seen in the graph in Figure 2. Based on the results of the analysis of the homogeneity test data, it shows a significance of 0.550 (p>0.05), which means that the data is homogeneous. Then a normality test was carried out with a significance value of 0.471 (p>0.05) which showed that the data was normally distributed so that it could be continued with the One Way Anova test. The results of the ANOVA test obtained a value of p = 0.004 (p> 0.05) where there was a significant difference between administration of the extract and induction of MSG for 28 days on liver SGOT levels. In further analysis with the Tukey test showed that there were significant differences in each group given Java bark extract (Lannea coromandelica) both doses of 250 mg/kg, 500 mg/KBB and doses of 750 mg/kg BW in MSG-induced rats. for 28 days.

The average value of SGOT levels in the healthy control group (HKS) where mice without MSG and Lannea coromandelica extract had the lowest SGOT levels showing a value of 150.60 ± 23.33 IU/L, and SGOT levels in the negative control (HKN) where rats induced with MSG at a dose of 3mg/gBW showed a value of 229.00 ± 30.07 IU/L which was slightly lower than healthy controls. In contrast to the treatment group (HKPA), where rats were given Lannea coromandelica extract at a dose of 250 mg/kgbb and induced MSG, SGOT levels began to decrease showing a value of 204.60 ± 24.27 IU/L, followed by the treatment group (HKPB) where rats were given Lannea coromandelica extract at a dose of 500 mg/kg and was induced by MSG so that the SGOT levels decreased significantly, showing a value of 173.75 ± 14.40 IU/L. In the treatment group (HKPC) rats given Lannea coromandelica extract at a dose of 750 mg/kg BW and induced MSG had SGOT levels slightly increased showing a value of 185.60 ± 40.94 IU/L.

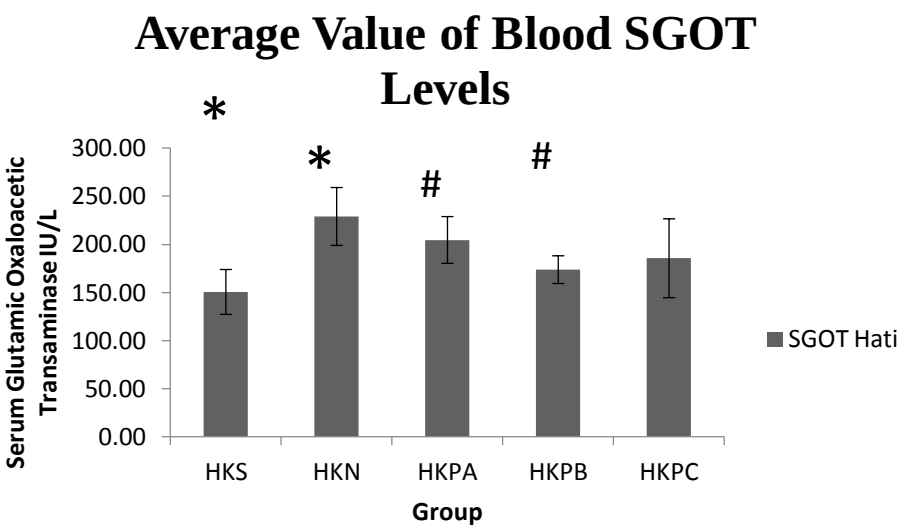

Figure 2. Graph showing the average value of blood SGPT levels in each group. Data are presented as mean ± SD (n = 5) and symbols * where p <0.05 is significantly different from healthy controls (KS). The symbol # where the value of p <0.05 is significantly different from the negative control (KN).

Liver SGPT levels

Comparison of SGPT levels for each group can be seen in the graph in Figure 3. Based on the results of the analysis of the homogeneity test data, it shows a significance of 0.144 (p>0.05), which means that the data is homogeneous. Then a normality test was carried out with a significance value of .085 (p>0.05) which showed that the data was normally distributed so that it could be continued with the One Way Anova test. The results of the ANOVA test obtained a value of p = 0.004 (p> 0.05) where there was a significant difference between administration of the extract and induction of MSG for 28 days on liver SGPT levels. In further analysis with the Tukey test showed that there was a significant difference in each group given Java bark extract (Lannea coromandelica) both doses of 250 mg/kg, 500 mg/kg, and doses of 750 mg/kg, in rats induced with MSG for 28 days.

The average value of SGPT levels for healthy controls (HKS) where mice without MSG induction and administration of Lannea coromandelica extract had the lowest SGPT levels showing a value of 55.60 ± 11.12 IU/L, and there was an increase in SGPT levels in the negative control (HKN) where rats induced with MSG 3mg/gBW showed a value of 107.80 ± 24.59 IU/L. In the treatment group (HKPA) where rats were given Lannea coromandelica extract at a dose of 250 mg/kg BW and induced by MSG SGPT levels increased so that it showed a value of 121.40 ± 31.67 IU/L. Then it decreased in the treatment group (HKPB) where the rats with Lannea coromandelica extract at a dose of 500 mg/kg BW and induced MSG showed a value of 79.25 ± 27.87 IU/L, and there was a slight increase in the treatment group (HKPC) where the rats were given Lannea extract coromandelica at a dose of 750 mg/kgBW and induced by MSG so that it showed a value of 80.40 ± 23.92 IU/L.

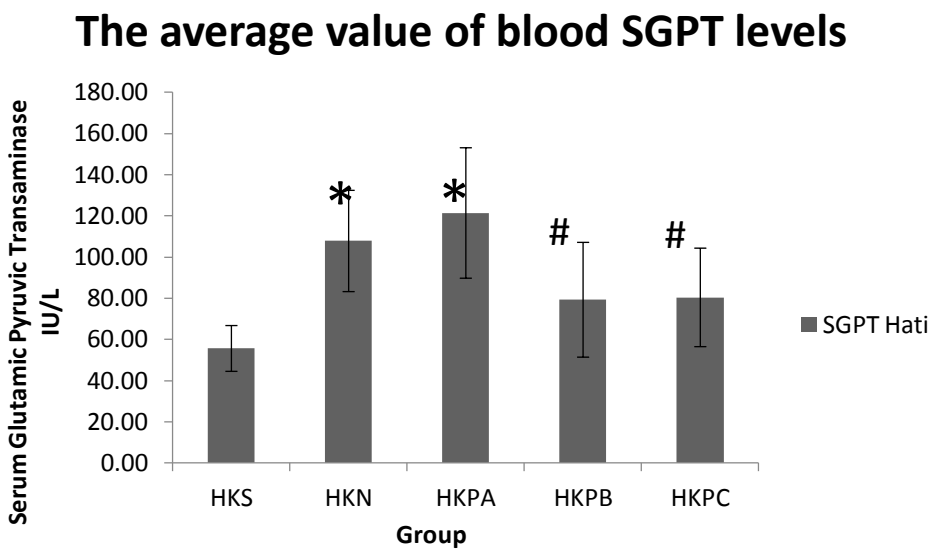

Figure 3**.** Graph showing the average value of blood SGPT levels in each group. Data are presented as mean ± SD (n = 5) and symbols * where p <0.05 is significantly different from healthy controls (KS). The symbol # where the value of p <0.05 is significantly different from the negative control (KN).

Results of Histopathological Analysis

The results of liver histopathological examination showed that healthy control (HKS) where there was no treatment of Lannea coromandelica extract and MSG induction had a degree of damage of 0, and mild damage occurred in the negative control (HKN) where rats were induced with MSG at a dose of 3 mg/gBB with a value 1.33 ± 0.57. In contrast to the treatment group (HKPA) where rats were given Lannea coromandelica extract at a dose of 250 mg/kg BW and MSG induction had a moderate degree of damage with a value of 2.00 ± 1.73, then in the treatment group (HKPB) rats that received Lannea coromandelica extract at a dose of 500 mg/kg BW and MSG induction had mild damage compared to the HKPA group with a value of 1.66 ± 1.52, and there was a decrease in damage in the treatment group (HKPC) in mice that were given Lannea coromandelica extract at a dose of 750 mg/kg BW and MSG induced had mild damage with a value of 0.66 ± 0.57 compared to the HKN, HKPA, and HKPB groups . Figure 4.6 shows the liver histology of the HKS group (healthy control) which was not given induction or Lannea coromandelica extract, it appears that the architecture of the hepatocyte cells looks normal. Based on the results of data analysis, it was obtained p = 0.247 (p> 0.05) where there was no significant effect on the histological appearance of the liver after being given MSG-induced Lannea coromandelica extract.

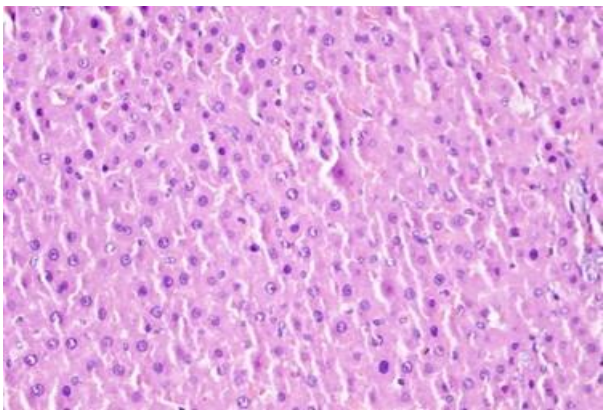

Figure 4. Histopathological picture of the liver of rats in the HKS (Healthy Control) group. The picture shows normal hepatocyte architecture

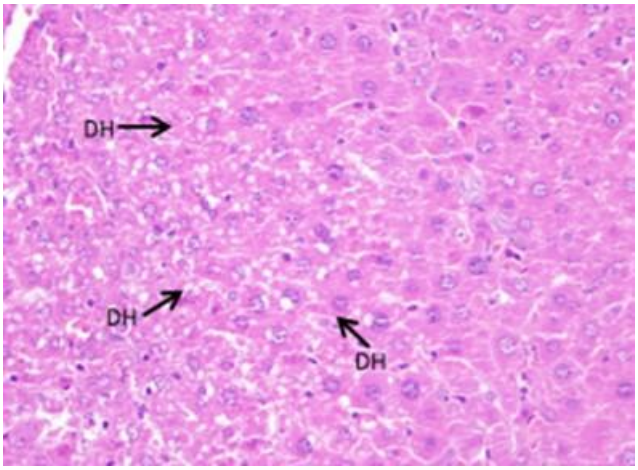

Figure 5. Histopathological picture of the liver of rats in the HKN group (Negative Control) The picture shows hydrophic degeneration. Mild category of damage

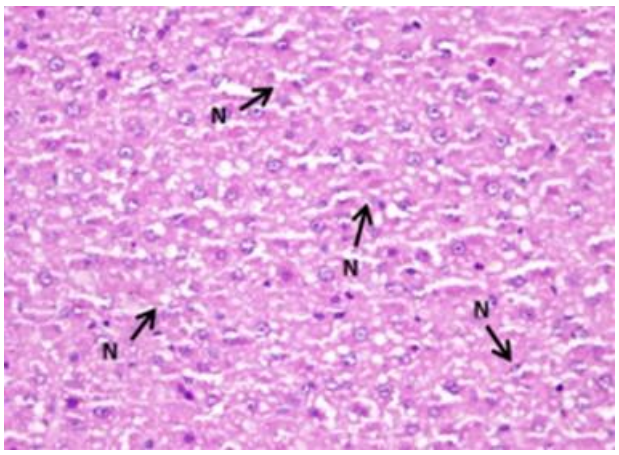

Figure 6. Histopathological picture of the liver of rats in the HKPA group (treated at a dose of 250 mg/kgBB) the picture shows nicrosis. Severe category of damage

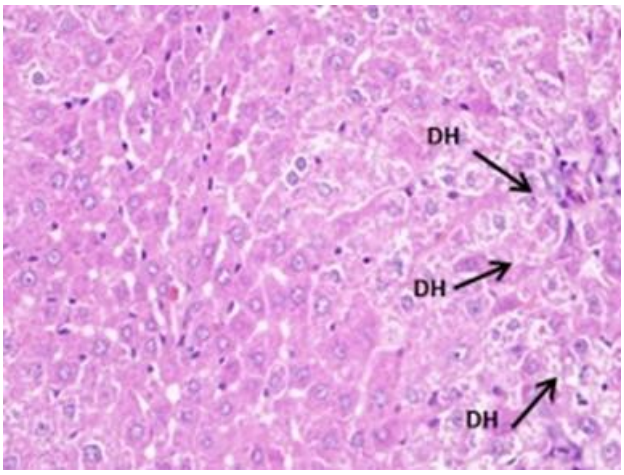

Figure 7. Histopathological picture of the liver of rats in the HKPB group (treated at a dose of 500 mg/kg BW) the picture shows hydrophic degeneration. Mild category of damage.

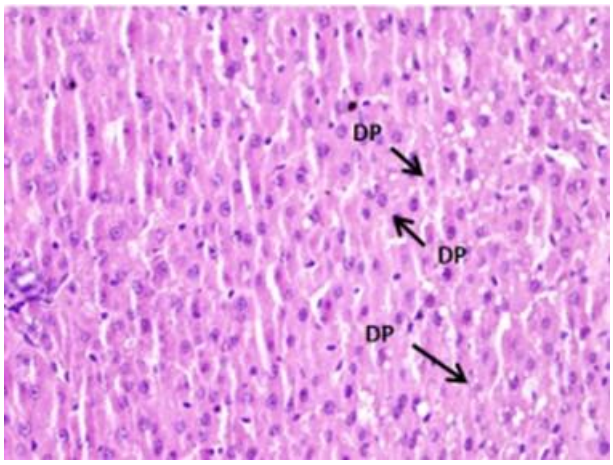

Figure 8. Histopathological picture of the liver of rats in the HKPC group (treated at a dose of 750 mg/kg BW) the picture shows parenchymatous degeneration. Mild category of damage.

4. Discussion

The ability of monosodium glutamate (MSG) can help regulate and coordinate functions in increasing the transmission of nerve impulses if used optimally. Excessive use can cause toxicity to the liver. This is because the liver has receptors for MSG so it is susceptible to damage due to oxidative stress (Lestari et al, 2021). Excessive consumption of MSG can cause the formation of free radicals in the body and cause accumulation of MSG in the liver which functions as a detoxification of toxic substances that enter the body (Legoh et al, 2017).

Lannea coromandelica plants, especially the bark, contain carbohydrate compounds, steroids, cardiac glycosides, terpenoids, tannins and flavonoids (Manic et al, 2013). This compound is a group of bioactive compounds that produce antioxidant, anti-inflammatory, antibacterial, anticancer and immunomodulatory activities (Pagarra and Sahribulan, 2022). Flavonoids are exogenous antioxidants that contain phenolic groups and have been shown to be useful in preventing cell damage due to oxidative stress.

In this study, experimental animals were given Java bark extract (Lannea coromandelica) with various doses, namely 250 mg/kg, 500 mg/kg and 750 mg/kg, which were administered orally through a cannula. MSG induction in negative control animals was carried out at a dose of 3 mg/gBW. The dose of Java bark extract (Lannea coromandelica) refers to previous research conducted by Calsum, Akhmad and Khilda (2018) concerning the Activity of the Ethanol Extract of Java Bark (Lannea coromandelica) on Healing Cuts in White Rats (Rattus Norvegicus L. .). Java bark extract (Lannea coromandelica) was administered 1 hour before MSG induction for 28 days . Assessment of the effect of Java bark extract (Lannea coromandelica) for liver damage, variables of MDA, SGOT, SGPT and SGPT levels were used histopathological analysis.

One indicator of damage to cell membranes due to ROS in the oxidative stress phase is shown by high levels of Malondialdehyde (MDA) (Kamala and Umi, 2019). High MDA levels are affected by lipid peroxidation which indirectly indicates a high number of free radicals and indicates an oxidation process in the cell membrane (Asad et al, 2022)

ANOVA test, it was shown that $p = 0.037$ ($p > 0.05$), which means that there was a significant difference between administration of the extract and induction of MSG compared to the healthy control group which was not treated for 28 days on liver MDA levels. Based on the average MDA levels obtained in each treatment group, it was shown that the group that was given Java bark extract (Lannea coromandelica) had lower liver MDA levels than the group that was not given Java bark extract (Lannea coromandelica).

Research conducted by Calsum et al (2018) found that Java bark extract has free radical scavenging activity, inhibits hydrolysis and oxidation of enzymes and is anti-inflammatory. If Lannea

coromandelica extract is able to ward off free radicals, it will affect MDA levels, where the higher the free radicals, the higher the MDA levels in the body.

The increase in SGOT levels in mice that were given MSG indicates a disturbance in the function of the liver which plays a role in secreting this enzyme. Liver abnormalities can be seen by changes in SGOT and SGPT levels because these two enzymes have high concentrations in liver cells.

Based on the results of statistical analysis using the ANOVA test , it was shown that $p = 0.004$ ($p > 0.05$), which means that there was a significant difference between administration of the extract and induction of MSG compared to the healthy control group which was not treated for 28 days on blood AST levels. Based on the data above, it can be concluded that there was an increase in the HKN group and a decrease was shown in the HKPA treatment group. Then the SGOT value began to decrease significantly in the HKPB group and slightly increased in the HKPC group.

Anova test, it was shown that the value of $p = 0.004$ ($p < 0.05$) which means that it has a significant effect on SGPT levels given Lannea coromandelica extract and induced by MSG. The lowest SGPT value was shown in the HKS Healthy Control group and then there was an increase in the HKN and HKPA groups. Then there was a significant decrease in the HKPB and HKPC groups

Damage to hepatocyte cells is characterized by cellular changes such as swelling of the cells, formation of vacuoles, fattening (steatosis) and ends with cell rupture. Liver damage can be seen in a short time in the form of inflammation and fatty degeneration of hepatocytes, which ends in cell death (Maulina, 2018).

Histopathological examination of the liver found a healthy group (HKS) in which mice without MSG and Lannea coromandelica extract microscopically showed no inflammation of hepatocyte cells, cell necrosis, parenchymatous degeneration and hydrophic degeneration so that this group had a degree of damage of 0 (Normal). In the negative control group (HKN) where rats were given MSG 3 mg/kgbb with a damage value of 1 (mild) showing a value of 1.33 ± 0.57, parenchymatous degeneration was seen in this group. In the treatment group (HKPA) where rats were given Lannea coromandelica extract 250 mg/kgBB and induced MSG with a degree of damage with a score of 2 indicating a value of 2.00 ± 1.73, visible cell damage, and cell hydropic degeneration. Treatment group (HKPB) where rats were given Lannea coromandelica extract 500 mg/kgBB and induced by MSG the degree of damage with a score of 1 showed a value of 1.66 ± 1.52, parenchymatous degeneration was seen, and the distance between inflammatory cells and swelling was seen. Treatment group (HKPC) where rats were given Lannea coromandelica extract 750 mg/kgBB and MSG-induced degree of damage with a score of 1 and showed a value of 0.66 ± 0.57, degeneration of hepatocyte cells was seen, but almost normal. The lowest mean liver damage was HKS then HKPC, HKN, HKPB compared to the HKPA group which had moderate damage. Histopathological analysis was carried out by statistical tests using the Shapiro-Wilk, then followed by the Kruskal-Wallis test. Found no significant difference because it has a value of 0.247 ($p < 0.05$).

5. Conclusion

Based on the research results obtained, it can be concluded that Administration of MSG-induced Lannea coromandelica extract had a significant effect on reducing hepatic MDA levels. MSG-induced Lannea coromandelica extract had an effect on reducing SGOT and SGPT levels in treatment with a high dose of 750 mg/kgBb. Based on histopathological examination, there were treatments that experienced mild damage after being given Lannea coromandelica extract , namely at a dose of 750 mg/kg BW so that it has the potential to repair liver histopathological damage in male Wistar rats due to MSG-induced

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