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**THE EFFECT OF LEMON JUICE (*Citrus limon*) IN LIVER HISTOPATHOLOGIC APPEARANCE OF MALE MICE (*Mus musculus* L.) EXPOSED BY MONOSODIUM GLUTAMATE (MSG)****Dela Destiani Aji\*, Ruri Eka Maryam Mulyaningsih, Ahmad Fariz MZZ***Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia*

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**ABSTRACT**

**Background:** Excessive consumption of MSG can cause the formation of free radicals in the body. Continuous use of MSG will cause free radical accumulation and oxidative stress in the liver. An antioxidant is one of the substances that has an ability to eliminate free radicals and protect the liver from oxidative stress. Lemon is a fruit that has benefits as a natural antioxidant. This study aims to determine the effect of administering lemon juice on the histopathological appearance of the liver exposed by MSG.

**Methodology:** This post-test only control group design experimental study used 30 male white mice which were randomly divided into five groups: NC group, C(-) group, D1 group, D2 group, and D3 group. After 7 days of adaptation and randomized grouping, the mice were treated differently based on their group. All groups, except the NC group, were exposed to 4mg/gBW dose of MSG orally for 14 days. On the 15<sup>th</sup> day, MSG exposure was stopped and after that D1 group, D2 group, and D3 group were given lemon juice with a dose of each group 3.33ml/kgBW, 6.67 ml/kgBW, 13.33 ml/kgBW. The lemon juice was given once a day for 14 days using the gastric sonde. Histopathology examination was analyzed by pathologist in Gunung Jati Hospital, Cirebon, Indonesia

**Results:** Significant differences of liver histopathology were obtained between the normal control group with the dose 3 group ( $p = 0.005$ ), the normal control group with the dose 2 group ( $p = 0.019$ ), the normal control group with the dose group ( $p = 0.019$ ) and between the negative control groups with dose 3 group ( $p = 0.027$ ). Dose 3 group has higher liver damage compared to a normal control group and negative control group characterized by the form of parenchymatous degeneration.

**Conclusion:** Lemon juice has a negative effect on the histopathology appearance of male white mice's (*Mus musculus* L.) liver exposed by monosodium glutamate (MSG). Further studies are needed to validate the effect of the active compound and toxicity of lemon juice and to reveal the precise mechanisms on how lemon juice affects the hepatocytes.

**Keywords:** Monosodium Glutamate (MSG), Citrus lemon, Histopathology Liver

**INTRODUCTION**

Monosodium glutamate (MSG) is a flavor enhancer that is widely being used in society. It can improve the perception of sweet and salty taste and reduce sour and bitter taste of food and it can give a new taste called umami, i.e. savory flavor [1]. Based on the 2013 Basic Indonesian Health Research, the results of risky food consumption behaviors in residents aged  $\geq 10$  years were mostly consumptions of seasoning foods (77.3%), followed by sweet foods and drinks (53.1%), and fatty foods (40.7%) [2].

The US Food and Drug Administration (FDA) shows that a mean daily intake of MSG per capita is 550 mg/d [3]. Excessive consumption of MSG can cause the formation of free radicals in the body. Several

studies on MSG in experimental animals results show that the accumulation of excessive MSG can cause negative effects such as nerve damage, eyes damage, increase of body fat, obesity, damage of intestinal mucosa, reduction of growth hormone in the blood, and damage of liver and kidney parenchyma [1].

Liver is an organ that serves to offer toxic substances entering into the body [4]. Excessive liver metabolism of glutamate as in chronic MSG intake can be a source of free radicals such as reactive oxygen species (ROS). The deamination of glutamate produces ammonium ions ( $\text{NH}_4^+$ ). Excess  $\text{NH}_4^+$  amount can damage hepatocytes' mitochondria through activation of the independent  $\text{Ca}^{2+}$  intrinsic apoptotic pathway. Increased free radicals can cause oxidative stress which is characterized by the presence of lipid peroxidation and increased glutathione-s-transferase activity. Therefore, accumulation of MSG can cause damage to hepatocytes due to the effects of free radicals caused [5].

Human body needs important substances such as antioxidants to reduce the negative effects of free radicals. Sha Li states that antioxidant has an ability to eliminate free radicals and protect the liver from oxidative stress [6]. Lemon is a very beneficial fruit for the human body. Lemon is a fruit that has more benefits as a natural antioxidant than lime because it contains vitamin C, citric acid, essential oil, bioflavonoids, polyphenols, coumarin, flavonoids, and volatile oils on the skin such as limonene ( $\pm 70\%$ ),  $\alpha$ -terpinene,  $\alpha$ -pinene,  $\beta$ -pinene, as well as coumarin, and polyphenols [7].

It is known that MSG can cause liver damage and can be prevented by giving antioxidants such as those found in lemon juice which has the potential as a protective material for the liver from the MSG effect. This study aimed to determine the effect of giving lemon juice to the histopathological appearance of the liver exposed by MSG.

## MATERIAL AND METHOD

This was an experimental study with post-test only control group design using male white mice (*Mus musculus* L.) as research subjects. The study was conducted in the Food and Nutrition Laboratory of the Inter-University Center (PAU) and Pathology Anatomy Laboratory of Gadjah Mada University, Yogyakarta, Indonesia. The experimental protocol and animal handling were approved by the Ethical Committee of the Faculty of Medicine, Swadaya Gunung Jati University (Approval number:52/EC/FK/XI/2018).

### Animal protocol

Thirty mice (aged three months old) weighted of 25-30 grams were divided randomly into 5 groups: NC group, C(-) group, D1 group, D2 group, and D3 group. After 7 days of adaptation (all mice were fed routinely with standard alimentation and the cages were cleaned daily) and randomized grouping, the mice were treated differently based on their group. All groups, except NC group, was exposed to 4mg/gBW dose of MSG orally for 14 days. On the 15<sup>th</sup> day, MSG exposure was stopped and after that D1 group, D2 group, and D3 group were given lemon juice with a dose of each group was 3.33ml/kgBW, 6.67 ml/kgBW, 13.33 ml/kgBW, respectively. The lemon juice was given once a day for 14 days using the gastric sonde [8].

Table 1. Treatment for each group of mice

Group	Treatment
Normal control (NC)	Non-induced by MSG and lemon juice
Negative control (C(-))	Induced by 4 mg/gBW
Dose 1(D1)	Induced by 4 mg/gBW and 3.33 ml/kgBW
Dose 2 (D2)	Induced by 4 mg/gBW and 6.67 ml/kgBW
Dose 3 (D3)	Induced by 4 mr/gBW and 13.33 ml/kgBW

### ***Liver histopathology***

After 28 days of treatment, all the mice were cervically dislocated and the livers were taken. Liver histopathology slide preparation was done by fixating, washing, dehydrating, clearing, impregnating, embedding, cutting, staining, and mounting. Hematoxylin and eosin (H&E) stained liver sections were prepared for each mouse in all groups. Histopathology examination was analyzed by pathologist in Gunung Jati Hospital, Cirebon, Indonesia. In examination process, the pathologist used a binocular light microscope and counted in 5 viewing field with 400x magnification. Liver histopathology was scored and graded using Manja-Roegnik score ranging from 1 to 4; Score 1 is for normal cells domination, score 2 is for cells with dominant parenchymatous degeneration, score 3 is for dominant hydropic degeneration, and score 4 is for dominant necrotic cells [8].

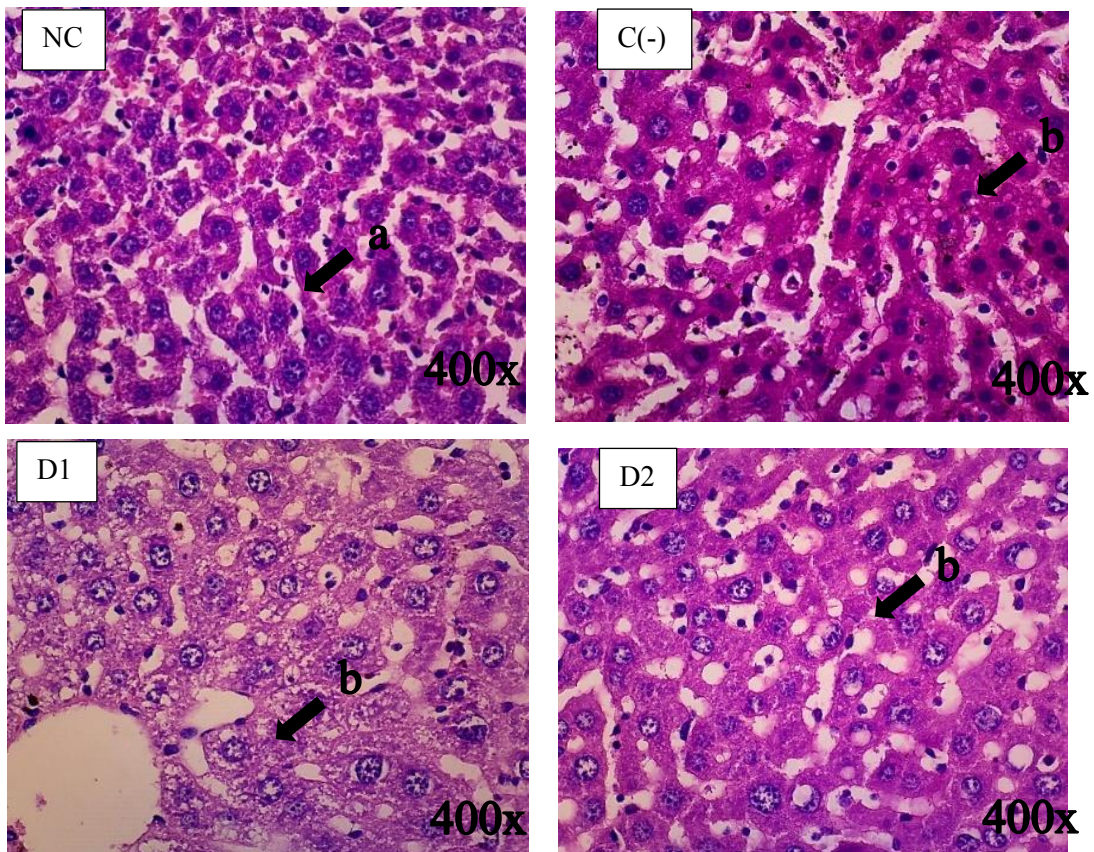
### ***Statistical analysis***

The results of the study were analyzed using Kruskal-Wallis test and Mann Whitney post-hoc test to find out which group had significant different.

## **RESULTS**

### ***Liver Histopathology***

In observing liver histopathology, changes can be found in the form of parenchymatic degeneration, hydropic degeneration, and necrosis. Observation results can be seen in the following figures.



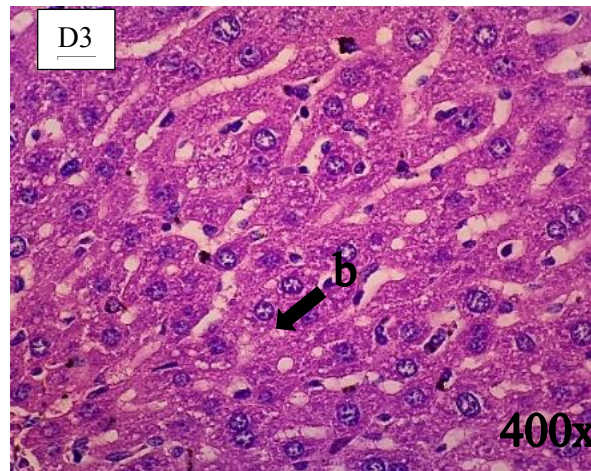


Fig. 1 shows observation result of liver histopathology appearance after 28 days of treatment in objective lens 400x. In this case, NC is normal control, C(-) is negative control, D1 is dose 1, D2 is dose 2, and D3 is dose 3. Arrow (a) shows normal hepatocyte and (b) shows parenchymatous degeneration.

Fig. 1 shows grade 1 (normal hepatocyte) appearance in NC group and grade 2 (parenchymatous degeneration) appearance in D1, D2, D3, and C(-) characterized by swelling and opacification of cytoplasm due to granules appearance in the cytoplasm.

Table 2. The average of histopathology appearance of hepatocytes in each group

Group	The Average number of hepatocytes DS ±	Minimum	Maximum
Normal control	1 ± 0.408	1	2
Negative control	1 ± 0.408	1	2
Dose 1	2 ± 0.516	1	2
Dose 2	2 ± 0.516	1	2
Dose 3	2 ± 0.408	1	2

From the table above, the average number of histopathology appearance of the normal control group is normal hepatocytes arranged radially, the central vein is clear, the normochromic nucleus, and the hepatocyte plates anastomose freely, which limits the gap occupied by the sinusoid. In the negative control, Dose 1, Dose 2, and Dose 3 groups, there were parenchymatous degeneration characterized by presence of swollen hepatocytes (more than normal size) and red granular cytoplasm as a sign of a buildup of protein.

**Statistical Analysis**

Distribution of scoring data of histopathologic analysis was not normal; thus, non-parametric test was performed to know whether there is significant difference between all groups.

**A. Kruskal Wallis Test**

Table 3. *Kruskal Wallis Test Results*

Group	N	p Value
NC	6	0,016
C (-)	6	
D1	6	
D2	6	
D3	6	

The analysis result of the histopathologic appearance of hepatocytes in each group of this study obtained a significant value of 0.016 ( $p < 0.05$ ). It means among those groups there are at least two groups showing significant difference in histopathologic scoring. Afterward, Mann-Whitney post-hoc test was performed to see which pairs of groups have a significant difference (Table 2).

**B. Post-Hoc Mann-Whitney Test**

Table 4. *Post-hoc Mann-Whitney test results*

Group	NC	C(-)	D1	D2	D3
NC	-	0.317	0.019*	0.019*	0.005*
C(-)	0.317	-	0.093	0.093	0.027*
D1	0.019*	0.093	-	1	0.523
D2	0.019*	0.093	1	-	0.523
D3	0.005*	0.027*	0.523	0.523	-

\*p-value < 0.05 meaning there is significant difference

The post-hoc Mann-Whitney test analysis result shows that several groups had significant differences that was indicated by significant value result below 0.05. The groups that had significant difference in liver histopathology appearance was between:

- 1) NC group and D3 group with p-value = 0.005 ( $p < 0.05$ )
- 2) NC group and D2 group with p-value = 0.019 ( $p < 0.05$ )
- 3) NC group and D1 group with p-value = 0.019 ( $p < 0.05$ )
- 4) C(-) group and D3 group with p-value = 0.027 ( $p < 0.05$ )

**DISCUSSION**

***The effects of administering MSG to Male Mice***

The research results show normal histopathology appearance found in negative control group administered with 4mg/gr MSG during 14 days, followed with standard feeding and aquades for the next 14 days [9].

Based on Agverianti's research result, gained effects of administering 4mg/gr MSG during 14 days to the appearance of male mice's liver histopathology in the form of liver cell degeneration. Consumption of MSG caused oxidative stress such as the increased peroxide lipid level and decreased antioxidant enzyme [10].

The observation results show that, in negative control group after 28 days of treatment, there was a normal liver histopathology appearance. This case may occur because liver is an organ which has high regeneration ability. Andreas shows that MSG consumption caused liver damage and liver regeneration occurred though MSG consumption was stopped after 14 days [11]. Gilgenkrantz found that when two third of mice's liver was exposed, the rest liver would be recovered like in normal condition in



approximately 10 days [12]. Based on the discussion above, it can be concluded that MSG exposure may cause liver damage, but liver cell regeneration is able to return in normal condition.

### ***The effects of administering Lemon Juice to Histopathology Appearance of Mice's Liver Exposed by MSG.***

The results of this research show that the administration of lemon juice to mice exposure by MSG influences the description of mice's histopathology liver. Degeneration parenchymatous was found in Dose 1, Dose 2 and Dose 3 groups. Degeneration of parenchymatous is the most minor degeneration in the form of puffy hepatocyte cell (abnormal measurement) and red granule cytoplasm as the signs of protein accumulation. This degeneration is reversible because it merely happens in mitochondria and reticulum endoplasm because of oxidation disturbance. If this degeneration continues, it will make hydroponic degeneration which is the most serious damage level. If the causes of injury remains, the injured cell may have a tear of membrane plasma and the changing of main cell; thus, the cell becomes dead or necrosis [13].

The results of this research also show the description change of liver histopathology to a dose groups with lemon juice treatment. This may happen because lemon contains a bunch of natural chemical components. Beside antioxidant contents, lemon also contains other substances such as citrate acid giving acid taste to lemon and has pH 2 to 3.

Citrate acid is an organic acid naturally contained in fruits and vegetables. The great amount of citrate acid can be in lemon fruit. Lemon contains high acid concentration, there is 8% from dry weight and around 47 g/L in lemon juice [14, 15].

Chen states that detection microscopically showed a series of histopathology changes to mice's liver induced by citrate acid such as hepatocyte cell random compose, thrombosis to central vein and lymphocyte infiltration. Furthermore, biochemical checking which showed decreased antioxidant enzyme of T- SOD and GSH-Px and decreased free radical H<sub>2</sub>O<sub>2</sub> and MDA [16].

Citrate acid has proven that it can cause oxidative damage to liver by decreasing antioxidant enzyme activity of T-SOD (*Superoxide Dismutase*) and GSH-Px (*Glutathione Peroxidase*). These enzymes have important role as antioxidant in detoxified H<sub>2</sub>O<sub>2</sub>. Decreased enzyme antioxidant has caused increased free radical activity H<sub>2</sub>O<sub>2</sub> (Hydrogen Peroxide). Furthermore, other free radical resulted during metabolism process can attack unsaturated fatty acid in bio-membrane to result peroxidation lipid of membrane cell in the end form of hydroperoxide lipid such as MDA (Malonyl dialdehyde). The decreased antioxidant enzyme activity of T-SOD or GSH-Px is able to accelerate the activity of caspase-3 and induction apoptosis to hepatocyte cell [16].

The limitation of this study was that the researcher did not terminate mice in K (-) group on the day the MSG treatment was stopped; thus, it could be one of the biases for the results of the study. In addition, researcher did not include the groups treated with lemon juice in the research; thus, the effect of lemon juice to the liver couldn't be seen.

## **CONCLUSION**

Lemon juice has a negative effect on the histopathology appearance of male white mice's (*Mus musculus* L.) liver exposed by monosodium glutamate (MSG). Dose 3 group has higher liver damage compared to normal control group and negative control group characterized by the form of parenchymatous degeneration. Further studies are needed to validate the effect of the active compound and toxicity of lemon juice and to reveal the precise mechanisms on how lemon juice affects the hepatocytes.

## CONFLICT OF INTEREST DECLARATION

The author states that there is no conflict of interest regarding the publication of this paper.

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