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# EFFECT OF TIGER SHRIMP SHELL EXTRACT (*PENAEUS MONODON*) ON MONOSODIUMGLUTAMATE-INDUCED MICE

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## ABSTRACT

**Background:** The shell is a part of the tiger shrimp (*Penaeus monodon*) body that often becomes waste and has not been utilized properly, however it contains secondary antioxidants that can prevent oxidative stress in the form of astaxanthin. Although several studies have examined the function of astaxanthin as an antioxidant, the role of it in tiger shrimp shells against hepar mice induced by monosodium glutamate (MSG) is not well known. **Objective:** to determine the effect of tiger shrimp shell extract in graded doses on the histopathological picture of hepatic mice induced by MSG. **Methods:** Extraction of tiger shrimp shell was carried out with palm head oil solventand analyzed with *Ultraviolet-Visible spectrophotometer (UV-Vis).* This study used 25 male mice classified into 5 groups. One group was negative control without any need and four other groups were induced with MSG 0.84 g/kgBB. Three of the MSG-induced groups were induced with tiger shrimp shell extract at dosesof 50 mg/kgBB, 100 mg/kgBB, and 200 mg/kgBB. The treatment was given for thirty five days. Mice were terminated on the 42nd day and hepatic histopathology was observed with Manja Roenigk score. **Results:** The group induced with tiger shrimp shell extract in graded doses showed a better picture of hepatic histology structure compared to the group that was only induced with MSG. Statistical tests using the *Kruskal Wallis* test showed a significant difference between each group with a P value of 0.005. **Conclusion:** Feeding of tiger shrimp shell extract in graded doses can prevent hepatic damage to MSG-induced mice.

***Keywords:*** *Astaxanthin, Hepatic histopathology, Monosodium Glutamate, Tiger shrimp shell extract*

## INTRODUCTION

Hepar is the body's largest metabolic center organ that is prone to damage. In addition to its function in detoxifying the body's waste substances, exposure to varioustoxic materials such as chemicals and food additives will exacerbate hepatic damage when consumed in excess.[1] MSG is one of the food additives in the form of food flavouring that is often consumed by the public.[2]

The study proved that MSG feeding at a dose of 6 g/b.wt for 45 days gave a picture of cytoarchitectural distortion of hepatocyte cytoplasm, an increase in the number of vacuoles, dilatation of central veins, and inflammatory cell infiltration with small fragmented nuclei that were many around the central vein in rat hepar.[3] Feeding of MSG at a dose of 6 g/kgb.wt for 21 days gives a picture of vacuolization, the presence of necrosis cells and inflammatory cells in the pericellular channels of rat hepar.[4]

The toxic effect on the liver is due to the activity of free radical compounds from MSG.[1] The toxic effects due to free radicals from MSG can be overcome by the body in the presence of endogenous antioxidants or antioxidants that come from within the body. However, if free radical compounds enter in excessive amounts, exogenous antioxidants are needed to fight them.[5]

Astaxanthin is one of the exogenous carotenoid antioxidant compounds that can be found in all marine life such as tiger shrimp.[6,7] Unlike the meat, the shell of tiger shrimp is often wasted and underutilized. The shells are left to waste until they eventually rot. Meanwhile, according to Sachindra et al, the shell has a high astaxanthin content compared to other parts of the tiger shrimp body.[8]

Although many studies have conducted research on the function of astaxanthin as an antioxidant, its role in preventing hepatic damage due to free radicals from MSG is not well known. Based on this background, a study was conducted on the effect of tiger shrimp shell extract feeding in graded doses on the histopathological picture of MSG-induced liver.

## METHODS

Extract samples were cleaned, dried, put in an oven at 70° C for 24 hours, blended and sieved with

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mesh 70 to produce mesh 80/100 results to become powder.[9,10] Tiger shrimp shell powder was then extracted using palm oil in a ratio of 1:2. Every 10 g of tiger shrimp shell powder wasmixed with 20 ml of palm oil then was heated at 70 °C, stirred using a magnetic stirrer for 2 hours. The extraction results were centrifuged at 4500 rpm for 10 minutes at 20°C. The supernatant was taken and screened for total astaxanthin measurements using a UV-Vis spectrophotometer.[11,12]

Prior to treatment, 25 male mice were adapted by caging and given standard food and drink for one week. Group treatment was carried out for 35 days. MSG concentration for all mice was 0.84 g/kgb.wt except the control group. The control group (K-) was only given standard feed, group (K+) induced only MSG, treatment group 1 (P1) induced MSG and tiger shrimp shell extract 50 mg/kgb.wt, treatment group 2 (P2) induced MSG and tiger shrimp shell extract 100 mg/kgb.wt, treatment group 3 (P3) induced MSG and with tiger shrimp shell extract 200 mg/kgb.wt. MSG feeding was carried out for three weeks after adaptation followed by tiger shrimp shell extract for 14 days.

Termination was carried out by putting the mice into a jar that had been given cotton containing an anesthetic substance. The hepatic organs were washed in physiological solution (NaCl 0.9%), weighed, and fixed in 10% formalin buffer solution. Hepatic slice preparations were made with paraffin method.

Hepatic slice preparations were scored using Manja Roenigk histopathology scoring with score criteria 1-4 in 5 fields of view. Each hepatic preparation was observed under a microscope in 5 different fields of view (at 4 angles and in the center) at 400x magnification. In each field of view, 20 cells were randomly counted and scored for each cell using the Manja Roenigk histopathology scoring model in Table 1.

**Table 1.** Manja Roenigk Scoring

| Value | Rate of Change |
|---|---|
| 1 | Normal |
| 2 | Parenchymatous degeneration |
| 3 | Hydropic degeneration |
| 4 | Necrosis |

## RESULTS

Extraction of tiger shrimp shells in this study produced astaxanthin of 56.75 µg/g. Based on it, the predicted doses of astaxanthin induced in mice are 0.003 mg/day (P1), 0.006 mg/day (P2), and 0.010 mg/day (P3).

The microscopic damage of mice hepar in five groups, positive control group (K+), negative control group (K-), treatment group 1 (P1), treatment group 2 (P2), treatment group 3 (P3) observed using the Manja Roenigk score is shown in Figure 1.

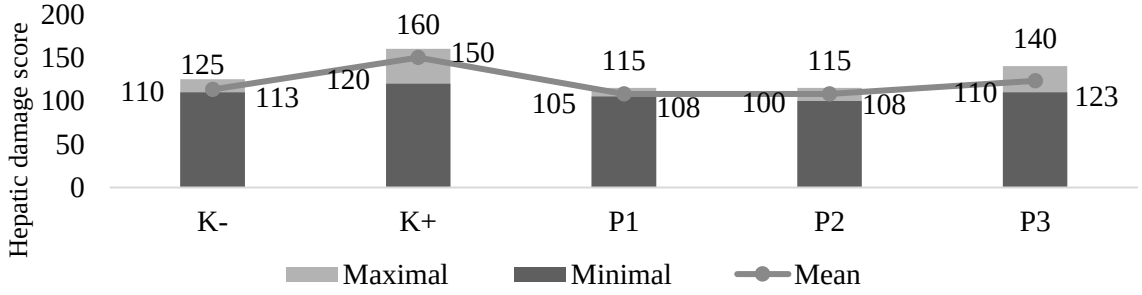


**Figure 1.** Manja Roenigk Score in Each Treatment

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Based on Figure 1, it can be seen that the microscopic picture of hepatic cells in the K+ group has a higher mean value of damage than the other four groups. Meanwhile, the mean P1 and P2 treatment group is higher than in the P3 treatment group

**Table 2.** Saphiro-Wilk Test Result

| Group | Shapiro-wilk |
|---|---|
| | P |
| K- | 0,000* |
| K+ | 0,005* |
| P1 | 0,046* |
| P2 | 0,814* |
| P3 | 0,032* |

Note : * Normal (p>0,05)

Table 2 shows the normality test using the Saphiro-Wilk test because the number of samples is less than fifty. Based on Table 2, it was found that the data was not normally distributed because in groups K-, K+, P1, and P3 the p-value was <0.05, so a non-parametric test was performed in the form of the Kruskal Wallis test (Table 3).

**Table 3.** Kruskal Wallis Test Result

| Group | Mean ± SD | P |
|---|---|---|
| K- | 113 ± 6,70[a] | 0,005 |
| K+ | 150 ± 16,95[b] | |
| P1 | 108 ± 4,47[ab] | |
| P2 | 108 ± 5,70[ab] | |
| P3 | 123 ± 15,65[ab] | |

Note: a = similar letter notation means there is nosignificant difference in the *Mann-Whitney* test with an Asymp.Sig value> 0.05, b = similar letter notation means there is a significant difference in the *Mann- Whitney* test with an Asymp.Sig value <0.05.

In the *Mann-Whitney* test, there was a significant difference between the K+ group and the P1, P2, and P3 groups with an with a significant value <0.05. Furthermore, the with a significant value >0.05 was obtained between the K- group and the P1, P2, P3 groups, so there was no significant difference between these groups (Table 3). In addition,the significant value >0.05 was also obtained between the P1 group and the P2 and P3 groups, as well as between the P2 group andthe P3 group so that no significant differencewas found between the P1 group and the P2 and P3 groups, as well as between the P2 group and the P3 group (Table 3).

## DISCUSSION

Astaxanthin is a group of carotenoids with a structure similar to β-carotene. The similarity of astaxanthin structure with β-carotene can be seen in Figure 2.

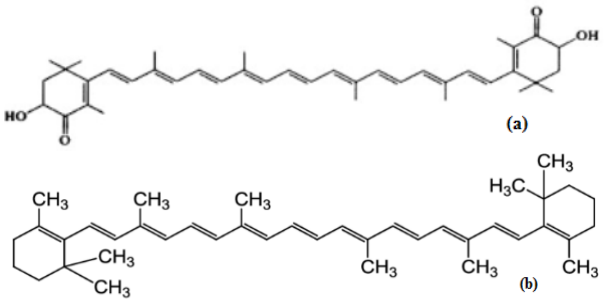


**Figure 2.** Structure of (a) Astaxanthin and (b) β-carotene

Based on the similarities seen in Figure 2, the solvent used to extract astaxanthine can be used to extract beta-carotene. Extraction of tiger shrimp shells was carried out using palm oil because it facilitates the application of carotenoid results compared to VCO oil. In addition, vegetable oils are known to release more carotenoids than animal oils.[11] The choice of temperature, time, and particle size in performing extraction are important things that affect the yield of astaxanthin.[14]

The extraction in this study used a temperature of 70 °C. In line with the research of Handayani, Banucaturananti and Sachindra, astaxanthin extraction with a temperature of 70 °C is able to produce optimal astaxanthin yield.[10,11,15] Heating is able to induce carotenoproteins to release free carotenoids bound to lipoproteins. The free carotenoids will then bind to fatty acids in vegetable oil.[16]

Extraction for 120 minutes supports the formation of higher astaxanthin yields compared to other lengths of time.[15] The longer extraction time will degrade the pigment significantly.[17] However, the higher temperature (close to 100 °C) and the longer extraction time will decrease the astaxanthin yield.[10] Meanwhile, the use of mesh 70 will produce mesh 80/100 which facilitates the penetration of palm oil into the tiger shrimp shell to be extracted.[10,11] The astaxanthin yield in this study was 56.75 μg/g.[9,11,15]

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Calculation of the degree of hepatic cell damage with Manja Roenigk scoring in statistical analysis shows differences in the degree of hepatic cell damage between groups. Based on Figure 3, the K+ group is the group with the greatest degree of damageand the P1 and P2 groups are the groups with the greatest degree of improvement in the picture of hepatic cell damage.

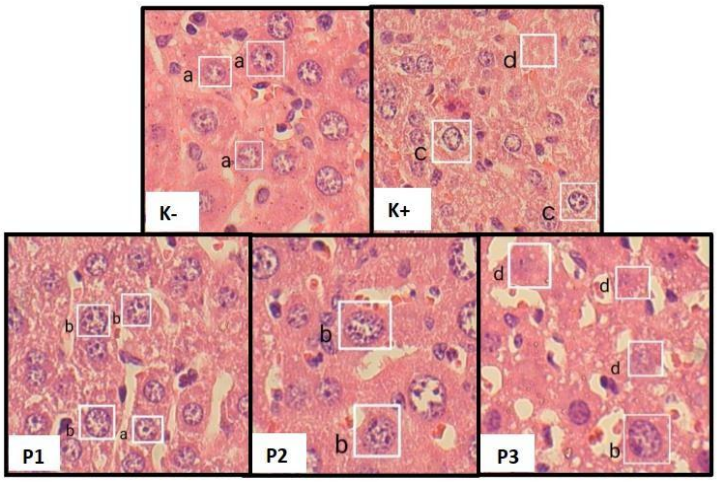


**Figure 3.** Microscopic picture of hepatic cell damage with Manja Roenigk score assessment at 400x magnification.
Description: a = normal, b = parenchymatous degeneration, c = hydropicdegeneration, d = necrosis

Based on Figure 3, the K- group showed a predominance of normal cell images and alimited amount of parenchymatous degeneration images with an average hepaticdamage of 113. The average hepatic cell damage in the K- group was lower than the P3 and K+ groups (Figure 1). This is in accordance with the research conducted by Rahmando who obtained normal hepatic cells with a limited amount of parenchymatous degeneration and hydropic degeneration. In general, any normal preparation of hepatic cells gives a picture of cell necrosis and is not a pathological process. However, a pathological process can occur when the amount of necrosis in the cells increases.[11]

Figure 3 also shows a hepar microscopic picture of the K+ group with the most severe damage, namely the presence of a picture of hydropic degeneration which is often found with a total damage of 150 (Figure 1). The existence of hydropic degeneration that lasts long and heavy enough to make cells can no longer compensate so that irreversible changes occur in the form of cell necrosis.[18] Therefore, a picture of cell necrosis can be found in the K+ group (Figure 3).

Microscopic examination of the hepatic cells in group P1 and group P2 showed a better histological structure compared to groups K+ and K- with a predominance of mild damage, namely parenchymatous degeneration (Figure 1). In this degeneration, cells look swollen with cloudy cytoplasm due to reversible protein deposits, so that normal cells can still be found in this group.[19] The mean improvement of hepatic cell damage ingroups P1 and P2 was 108, higher than in group P3 (Figure 1).

Then, Figure 3 shows the histological structure of the P3 group which is better than the K+ and K- groups with the dominance ofthe hydropic degeneration picture, which shows clear vacuoles in the cytoplasm due to incoming fluid so that the cytoplasm appearsswollen, pale, clear, contains a lot of water and does not contain fat or glycogen.[18,20] The mean improvement of hepatic cell damage in group P3 was 123, lower than the mean improvement of damage in groups P1 and P2 (Figure 1).

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The difference in the degree of damage indicates differences in the effect of preventing hepatic cell damage due to the toxic effects of MSG. This is in accordance with research conducted by Mezzomo who successfully reduced serum cholesterol levels in mice that can induce oxidative stress and lipid peroxidation by using pink shrimp residue extract at doses of 50 mg/kgb.wt, 100 mg/kgb.wt, and 200 mg/kgb.wt. In the study, the optimal dose was 100 mg/kgb.wt using the Soxhlet extraction method. This shows that, the higher the dose of astaxanthin does not produce an improvement effect on the picture of better hepatic cell damage.

Based on the amount of astaxanthin dose induced to mice, Han's research shows results that are in accordance with this study. In that study, giving a dose of 0.2 mg/kgb.wt astaxanthin to mice which is equivalent to 0.006 mg/30gb.wt astaxanthin can reduce the picture of hepatic cell damage in the form of infiltration of fat molecules, inflammatory cells, and necrosis of hepatic cells, and reduce AST and ALT levels to very low. The damage in the study occurred due to ethanol administration which triggered oxidative stress.[22] In addition, this study is in line with Wu's research, namely at a dose of mg/day astaxanthin in mice is able to prevent liver fibrosis due to alcohol induction. These results were supported by an improvement in serum ALT and AST values.[23]

Damage to the liver is basically caused by the glutamate content in MSG which triggers oxidative stress and lipid peroxidation due to free radicals.[24] This is due to an imbalance between the formation of free radicals and the amount of anti-radical compounds or antioxidants in the body.[5] An increase in free radicals can reduce the amount of oxygen, thus reducing ATP production. Decreased ATP can interfere with the active transport process and cause the accumulation of sodium ions in the intracellular fluid to form edema. If this situation continues, it will cause swelling of organelles, including mitochondria and endoplasmic reticulum. As a result, there is a decrease in protein and lipid synthesis so that the cell membrane regeneration process is disrupted.[25]

In addition, polyunsaturated fatty acids in the cell wall due to the presence of free radicals undergo lipid peroxidation in the form of long chains that damage the organization of the cell membrane. The toxic effect initially only damages the cell membrane but the damage continues to the cell nucleus.[25] The damage triggers hydropic degeneration and parenchymatous degeneration, and if the condition continues, it results in cell necrosis.[18]

Feeding of tiger shrimp shell extract containing astaxanthin showed the results of preventing hepatic cell damage dueto free radicals. This is because astaxanthin as a secondary antioxidant is able to neutralize free radicals by giving one electron to free radicals so that they become stable molecules.[26] In addition, astaxanthin can clean radical lipid peroxidation by breaking the long chain that has been formed and scavenge free radicals effectively by reaching the terminal ring membrane layer.[27] Thus, the feeding of tiger shrimp shell extract in graded doses in this study proved to be able to prevent hepatic damage as indicated by the improvement in the picture of hepatic histopathology damage to mice due to MSG induction.

## ETHICAL APPROVAL

Ethical clearance was obtained with the approval and consideration of the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University. The subject was willingly join the study by signing informed consent.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest

## ACKNOWLEDGMENTS

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## AUTHOR CONTRIBUTIONS

Conceptualization, Indah Saraswati and Desy Armalina; methodology, Indah Saraswati and Desy Armalina; software, Fadila Risang Ayu; validation, Indah Saraswati, Desy Armalina, and Fadila Risang Ayu; data curation, Indah Saraswati and Desy Armalina; writing—original draft preparation, Fadila Risang Ayu and Indah Saraswati; writing—review and editing, Desy Armalina; supervision, Ratna Damma Purnawati;

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