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# Subchronic toxicity test of black garlic (*Allium sativum*) ethanol extract in liver histology of female mice (*Mus musculus*)

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## ABSTRACT

Black garlic is an innovative product from garlic which contains S-allyl cysteine (SAC), flavonoids, and polyphenols during the aging process. The use of black garlic as a medicine has not been studied regarding its toxicity to the liver, so research is needed regarding the safety of its use through toxicity tests. The purpose of this study was to determine the effect of giving various doses of black garlic ethanol extract on liver histology and to determine the safe dosage of black garlic ethanol extract for consumption. The method used was the treatment of black garlic ethanol extract in the treatment group at doses of 5, 50, 100, 300, 600, 1000, and 2000 mg/kg BW orally in mice to determine the effect on the histology of the mice's liver. The results showed that the greater the treatment dose of black garlic ethanol extract, the greater the percentage of damage to hepatocyte cells in the form of necrosis in the histology of the mice liver. The highest percentage of damaged cells experiencing necrosis was 49% at a dose of 2000 mg/kg BW. The use of doses that are safe for consumption has not been found in this study due to cell damage found > 30% in the category of moderate damage. So further testing is needed using a lower dose or by administering the extract over a shorter period of time to determine the safe dose for consumption.

**Keywords:** Hepar, histology, toxicity subchronic

## INTRODUCTION

Garlic has other innovations to be made into products that are used for consumption as medicine. This is because garlic has a unique taste and aroma pungent, so another innovation was created that made garlic consumable directly. Black garlic is an innovation from garlic which is believed to have properties that exhibit anticancer, antiobesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects (Wang et al., 2010).

Black garlic is rich in polysaccharides, reducing sugars, proteins, phenolic compounds and sulfur compounds. The amounts of polyphenols increased 6-fold in peeled black garlic. In addition, the total polyphenol and flavonoid content of black garlic increased significantly during the heating process. (Lu et al., 2017; Agustina et al., 2020). The heating process was carried out for 35 days. Based on value IC50, heating time for 35 days is 2.27 ppm with IC value50the smallest compared to the heating time of 15 and 25 days, namely 2.41 ppm and 2.93 ppm. The smaller the value IC50 extract, the more optimal the ability to scavenge free radicals by the active compounds in the extract. Flavonoid content during the 35 days heating period was higher than the 15 until 25 days heating periods (Agustina et al., 2020).

The use of black garlic for medicine that is widespread requires research related to its safety in the short term. To check the safety of a drug, it is necessary to have a toxicity test. According to Hairunnisa (2019), toxicity test is a test that uses preparations in the form of mice, rats, guinea pigs, rabbits, dogs, hamsters, and other animals. These experimental animals are instrumental in developing drugs that aim to demonstrate human biochemical, physiological and pathological reactions. Although toxicity tests cannot be used as a

reference as absolute proof of the safety of a substance or substance, toxicity tests can determine the presence of relative toxicity and identify the appearance of toxic effects if exposure occurs to humans.

Toxicity tests can be divided into 2 groups including the general test group which consists of acute, subchronic, and chronic and the special test group (Lu, 1995; Eriadi et al., 2016). The research to be carried out is a subchronic toxicity test. The subchronic toxicity test is for repeated dosing of experimental animals for 28 or 90 days to detect toxic effects after administration of the test substance (OECD, 2008). The principle of the subchronic toxicity test is that the dose level in the test preparation is given every day for several groups of experimental animals. During the time of administration, the experimental animals must be observed every day. These observations are to determine when a given test formulation develops toxicity (BPOM RI, 2011). The purpose of the subchronic toxicity test is to obtain information about the presence of toxic effects obtained after exposure to the test preparation, determine the dose that does not cause toxic effects, and information about the toxic effects of substances that are not detected in acute toxicity tests (OECD, 2008).

Research to test the toxicity of black garlic in experimental animals, has been carried out by Handayani et al. (2018) where research on the acute toxicity of black garlic was carried out on shrimp larvae. This study produced data of 90% where shrimp larvae died at a concentration of 1000 ppm after being given a solution of black onion extract.

## METHOD

### Time and place of research

The research was conducted from April 2022 to March 2023. The research location was carried out at the Chemistry Laboratory and Taxonomy Laboratory, Universitas Islam Negeri Sunan Ampel, Surabaya, Campus 2 Gunung Anyar.

### Variables and types of research samples

The independent variable in this study was the dose level of the extractblack garlic (*Allium sativum*) by giving orally to mice (*Mus musculus*) at doses of 5, 50, 100, 300, 600, 1000, 2000 mg/kg BW. The dependent variable in this study was the histopathological appearance of the liver in mice with parameters of inflammatory cell infiltration and necrosis. The control variables in this study were the cages of the mice, the age of the mice, the body weight of the mice, and the sex of the mice. This research is an experimental method using a completely randomized design (CRD). The treatments were grouped based on Federer's formula into 8 groups with 3 repetitions each consisting of a control group with distilled water and a treatment group.

### Research procedure

#### *Making black garlic*

Making black garlic begins with preparing the tools (scales and oven) and ingredients in the form of garlic. Then the garlic is weighed as much as 1000 grams. After that, the garlic was placed in the oven for 35 days at 70°C. Once in the oven the garlic turns black then the black garlic is removed from the oven.

#### *Making black garlic extract*

Cut the dried black onion bulbs after baking them into thin slices. After that, the black garlic is crushed with a blender and then put into the Erlenmeyer. Then 70% ethanol solution was poured in the erlenmeyer and covered with aluminum foil. After that, the solution was sonicated for 1 hour to homogenize. Then the solution was macerated for 48 hours. Furthermore, the maceration results were filtered using a funnel and filter paper to separate the residue and the filtrate.

The collected filtrate was evaporated withrotary evaporatorsat 55℃. The end result is a thick extract.

***Determination of black garlic extract dosage***

The dose used for treatment of mice refers to research of Rumaseuw et al. (2022), tested the acute toxicity of the extractblack garlictested on female mice using graded doses of 5, 50, 300, 2000 mg/ kg BW. This study used a dose that referred to that study because the results of the acute toxicity test with the highest dose did not give a lethal effect, so the subchronic toxicity test was continued to obtain information on the histopathology of the mice organs. So that the doses used in this study were 5, 50, 100, 300, 600, 1000, and 2000 mg/ kg BW.

***Preparation of experimental animals***

This research uses female mice (*Mus musculus*) of the DDY type as experimental animals, provided that the mice are healthy and there are no visible abnormalities in their body parts. Prior to treatment, mice were acclimatized for 1 week to the same conditions including drinking, cages, feed, and husks. Acclimatization aims to allow mice to adapt to their new environment.

***Giving black garlic extract***

Giving black garlic extract by injecting it with a syringe gavage/orally according to the treatment group was carried out for 28 days. The way to give the extract is by attaching a sonde to the upper palate of the mice.

***Subchronic toxicity test treatment***

The subchronic toxicity test treatment, based on BPOM RI (2014), included female mice divided into 8 treatment groups with 3 repetitions. Group I (control) was treated with distilled water, groups II, III, IV, V, VI, VII, VIII were treated with doses of 5, 50, 100, 300, 600, 1000, 2000 mg/kg BW orally, administration The ethanol extract of black garlic was carried out for 28 days, and on the 29th day the experimental animals were anesthetized after being fasted for 1 night which were then operated on to observe the histopathology of the liver of female mice (Rofiqoh, 2015).

***Observation of each parameter***

Histological preparations were then observed under a microscope each repetition at 5 microscopic fields of view with a magnification of 1000x for observation of necrosis and 400x for observation of inflammatory cell infiltration. Observation consists of identification of normal cells and damaged cells. Then scoring is done by calculating the percentage of damage according to Januar (2014):

$$(\%) = \frac{number\ of\ damaged\ cells}{number\ of\ cells\ found}\ x\ 100\%$$

Then the percentage is categorized based on the level of damage with the percentage of damage 50% in the category of heavy damage (Arsad et al., 2014; Jannah & Budijastuti, 2022).

**Data analysis technique**

Collecting data from this study aims to examine the subchronic toxicity of black garlic ethanol extract to the histology of mice livers. Statistical analysis using SPSS computer program. The results of the study were analyzed by using the normality test shapiro-wilk to determine the normality of the distribution of the data used. Then proceed with the homogeneity test using the testlevel test to find out the variation of the data group. P-value > 0.05 indicates that the data is normally distributed and homogeneous and is continued with the testone-way Analysis of Variance (ANOVA).

## RESULTS AND DISCUSSION

Based on the observations made, it showed signs of damage to hepatocyte cells with necrosis damage on a microscope with a magnification of 1000x with hematoxylin eosin staining in the image shown in Figure 1. It showed the liver histology of mice with

necrosis in the control group and treated with black garlic extract at doses of 5, 50, 100, 300, 600, 1000, and 2000 mg/kg BW. The percentage of cell damage due to necrosis can be seen in (Figure 5). When observed under a microscope, changes can be seen in the form of disappearance of chromatin, the nucleus is wrinkled, no longer vascular, the nucleus looks denser, the color is darker/pyknosis, the nucleus is divided intoseveral parts, torn/ karyorexis, the cell nucleus no longer needs a lot of color because of pale/unreal/ karyolysis (Himawan, 1992; Adinata et al., 2012).

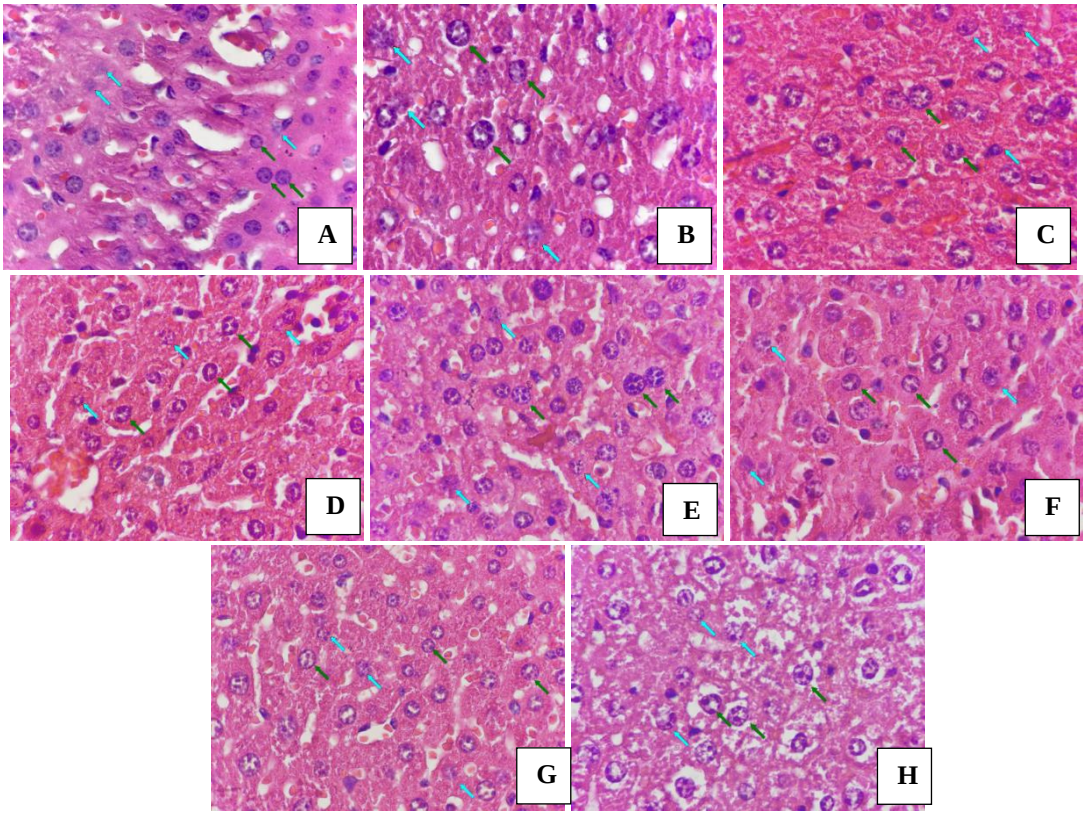


Figure 1. Histology of liver with necrosis group: (A) Control, (B) Dose of 5 mg/kg BW, (C) Dose of 50 mg/kg BW, (D) 100 mg/kg BW, (E) 300 mg/kg BW, (F) 600 mg/kg BW, (G) 1000 mg/kg BW, and (H) 2000 mg/kg BW (Necrosis cells: blue arrows, normal cells: green arrows).

Histological changes in the liver of mice in the form of necrosis could be due to the influence of excessive substance content from the treatment of black onion extract, so that it becomes a toxic substance for hepatocyte cells which results in damage such as necrosis. Necrosis is the death of tissue cells after injury while the individual is still alive. This is in line with the research of Nursofia & Yuliawati (2021) regarding the acute toxicity test of the ethanol extract of cinnamon leaves (*Cinnamomum burmanii*) on the liver function of white rats (*Mus musculus* L.) female. The study used doses of 250, 500, 1000 and 2000 mg/kg BW. The results showed that at a dose of 2000 mg/kg BW, there was a lot of parenchymal degeneration damage, with a small number of normal hepatocyte cells. Cells that undergo degeneration experience tears in the plasma membrane and undergo changes in the cell nucleus, so that cells become necrosis.

Cell damage at the control dose could occur thought to be caused by apoptosis and external factors. According to Juan et al. (2011), untreated cell cultures detected caspase-3 activity which is an apoptotic activation protein that causes apoptosis by 2-5%. This is in line with the results of research from Kurniawan et al. (2016) regarding mouse liver histology (*Mus musculus* L.) which

was given lamtoro leaf extract (*Leucaena leucocephala*), showed the highest cell damage due to apoptosis of 1.52%. Therefore, if the cell damage results by a percentage exceeding the amount of damage in general it can be caused by external factors.

Fixation of one of the external factors that may affect liver histology. At the stage of making histological preparations when the fixation process with 10% formalin was of poor quality. is the stage that becomes a success factor in the manufacture of histological preparations. If the fixation process has an error, then the next stage will be useless. This is because the results of preparations with the wrong fixation process can damage the cells in the preparation, so that when observed there is a lot of damage (Erick & Dewi, 2017; Ramadhani, 2021). This is evidenced by the presence of erythrocyte cells on the outside of the blood vessels in liver histology with control doses up to 2000 mg/kg BW, which is thought to be due to imperfect fixation. These results are in line with the research of Fajrina et al. (2018) regarding the description of the quality of liver tissue preparations using a 10% NBF fixative solution and 70% alcohol in HE (Hematoxylin-Eosin) staining. The results of the research in this study showed good fixation results with the appearance of hepatocyte cells evenly distributed in the blue cell nucleus and clearly visible red in the cytoplasm. While the picture of hepatocyte cells that are not fixed properly shows hepatocyte cells that look rough and look uneven, the blue color of the cell nucleus and red cytoplasm is clearly visible with a thick color, and the erythrocyte cells are spread outside the blood vessels.

External factors that may cause cell damage include the health of the mice. This is in line with the research of Nugroho et al. (2014) regarding the protective effect of the ethanol extract of binahong leaves (*Anredera cordifolia* (Tenore) Steenis) on the liver histopathology of white rats induced by ethanol using doses of 50, 100, and 200 mg/kg BW. The results of this study indicated that there was cell damage at the control dose in the form of cell swelling in the mild damage category with the possible cause being stress or health problems.

The results of observations at the doses of 5, 50, 100, 300, 600, 1000 and 2000 mg/kg BW showed a higher level of cell damage than the control group. This could be caused by the treatment of black onion extract which resulted in the cells becoming damaged due to the effect of the substances contained in the black onion extract on the liver of mice. These substances can be in the form of flavonoids and tannins contained in black onion extract. Necrosis is the death of cells or tissues in living organisms which is characterized by changes in morphology due to progressive degradation by enzymes in cells that cause damage (Pramono, 2012; Oktarian et al., 2019). Cell death is damaged whose nature cannot return to normal. Factors in the occurrence of cell death can be through the process of apoptosis and cell necrosis. Apoptsis is the process by which cells die in a planned or programmed manner, while necrosis is characterized by the presence of inflammatory cells. Necrosis can be local or diffuse due to lack of oxygen, ischemia, anemia, and peptide disturbances, as well as free radicals (Lu, 1995; Oktarian et al., 2019).

According to Ressang (1984), cell damage in the form of necrosis in the liver can be caused by the effects of the entry of substances that have toxic properties such as chemicals or germ toxins (Guyton & Hall, 1997). The result of a toxic substance that enters the liver for a long period of time can cause damage in the form of cell death in the lobules. The stage of necrosis starts from the morphology of the cell nucleus which undergoes changes, namely pyknosis. Next is the process of the cell nucleus breaking apart (karyorexis) then the cell nucleus disappears

(karyolysis) (Robbins, 1992; Adikara et al., 2013). Apart from being influenced by the period of time, cell damage is also influenced by the large amount of chemicals that enter the liver organs, which can cause cell damage, such as inflammatory cell infiltration and and necrosis (Guyton & Hall, 1997). Following are the results of histological observations in the form of inflammatory cell infiltration at 400x magnification with hematoxylin eosin staining.

Based on Figure 2 shows liver histology images of mice experiencing cell infiltration in the control group and treatment with black garlic extract at doses of 5, 50, 100, 300, 600, 1000, and 2000 mg/kg BW. The degree of infiltration of inflammatory cells is determined by their presence in each field of view. At the control dose and at 5 mg/kg BW, inflammatory cell infiltration was found in several fields of view, while at doses of 50, 100, 300, 600, 1000, and 2000 mg/kg BW, inflammatory cell infiltration was found throughout the field of view.

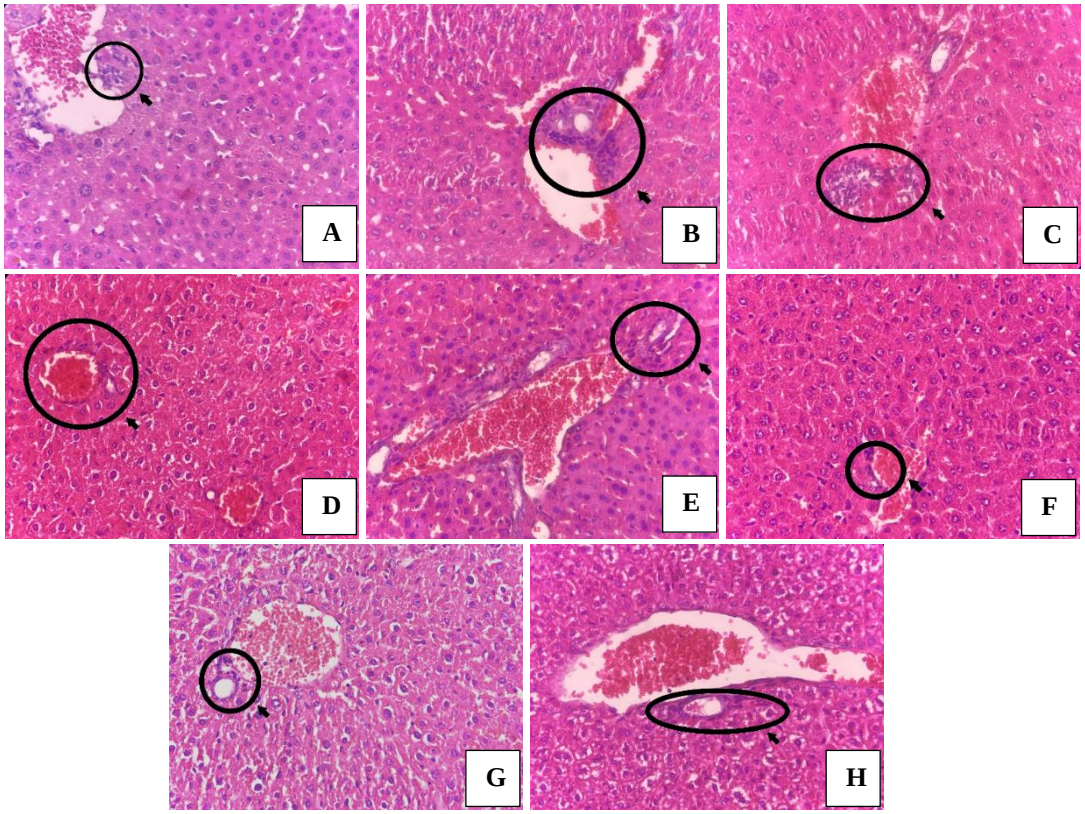


Figure 2. Histology of the liver experiencing inflammatory cell infiltration group: (A) Control, (B) Dose of 5 mg/kg BW, (C) Dose of 50 mg/kg BW, (D) 100 mg/kg BW, (E) 300 mg/kg BW, (F) 600 mg/kg BW, (G) 1000 mg/kg BW, and (H) 2000 mg/kg BW (Cell infiltration: black arrow).

According to Wiralaga et al. (2015) inflammatory cell infiltration in several fields of view is categorized as moderate, whereas if inflammatory cell infiltration is found throughout the field of view it is categorized as severe. This indicates that the greater the cell damage, the greater the infiltration of inflammatory cells. The state of inflammatory cell infiltration is a response mechanism that shows signs of damaged tissue and acts as a protective response to causes of tissue damage or cellular debris (Kumar et al., 2004; Azzahra, 2020). As shown in (Figure 2.), inflammation of the liver is characterized by the presence of inflammatory cells in the form of phagocytes, namely infiltrating lymphocytes and polymorphonuclear leukocytes which can be seen microscopically in liver tissue.

Microscopically, all cell infiltration is shown by the presence of inflammatory cells with purple color characteristics (Swarayana et al., 2012; Cahyani et al., 2021). Inflammation, also known as an inflammatory reaction, is an important mechanism for the body as a form of self-defense from hazards that cause balance disturbances and has a function to repair the structure and function of tissues that are disturbed due to danger (Baratawidjaya, 2002; Adikara et al., 2013). The mechanism of the inflammatory response is to attract phagocytes and plasma proteins to the site of tissue damage to isolate, destroy and inactivate the cause of the damage, as well as prepare the tissue for the regeneration process. Causes of inflammation include chemical compounds, physical trauma and microbiological agents (Azzahra, 2020; Corwin, 2008).

Substances that cause histological changes in the liver are thought to be due to the presence of too much of these substances, which results in disruption of metabolism in the liver to absorb substances that enter from outside the body. These substances can be in the form of flavonoids and tannins contained in black onion extract. Both of these compounds are antioxidant compounds which, if they enter the body, processes of absorption, metabolism, distribution, and excretion will occur. This applies to black garlic where the extract contains antioxidants. According to research of Agustina et al. (2020) regarding the antioxidant activity of black garlic, showed that black garlic extract with a heating time of 15, 25, and 35 days had very strong antioxidant potential. When it enters the body, the compound will undergo a metabolic process in the liver (Lu, 2010).

High antioxidants can also have an effect on the rate of oxidation so that antioxidants become pro-oxidants. The high concentration of incoming antioxidants can affect the antioxidant activity of the phenolic groups which can be lost so they can become pro-oxidants (Gordon, 1990; Suaniti et al., 2017). Antioxidants are one of the pro-oxidant groups because they can act as pro-oxidants in their mechanism under certain conditions, the central nervous system has a high sensitivity to this damage which causes damage to cell lipids due to the small amount of antioxidants (Rahal et al., 2014) (Figure 3). The following is the mechanism by which antioxidants become pro-oxidants.

$$AH + O_2 \text{ ---------- } A\bullet + HOO\bullet$$
$$AH + ROOH \text{ ---------- } RO\bullet + H_2O + A\bullet$$

Figure 3. Antioxidants at high concentrations act as prooxidants
(Source: Rahal et al., 2014)

Radical compounds cause peroxidation of membrane lipids, especially unsaturated fatty acids. Lipid peroxidation is a complex chemical process due to the reaction of PUFA (Polyunsaturated Fatty Acid), a component of cell membrane phospholipids, with ROS (Reactive Oxygen Species) compounds to form hydroperoxides (Yuliandari et al., 2023). The mechanism of lipid peroxidation to unsaturated fatty acids is shown in Figure 4.

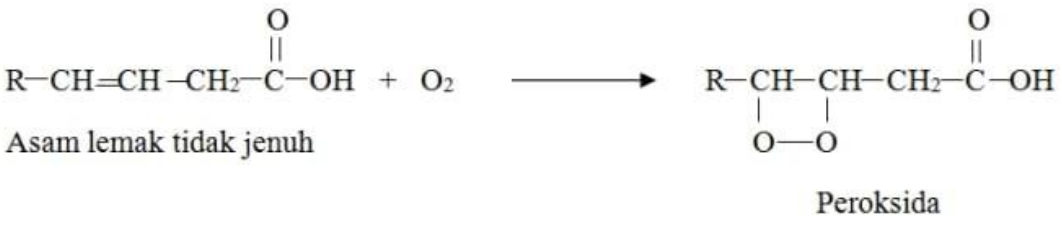


Figure 4. Mechanism of Pro-Oxidant Against Unsaturated atty Acids
(Source: Suaniti et al., 2017)

Research conducted by Eghbaliferiz & Iranshahi (2016) states that there is prooxidant activity from polyphenol and flavonoid compounds. Low doses of polyphenol compounds function to prevent lipid peroxidation and oxidative stress. Conversely, if in large quantities, alkaline pH, and the presence of oxygen molecules, these antioxidants can become pro-oxidants. Exposure to these substances if it enters the body continuously will cause greater toxic effects. This was proven by some hepatocyte cells experiencing cell death/necrosis. Necrosis is a continuation of the degeneration stage because the tubular cells absorb too much material which results in cell death (Nurdiniyah et al., 2015).

Based on observations of hepatocyte cells in mice liver histology preparations showing damage in the form of necrosis in the control group and the treatment group given black garlic extract doses of 5, 50, 100, 300, 600, 1000, and 2000 mg/kg BW. Furthermore, the results of calculating cell damage are obtained from calculating damaged cells and then totaling them by percentage of the score. Then, the total score was averaged to see the lowest and highest levels of cell damage. After getting the average total percentage of the control group and the treatment group given black garlic extract doses of 5 50, 100, 300, 600, 1000, and 2000 mg/kg BW on Figure 5, then the normality test and homogeneity test will be continued to determine the normality and homogeneity of the data obtained. After the normality and homogeneity tests were carried out, the data were normally distributed and homogeneous, then the test was continuedone-way anova to determine whether there is a significant effect of black garlic extract on liver histopathology.

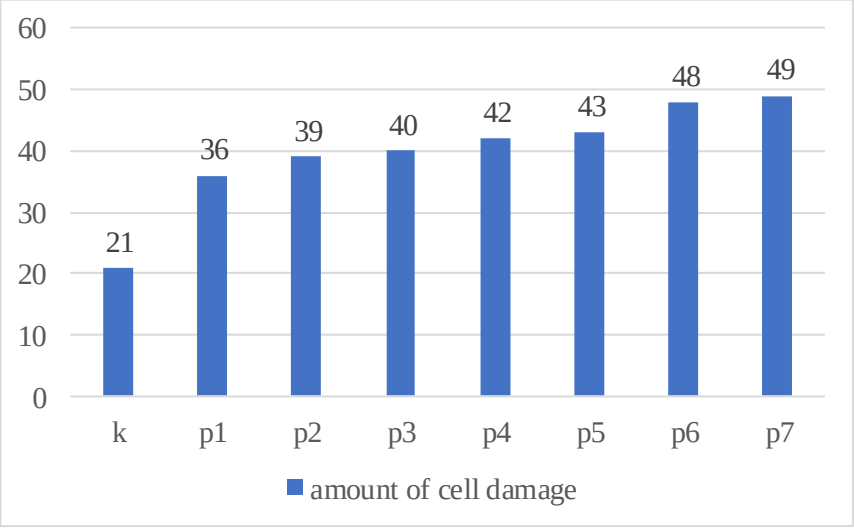


Figure 5. Average percentage of cell damage (k: control, p1: 5 mg/kg BW, p2: 50 mg/kg BW, p3: 100 mg/kg BW, p4: 300 mg/kg BW, p5: 600 mg/kg BW, p6: 1000 mg/kg BW, p7: 2000 mg/kg BW).

Based on Figure 5 shows the percentage results of the control group and the treatment group given black garlic extract doses of 5, 50, 100, 300, 600, 1000, and 2000 mg/kg BW. The control group showed an average percentage with a level of damage in the form of necrosis of 21% damage from all histological fields of the liver observed under a microscope, so it was categorized as mild damage. Whereas in the treatment group the black garlic extract showed that the higher the dose given, the average percentage of damage also increased. the cell. The highest damage was found in the treatment group given black garlic extract at a dose of 2000 mg/kg BW of 49% which was included in the moderate damage category. Test results one-way anova got the P-value with a value of 0.000, which means a P-value.

Table 1. Significant difference test results for differences in cell damage between doses.

| Groups | Average amount of cell damage ± SD |
|---|---|
| Control | 21.3333 ± 1.52753[a] |
| 5 mg/kg BW | 36.3333 ± 3.51188[b] |
| 50 mg/kg BW | 38.6667 ± 1.52753[b] |
| 100 mg/kg BW | 40.3333 ± 1.52753[b] |
| 300 mg/kg BW | 41.6667 ± 4.04145[bc] |
| 600 mg/kg BW | 42.6667 ± 2.51661[bcd] |
| 1000 mg/kg BW | 48.0000 ± 3.60555[cd] |
| 2000 mg/kg BW | 49.3333 ± 7.37111[d] |

Note: alphabet abcd is a sign of whether there is a significant difference

Based on the results (Table 1) for the control doses showed significant differences in average values with treatment of doses 5, 50, 100, 100, 300, 600, 1000, and 2000 mg/kg BW. The highest average result for cell damage in the Duncan test was at a dose of 2000 mg/ kg BW which resulted in an average value of 56.70441. The treatment was stated to be significantly different from the control doses, 5, 50, 100, and 300 mg/kg BW. These results are in line with the research of Maliza et al. (2019) who investigated the subchronic toxicity test of the methanol extract of arabica coffee fruit skin (*Coffea arabica* L.) in the kidney of mice (*Mus musculus* L.) BALB/c strain for 28 days. The results of the Duncan follow-up test showed a significant difference in the effect on organ histology between doses, the highest value obtained was 0.576 ± 0.288 at the largest dose, namely a dose of 1000 mg/kg BW with subsequent results followed by a dose of 500 mg/kg BW with a value of 0.313 ± 0.020 .250 of 0.213 ± 0.005 and 75 mg/kg BW of 0.170 ± 0.000. The occurrence of cell damage is thought to be due to the high concentration of the active compound content in the methanol extract of Arabica coffee fruit skin (*Coffea arabica* L.). such as tannins, alkaloids, and saponins, so that it is in accordance with Rasyid's statement (2012) The larger the dose, the greater the active substance contained in the extract suspension, this is because a dose is important in determining a chemical substance that is toxic to organs (Ginting, 2018).

The damage that occurred in the liver cells of mice showed that the doses given for 28 days were not safe, this was because all doses treated with black garlic extract were in the medium category, namely >30%, where the safe dose should be in the category of low damage or no damage to the liver cells of mice. According to (Fitmawati et al., 2018) the percentage of cell damage that is categorized as mild does not include pathology, but damage under normal circumstances. Therefore, further tests are needed using lower doses or by administering extracts with a shorter time span to find out which doses are safe for consumption. This is in line with research by Amaliyah (2015) regarding the subchronic toxicity test of katuk leaf aqueous extract (*Sauropus androgynus* (L.) Merr.) on heart weight and cardiac histology in white rats. The results showed that there was damage to the rat heart muscle cells (*Rattus norvegicus*) at a dose of 45 mg/kg BW which resulted in cell damage of 12.47%, at a dose of 60 mg/kg BW of 22.6%, and at a dose of 75 mg/kg BW of 27.43%, while at the control dose there was damage of 7.2%. So that a safe dose to use is a dose of 45 mg/kg BW which results in cell damage of 12.47% which is included in the mild damage category.

Another study by Traesel et al. (2016) regarding the acute and subchronic toxicity test of pequi oil in female Wistar rats orally, the results of the acute toxicity test with a single dose of 2000 mg/kg BW observed for 14 days showed no change or death were observed, with an LD value 50 higher than 2000 mg/kg BW.

While the results of the subchronic toxicity test, male and female Wistar rats at doses of 125, 250, 500 or 1000 mg/kg BW for 28 days did not produce significant changes in behavior, histopathological parameters in animals. Several haematological abnormalities were found after subchronic test treatment. These results indicate low acute and subchronic toxicity to pequi oil in rats.

Another study by Maliza et al. (2019) with the results of observations of the kidney of mice which showed cell necrosis, cell infiltration and hemorrhage. This is caused by toxic substances from the treatment of methanol extract of Arabica coffee fruit skin. Duncan's follow-up test showed a significant difference in the effect on organ histology between doses, the highest value obtained was 0.576 ± 0.288 at the largest dose, namely a dose of 1000 mg/kg BW with subsequent results followed by a dose of 500 with a value of 0.313 ± 0.020, 250 at 0.213 ± 0.005 and 75 mg/kg BW at 0.170 ± 0.000.

Another study by Ginting (2018) examined the study of the toxicity of the ethanol extract of purple passion fruit peel (*Passiflora edulis* Sims.) on the liver of mice for 14 days, with the results of the study in the control group, doses of 500 and 1000 mg/kg BW did not show toxic symptoms, but at doses of 2000 and 5000 mg/kg BW showed toxic symptoms in the form of cell necrosis and widespread hemorrhage.

Another study by Auta (2021) research on the subchronic toxicity of extracts of wood ash solutions from *Parkia bigbilosa* on *Mus musculus*. With doses of 5, 50, 100, 300, 500, 1000, 1500 and 2000 mg/kg BW. The results showed that mice died by 10-70% in the treatment group within 48 hours after administration of the extract, death by 30-70% in the treatment group at doses of 5, 50, 100, 300, 500, 1000, 1500 and 2000 mg/kg BW within 14 days after administration of the extract, and death by 40-50% in the treatment group at doses of 5, 50 and 100 mg /kg BW in 90 days. The results of histological observations showed that in the treatment group at doses of 5, 50, and 100 mg/kg BW within 90 days there were significant changes to the kidneys. This study concluded that the extract of wood ash solution from *Parkia bigbilosa* can be harmful to health if consumed for a long time because it affects liver and kidney function. This is evidenced by an increase in dose which also increases ASP, ALT, and ALP in the wood ash solution extract treatment group *Parkia bigbilosa*.

Another study by Donkor et al. (2014) regarding the Study of Acute and Subchronic Toxicity of Aqueous Extract of Bark Root *Cassia sieberiana* D. C. in Rodents. The results showed that giving 5000 mg/kg BW for 14 days and giving (*C. sieberiana*) NPK for 3 months in animals does not result in death. The results of the subchronic test based on the hematological test showed no significant difference ($p>0.05$) between the control and treatment. The results of observing liver micrographs when compared to controls showed necrosis in the centrilobular section at a dose of 750 mg/kg BW. Therefore, the calculation results from LD50 showed that the oral toxicity of NPK in rodents was categorized as low (LD50 orally >5000 mg/kg). However, the extract may have an adverse effect on the liver at high doses on long-term administration. This concludes that further research needs to be carried out to determine the safe dose of bark root *Cassia sieberiana* D. C extract.

## CONCLUSION

The results showed that the greater the treatment dose of black garlic ethanol extract, the greater the percentage of damage to the liver histopathology of mice with the highest percentage of necrosis cell damage, which was 49% at a dose of 2000 mg/kg BW. The use of doses that are safe for consumption has not been found in this study due to cell damage found >30% in the category of moderate damage.

## REFERENCES

Adikara, I. P. A., Winaya, I. B. O., & Sudira, I. W. (2013). Studi histopatologi hati tikus putih (*Rattus norvegicus*) yang diberi ekstrak etanol daun kedondong (*Spondias dulcis* G.Forst) secara oral. *Buletin Veteriner Udayana*, *5*(2), 107–113.

Adinata, M.O., Sudira, I.W. & Berata, I.K. (2012). Efek Ekstrak Daun Ashitaba (*Angelica keiskei*) Terhadap Gambaran Histopatologi Ginjal Mencit (*Mus musculus*) Jantan. *Buletin Veteriner Udayana*, *4*(2), 55–62.

Agustina, E., Andiarna, F., & Hidayati, I. (2020). Uji Aktivitas Antioksidan Ekstrak Bawang Hitam (Black Garlic) Dengan Variasi Lama Pemanasan. *Al-Kauniyah: Jurnal Biologi*, *13* (1), 39–50. https://doi.org/10.15408/kauniyah.v13i1.12114.

Ahada, A.H.U. (2018). *Efek Pemberian Ekstrak Daun Semanggi Air (Marsilea crenata) Pada Tikus Putih (Rattus norvegicus) Betina Terhadap Kadar Serum Glutamic Oxaloacetyc Transaminase (SGOT) Dan Serum Glutamic Piruvyc Transaminase (SGPT) Serta Histopatologi Hepar* . Universitas Brawijaya.

Ahmed, R.A. (2018). Hepatoprotective and antiapoptotic role of aged black garlic against hepatotoxicity induced by cyclophosphamide. *The Journal of Basic and Applied Zoology*, *79*(1). https://doi.org/10.1186/s41936-018-0017-7

Amaliyah, F.R. (2015). *Uji toksisitas subkronik ekstrak air daun katuk (Sauropus androgynus L. Merr.) terhadap berat jantung dan histologi jantung pada tikus putih (Rattus norvegicus) betina* (Issue 1). Universitas Islam Negeri Maulana Malik Ibrahim.

Arsad, S.S., Esa, N.M. and Hamzah, H. (2014). Histopathologic changes in liver and kidney tissues from male Sprague Dawley rats treated with *Rhaphidophora decursiva* (Roxb.) Schott extract. *J Cytol Histol S*, *4*(1), 1-6.

Auta, T. (2021). Sub-chronic toxicity of the aqueous wood-ash extract of Parkia biglobosa in *Mus musculus. Life Res*, *4*(1), 1–9. https://doi.org/10.12032/life2021-0118-304

Azzahra, H. (2020). *Pengaruh pemberian Natrium Nitrit terhadap fungsi enzimatis kadar Alanine Transaminase (ALT) dan Aspartate Transaminase (AST) serta gambaran histopatologis organ hati Tikus Putih (Rattus norvegicus)*. UIN Sunan Ampel Surabaya.

Baratawidjaya, K.G. (2002). *Imunologi Dasar*. Balai Penerbit Fakultas Kedokteran UI.

BPOM RI. (2011). *Pedoman Uji Toksisitas Nonklinik Secara In Vivo*. Pusat Riset Obat dan Makanan BPOM RI.

Cahyani, D., Maliza, R. & Setiawan, H. (2021). Pengaruh Pemberian Ekstrak Kulit Buah Kopi Arabika (*Coffea arabica* L.) terhadap Histopatologi Hati Mencit (Mus musculus L.) yang Diinduksi dengan Etanol. *JBNS (Journal of Biotechnology and Natural Science)*, *1*(1), 12–20.

Corwin, E.J. (2008). *Handbook of Pathophysiology 3th Edition*. Wolters Kluwer Health/ Lippincott Williams & Wilkins.

Donkor, K., Okine, L.N., Abotsi, W.K. and Woode, E. (2014). Acute and sub-chronic toxicity studies of aqueous extract of root bark of Cassia Sieberiana D.C. in rodents. *Journal of Applied Pharmaceutical Science*, *4*(4), 84–89. https://doi.org/10.7324/JAPS.2014.40415

Eghbaliferiz, S., & Iranshahi, M. (2016). Prooxidant Activity of Polyphenols, Flavonoids, Anthocyanins and Carotenoids: Updated Review of Mechanisms and Catalyzing Metals. *Phytotherapy Research*, *1391*(March), 1379–1391. https://doi.org/10.1002/ptr.5643

Eriadi, A., & Arifin, H. (2016). Uji toksisitas akut ekstrak etanol daun kirinyuh (*Chromolaenodorata* L. R.M.King & H. Rob) pada mencit putih jantan. *Jurnal Farmasi Higea*, *8*(2), 122–132.

Erick, K. & Dewi, I. (2017). *Sitohistoteknologi* (Bahan Ajar). Kementerian Kesehatan Republik Indonesia.

Fajrina, S.N., Ariyadi, T., & Nuroini, F. (2018). Gambaran Kualitas Sediaan Jaringan Hati Menggunakan Larutan Fiksatif NBF 10 % dan Alkohol 70 % pada Pewarnaan HE ( Hematoksilin-Eosin). *Prosiding Mahasiswa Seminar Nasional Unimus*, *1*, 60–65.

Fitmawati, F., Titrawani, T. & Safitri, W. (2018). Struktur Histologi Hati Tikus Putih (*Rattus norvegicus* Berkenhout 1769) Dengan Pemberian Ramuan Tradisional Masyarakat Melayu Lingga, Kepulauan Riau. *Ekotonia: Jurnal Penelitian Biologi, Botani, Zoologi Dan Mikrobiologi*, *03*(1), 11–19.

Ginting, H., Dalimunthe, A., & Pratama, E.K. (2018). Kajian Ketoksikan Ekstrak Etanol Kulit Buah Markisah Ungu (*Passiflora edulis* Sims.) Terhadap Hati Mencit. *Talenta Conference Series: Tropical Medicine (TM)*, *1*(1), 257–263. https://doi.org/10.32734/tm.v1i1.85

Gordon, M.H. (1990). The Mechanism of Antioxidant Action in Vitro. *Food Antioxidants*, *1*, 1–18. https://doi.org/10.1007/978-94-009-0753-9_1

Guyton, A.C. & Hall, J. E. (1997). *Buku ajar fisiologi kedokteran* (I. Setiawan (ed.); ed. 9). EGC.

Guyton, A.C. & Hall, J. E. (2007). *Buku Ajar Fisiologi Kedokteran*. EGC.

Hairunnisa, H. (2019). Sulitnya Menemukan Obat Baru di Indonesia. *Majalah Farmasetika*, *4*(1), 16–21. https://doi.org/10.24198/farmasetika.v4i1.22517

Handayani, S.N., Bawono, L.C., Ayu, D.P. & Pratiwi, H.N. (2018). Isolasi Senyawa Polifenol Black garlic Dan Uji Toksisitasnya Terhadap Larva Udang (*Artemia salina* Leach). *Jurnal Ilmu Kefarmasian Indonesia*, *16*(2), 145. https://doi.org/10.35814/jifi.v16i2.561

Himawan, S. (1992). *Kumpulan Kuliah Patologi*. UI Press.

Jannah, D. R. and, & Budijastuti, W. (2022). Gambaran Histopatologi Toksisitas Ginjal Tikus Jantan (*Rattus norvegicus*) yang diberi Sirup Umbi Yakon (*Smallanthus sonchifolius*). *LenteraBio : Berkala Ilmiah Biologi*, *11*(2), 238–246. https://doi.org/10.26740/lenterabio.v11n2.p238-246

Januar, R., Yusfiati, Y. & Fitmawati, F. (2014). *Struktur Mikroskopis Hati Tikus Putih (Rattus Novergicus) Akibat Pemberian Ekstrak Tanaman Tristaniopsis Whiteana Griff*. *1*(2), 392–401.

Juan, G., Zoog, S.J. & Ferbas, J. (2011). Leveraging image cytometry for the development of clinically feasible biomarkers: Evaluation of activated caspase-3 in fine needle aspirate biopsies. *Methods in Cell Biology*, *102*, 309–320. https://doi.org/10.1016/B978-0-12-374912-3.00012-2

Kumar, V., Fausto, N., & Abbas, A. (2004). *Robbins & Cotran Pathologic Basis of Disease* (7th Editio). Saunders.

Kurniawan, W.A.Y., Wiratmini, N.I. & Sudatri, N. W. (2016). Histologi hati mencit (*Mus musculus* L.) yang diberi ekstrak daun lamtoro (Leucaena leucocephala). *Jurnal Simbiosis*, *IV*(2), 46–49.

Lu, X., Li, N., Qiao, X., Qiu, Z. & Liu, P. (2017). Composition analysis and antioxidant properties of black garlic extract. *Journal of Food and Drug Analysis*, *25*(2), 340–349. https://doi.org/10.1016/j.jfda.2016.05.011

Lu, F. C. (1995). *Toksikologi Dasar; Asas, Organ Sasaran, dan Penilaian Resiko* (Penerbit Universitas Indonesia (ed.); 2nd ed.). UI Press.

Lu, F. C. (2010). *Toksikologi Dasar*. UI Press.

Maliza, R., Tofrizal, A., Setiawan, H., Fadilatun, S., & Piliang, H. (2019). *Uji Toksisitas Subkronis Ekstrak Metanol Kulit Buah Kopi Arabika ( Coffea Arabica L .) pada Ginjal Mencit ( Mus*

*Musculus L .) Galur BALB / c*. *November*, 137–146.

Meles. (2010). *Peran Uji Praklinik Dalam Bidang Farmakologi* (Pusat Penerbitan dan Percetakan Unair (AUP). (ed.)). Perpustakaan Universitas Airlangga.

Mustapa, M.A., Tuloli, T.S. & Mooduto, A. M. (2018). Uji Toksisitas Akut Yang Diukur Dengan Penentuan LD50 Ekstrak Etanol Bunga Cengkeh (*Syzygium aromaticum* L.) Terhadap Mencit (*Mus musculus*) Menggunakan Metode Thompson-Weil. *Frontiers: Jurnal Sains Dan Teknologi*, *1*(1), 105-117

Mustika, A. (2014). *Keamanan Penggunaan Ekstrak Etanol Singawalang (Petiveria alliacea) Pada Fungsi Ginjal Mencit*. Bagian Biokimia Fakultas Kedokteran Universitas Gajah Mada.

Nugroho, M. D., Busman, H., & Fiana, D. N. (2014). Efek Protektif Ekstrak Etanol Daun Binahong (*Anredera Cordifolia* (Tenore) Steenis) Terhadap Gambaran Histopatologi Hati Tikus Putih yang Diinduksi oleh Etanol. *Jurnal Majority*, *10*, 109–118.

Nurdiniyah, N., Nazaruddin, N., Sugito, S., Salim, M.N., Fahrimal, Y. & Aisyah, S. (2015). Pengaruh pemberian ekstrak kulit batang jaloh terhadap gambaran mikroskopis ginjal tikus putih (*Rattus novergicus*) yang diinfeksi (*Trypanosoma evansi)*. *Jurnal Medika Veterinaria*, *9*(2), 2–6.

Nursofia, Y. & Yuliawati, Y. (2021). Uji Toksisitas Akut Ekstrak Etanol Daun Kayu Manis (*Cinnamomum burmanii*) pada Fungsi Hati Tikus Putih (*Mus musculus* L.) Betina. *Jurnal Ilmiah Ibnu Sina*, *6*(2), 272–281.

OECD. (2008). *Organization for Economic Cooperation and Development Guidelines for the Testing of Chemicals*. tg 407.

Oktarian, A., Budiman, H., & Aliza, D. (2019). Histopatologi Hati Tikus Putih (*Rattus Novergicus*) Yang Diinjeksi Formalin. *Jimvet*, *01*(3), 316–323

Pramono, S., Soeharto, G., & Margawati, A. (2012). Pengaruh Formalin Peroral Dosis Bertingkat Selama 12 Minggu Terhadap Gambaran Histopatologis Hepar Tikus Wistar. *Jurnal Media Medika Muda,* 1-16.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/761264

Ramadhani, N. (2021). *Potensi Ekstrak Black Garlic (Allium sativum, Varietas Lumbu Kuning) terhadap kerusakan Histologi Ginjal dan Lambung Mencit Betina yang diinduksi Aspirin*. http://digilib.uinsby.ac.id/48802/

Rasyid, M. (2012). Uji Toksisitas Akut Ekstrak Etanol Lempuyang Wangi (*Zingiber aromaticum* Val.) Pada Mencit. *Majalah Farmasi Dan Farmakologi*, *16*(1), 13–19.

Ressang, A.A. (1984). *Patologi Khusus Veteriner* (Edisi ke-2). Percetakan Bali.

Robbins, S. L., & K. (1992). *Buku Ajar Patologi I (Basic Pathology Part II)*. EGC Penerbit Buku Kedokteran.

Rofiqoh, A. D. (2015). *Uji Toksisitas Subkronik Ekstrak Air Daun Katuk (Sauropus androgynous) Terhadap Kadar Bilirubin Serum dan Histologi Hepar Tikus (Rattus norvegicus) Betina*. Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Rumaseuw, E.S., Iskandar, Y., & Halimah, E. (2022). *Acute Toxicity Test of Black Garlic Ethanol Extract Uji Toksisitas Akut Ekstrak Etanol Bawang Hitam*. *2*(1), 1–9.

Suaniti, N.M., Manurung, M. & Utari, N.M.M. (2017). Efek penambahan antioksidan ekstrak metanol kulit buah manggis (*Garcinia mangostana* L.) terhadap perubahan kadar FFA, bilangan asam, dan bilangan peroksida biodiesel. *Jurnal Kimia*, *11*(1), 49–55.

Swarayana, I.M.I., Sudira, I.W. & Berata, I.K. (2012). Perubahan Histopatologi Hati Mencit (*Mus musculus*) yang Diberikan

Ekstrak Daun Ashitaba (*Angelica keiskei*). *Buletin Veteriner Udayana*, *4*(2), 119–125.

Traesel, G.K., Menegati, S.E.L.T., Dos Santos, A.C., Souza, R.I.C., Boas, G.R.V., Justi, P.N., Kassuya, C.A.L., Argandoña, E.J.S. & Oesterreich, S. A. (2016). Oral acute and subchronic toxicity studies of the oil extracted from pequi (*Caryocar brasiliense*, Camb.) pulp in rats. *Food and Chemical Toxicology*, *97*, 224–231. https://doi.org/10.1016/j.fct.2016.09.018

Wang, D., Feng, Y., Liu, J., Yan, J., Wang, M., Sasaki, J.I. & Lu, C. (2010). Black Garlic ( *Allium sativum* ) Extracts Enhance the Immune System. *Medicinal and Aromatic Plant Science and Biotechnology*, *4*(1), 37–40.

Wiralaga, I.P.A., Sudira, I.W., Kardena, I.M. & Dharmayudha, A. A. G. O. (2015). Pengaruh Pemberian Ekstrak Etanol Daun Ashitaba (*Angelica keiskei*) Terhadap Histopatologi Lambung Mencit (*Mus musculus*) Jantan. *Buletin Veteriner Udayana*, *7*(1), 26–33.

Yuliandari, M., Mushawwir, A., & Permana, R. (2023). Aktivitas peroksidasi lipid ayam broiler dengan dan tanpa penambahan glutathione dalam ransum. *Jurnal Sains Dan Teknologi Industri Peternakan*, *3*(1), 18–23.