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EFFECTIVENESS OF KELAKAI (STENOCHLAENA PALUSTRIS) EXTRACT ON THE HISTOLOGICAL LIVER ORGAN OF HARUAN SNAKEHEAD FISH (CHANNA STRIATA) AFTER AEROMONAS HYDROPHILA BACTERIAL INFECTION

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ABSTRACT
After being infected with *A. hydrophila* bacteria, haruan snakehead fish made efforts to restore their condition through recovery. The secondary metabolites found in kelakai leaves, namely alkaloid compounds, steroids, flavonoids, and iron, are believed to act as blood-boosting supplements. This study aims to analyze the histological response of the liver organs of haruan snakehead fish after being infected with *A. hydrophila* bacteria and fed with kelakai extract. The treatments provided for recovery were as follows: Treatment A (Control), Treatment B (50ml/kg Kelakai Extract), Treatment C (100ml/kg Kelakai Extract), and Treatment D (150ml/kg Kelakai Extract). The histological response of the liver organs of haruan snakehead fish with the addition of kelakai extract has been shown to effectively improve the histological structure of the liver post-infection with *A. hydrophila* bacteria, as indicated by an increase in the number of melanomacrophages.

KEY WORDS
Kelakai, histological, liver, haruan snakehead fish.

Kelakai is a typical swampy plant that grows in South Kalimantan. Empirical studies of young kelakai leaves and stems are used by the Dayak people as a blood booster supplement, youth medicine, breast milk enhancer for breastfeeding mothers, high blood pressure medication, fever reliever and skin ailments such as itching and allergies (Maharani *et al*., 2005). Kelakai plants contain secondary metabolites in the form of leaf and stem samples, namely: water content of 8.56% and 7.28%, ash content of 10.37% and 9.19%, crude fiber content of 1.93% and 3.19%, protein content was 11.48% and 1.89%, fat content was 2.63% and 1.37%, the results of the analysis of Ca minerals were higher in leaves than stems, namely 182.07 mg per 100 g, as well as the highest Fe 291, 32 mg per 100 g. The highest phytochemical content of flavonoids, alkaloids and steroids was found in the stem, amounting to 3.010%, 3.817% and 2.583% (Maharani *et al*., 2005). The content of secondary metabolites contained in kelakai leaves, namely alkaloid compounds, steroids, flavonoids and iron (Anggraeni at al, 2015). Kelakai plants contain iron minerals of 291.316 mg / 100 g on the leaves and as large as 221.443 mg / 100 g on the stems. Iron content in the leaves of fresh kelakai plants was 3.285% or equivalent to 3285 mg / 100 g (Maharani *et al*, 2005). The addition of kelakai extract which contains iron into pelleted feed can contribute positively to the immunustimulant of haruan snakehead fish (Norhayati *et al*, 2019).

Cultivation of haruan snakehead fish (*Channa striata*) is currently growing, because the maintenance of this fish is relatively easy and very profitable. Cultivation there are still obstacles to the biological response which will later have haematological consequences. Haematological response is one of the most important factors that must be considered in the maintenance of haruan snakehead fish (Sofian *et al*, 2019). The haematological condition of haruan snakehead fish is low when there is stress, making it easier to get sick and even result in death. the average number of leukocytes is above the normal range (Hidayat *et al*., 2014).

Wikiandy et al. (2013) stated that when fish suffer from disease disturbances, there will be changes in histological tissues characterized by visible damage and changes in cells and cell nuclei becoming less clear or even disappearing. Damage to the structural organs of haruan snakehead fish can be caused by disease attacks. This damage is illustrated by necrosis, characterized by a decrease in tissue activity, causing the gradual disappearance of several tissue parts, ultimately leading to the death of the fish in the near future (Tresnati et al., 2007). Common causes that can infect fish are divided into several factors: infectious diseases (parasites, bacteria, fungi, and viruses), and non-infectious diseases (stress, intoxication, deficiencies). One of them is bacterial diseases, especially Motile Aeromonad Septicaemia (MAS) disease caused by *Aeromonas hydrophila* bacteria (Allan & Stevenson, 1981).

Aeromonas hydrophila is a type of bacteria that causes illness in several freshwater fish species. This bacterium is generally found in freshwater environments with high organic content. Characteristics of *A. hydrophila* bacteria include a rod-shaped form, with dimensions of 0.3-1.0 µm in width and 1.0-3.5 µm in length. *A. hydrophila* bacteria have gram-negative properties, exhibit facultative aerobic life traits that can live with or without O2, do not have spores, and are actively motile due to possessing a single flagellum (monotrichous flagella) which protrudes from one end of their body (Aminah, et al., 2021).

After being infected with *Aeromonas hydrophila* bacteria, haruan snakehead fish are treated. However, if they are not completely healthy, further treatment with recovery methods is needed. The recovery process is a way to restore the condition of haruan snakehead fish after stress due to illness, to regain their health. For the recovery process to proceed smoothly, it needs to be supported by several factors, including environmental factors, water quality, and nutritional factors in the fish's diet (Sarjito et al., 2012).

Diet plays an essential role in nourishing the fish's body as a source of energy to improve immunity, growth, and reproduction (Aminah, et al., 2021). The nutritional content of fish feed must meet the fish's body needs and be well-controlled. This study aims to analyze the histological response of the liver organs of haruan snakehead fish after being infected with *Aeromonas hydrophila* bacteria and fed with kelakai extract.

MATERIALS AND METHODS OF RESEARCH

This research was conducted at the Fish Disease Laboratory for bacterial rejuvenation, and the Wet Laboratory of the Faculty of Fisheries and Marine Sciences at Lambung Mangkurat University (ULM), Banjarbaru, for maintenance purposes. Histological examinations were performed at the Mandiangin Aquatic Biotechnology Research and Development Center (BPBAT) in Banjar Regency, South Kalimantan Province.

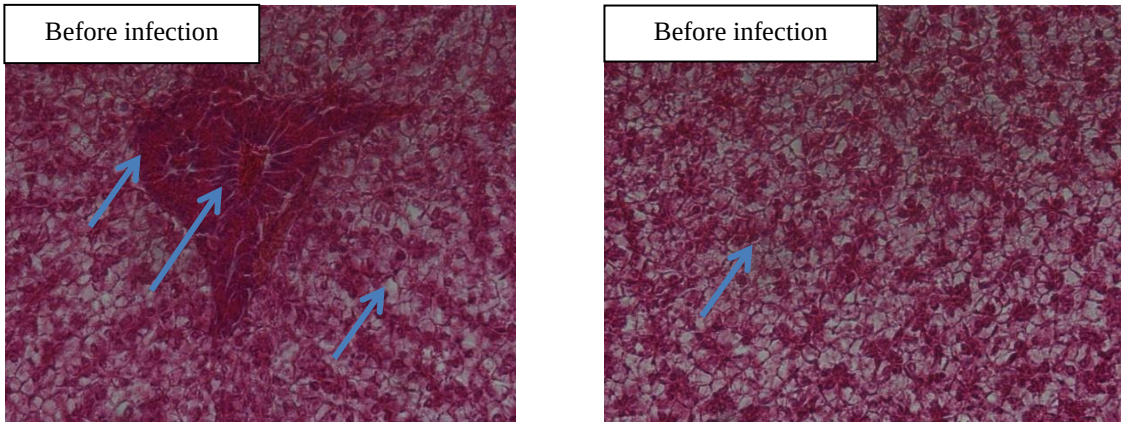
The design used in this study was a 4 treatments namely:

- Commercial feed kelakai extract 0 ml/kg;
- Commercial feed + addition of 50 ml/kg kelakai extract;
- Commercial feed + addition of 100 ml/kg kelakai extract;
- Commercial feed + addition of kelakai extract as much as 150 ml/kg.

The observation of histological tissue preparations of liver organs was conducted using an Olympus BX41 microscope with magnifications of 40x, 100x, and an eyepiece of 10x. The diagnosis of tissue damage was determined using the Histological Activity Index (HAI) (Jusuf, 2009), and the level of damage was categorized accordingly.

RESULTS AND DISCUSSION

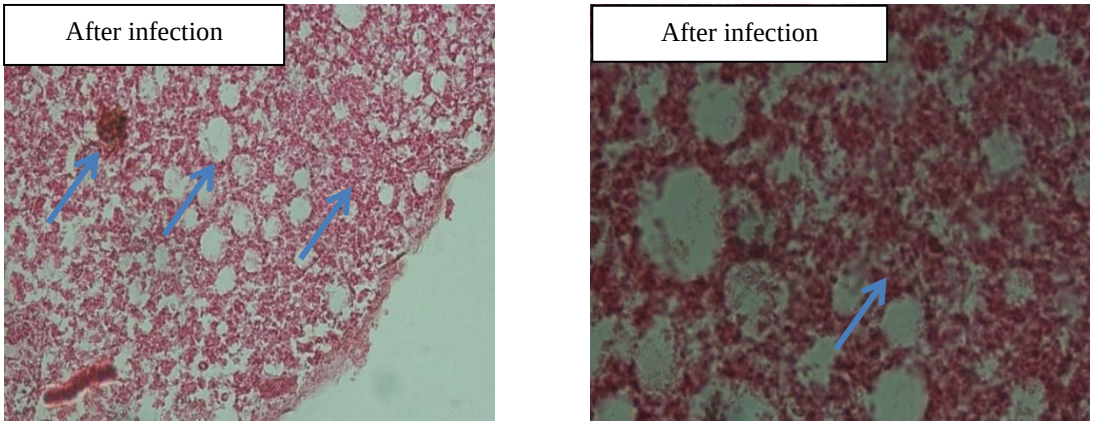
Histological measurements of the liver were conducted at the beginning, during fish infection, and after 15 days of maintenance following infection with *A. hydrophila* bacteria in haruan snakehead fish. Measurement data are presented in the figure. The results of the identification of haruan snakehead fish preparations before infection with *Aeromonas hydrophila* bacteria showed normal liver tissue. The normal liver of the fish appeared reddish-brown with the presence of red blood cells and hepatocytes. The results of the identification of haruan snakehead fish preparations after infection with *Aeromonas hydrophila* bacteria showed abnormal liver tissue characterized by degeneration and hemorrhage. The damaged liver exhibited suspected bacterial cells. Inflammatory responses, necrosis, and hematoxylin and eosin staining can be observed in the liver tissue, as shown in the following figure.



Erythrocytes

Hepatocytes

Figure 1 – Liver of Haruan snakehead fish Before Infection



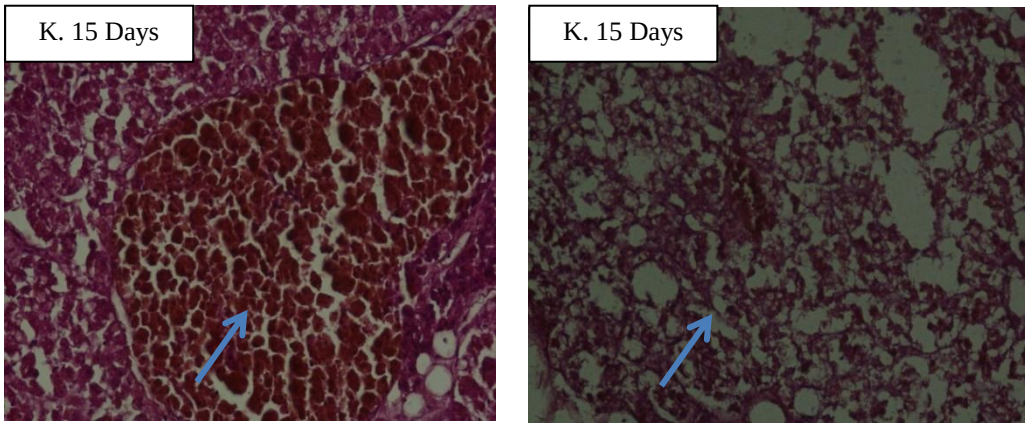
- An area of liver inflammation appears
- Liver hepatocyte necrosis is visible
- Fatty liver degeneration appears
- Hematoxyllin and Eosin 400x

- Suspected bacterial cells are visible
- Hematoxyllin and Eosin 1000x

Figure 2 – Liver of Haruan snakehead fish After Infection

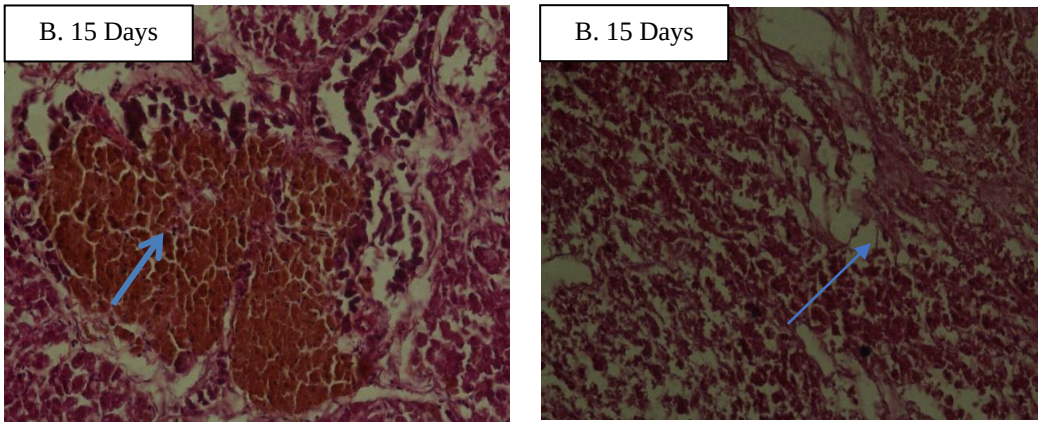
The results of identification of preparations in the liver of the Haruan snakehead fish after 15 days of recovery from *A. hydrophila* bacterial infection can be seen in the following figures. The histological examination of haruan snakehead fish liver showed that each treatment resulted in different changes in tissue structure. The liver tissue structure exhibited melanomacrophage centers (MMC) and hepatocyte lysis in treatment A (Control), treatment B (50 ml Kelakai Extract/kg feed), treatment C (100 ml Kelakai Extract/kg feed), and

treatment D (150 ml Kelakai Extract/kg feed). These changes were caused by the exotoxin enzymes produced by *A. hydrophila* bacteria infecting the haruan snakehead fish, resulting in abnormalities in the liver organ. Treatment A (Control) showed fewer melanomacrophages compared to treatments B (50 ml Kelakai Extract/kg feed), C (100 ml Kelakai Extract/kg feed), and D (150 ml Kelakai Extract/kg feed), suggesting that the addition of kelakai extract to the haruan snakehead fish feed exerted resistance against *A. hydrophila* bacterial infection, leading to an increase in liver melanomacrophages.



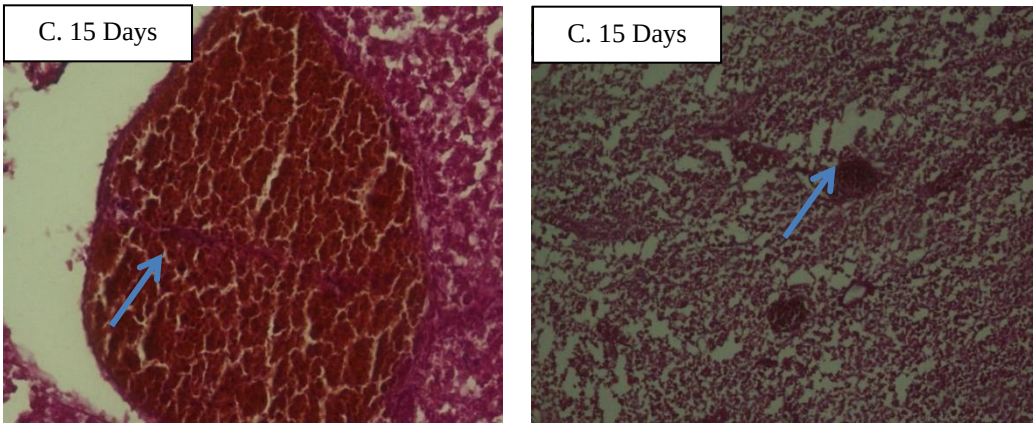
Melanomacrophage center Hepatocyte lysis

Figure 3 – Haruan snakehead fish Liver after 15 Days Recovery (Control/A)



Melanomacrofage center Fatty degeneration

Figure 4 – Haruan snakehead fish Liver after 15 Days Recovery (50ml Kelakai Extract/Kg feed /B)



Melanomacrophage center Lysis

Figure 5 – Haruan snakehead fish Liver After 15 Days Recovery (100 ml Kelakai Extract/Kg feed/C)

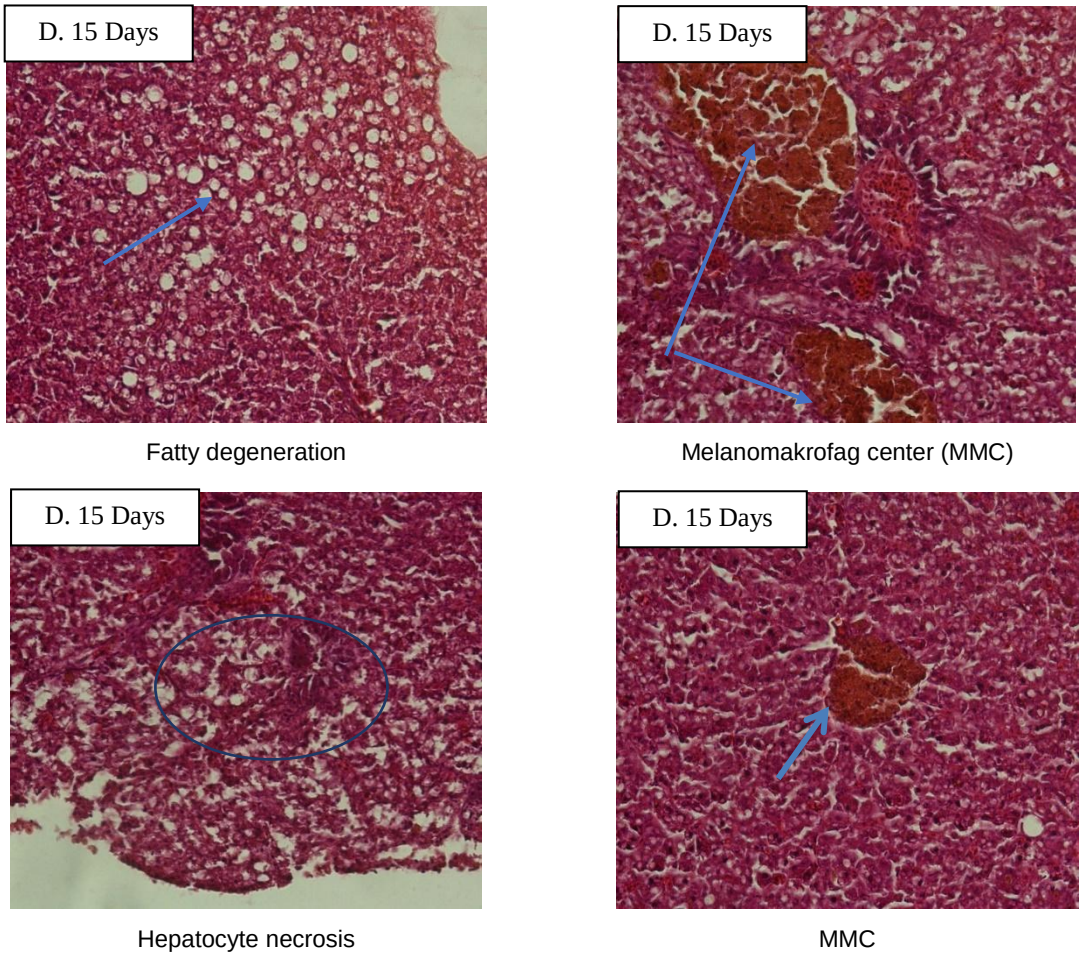


Figure 6 – Haruan snakehead fish Liver After 15 Days Recovery (150 ml Kelakai Extract/Kg feed/D)

Another abnormality found in the liver organ was melanomacrophages. Melanomacrophages are a type of macrophage with abundant melanin pigment in their cytoplasm (Wakita et al., 2007). Melanomacrophages are solid and round-shaped cells with varying pigment amounts, usually present in healthy fish but increasing in number during times of stress, serving as indicators of chronic stress (Noga, 2010). Necrosis and melanomacrophages are liver tissue abnormalities, while congestion is also evident. Congestion refers to the accumulation of blood in the tissue. Liver abnormalities, such as congestion, are considered moderate (Darmono, 1995). Congestion involves blood clotting in the sinusoid glands or small blood vessels in the liver.

Structural changes, such as congestion caused by *A. hydrophila* infection, predominantly experienced congestion (Priyatna et al., 2011). Histological examination of haruan snakehead fish liver tissues across all treatments revealed that treatment D (150 ml Kelakai Extract/kg feed) exhibited the best tissue structure compared to the other treatments. This was evident in the clarity of cell nuclei and hepatocytes in the liver tissue. Based on this research, it is evident that the use of kelakai extract in fish feed results in lighter structural changes in liver tissue.

These liver abnormalities are caused by the liver's highly potential susceptibility to cell abnormalities compared to other internal organs. Explain that the liver functions in detoxification and synthesizing several components of blood plasma, making it vulnerable to toxic substances. According to Prince and Wilson (2006), necrosis refers to cells with very low activity that eventually undergoes tissue cell death, resulting in the loss of cell function in the affected area.

Takashima and Hibiya (1995) mention that liver cell necrosis is caused by cytolysis or phagocytosis activity by lymphocytes or histiocytes, resulting in overall nuclear shrinkage.

Another tissue abnormality found in the liver is vacuolar degeneration. Degeneration is an inflammation response that occurs when cell abnormalities do not immediately cause cell death. These changes are reversible (recoverable after the source of abnormality is eliminated) and are caused by bacterial injuries (Tresnati et al., 2007). Degeneration occurs gradually in connective tissues and fine fibers, eventually thickening into homogeneous eosinophilic substances. Fibrocytes usually disappear, and parenchymal cells undergo atrophy when connective tissue degrades (Takashima and Hibiya, 1995).

Darmono (1994) categorizes liver damage in fish into three levels: mild damage characterized by liver steatosis marked by cell swelling, moderate damage including congestion and hemorrhage, and severe damage indicated by necrosis. Based on this classification, the liver damage observed in all observation stations experienced severe damage. Tissue abnormalities identified in the liver include degeneration, hemorrhage, and necrosis. Indarti et al. (2012) state that pigmentation can be influenced by internal factors such as age, size, genetics, and gender of the fish. Meanwhile, abnormal glomerulus structures were found in the liver tissue of fish from the Sibam River. Many glomerulus cells were damaged, some enlarged, some with lost cell nuclei, and some with necrotic spots.

Necrosis depicts a state of decreased tissue activity characterized by the gradual loss of some cell parts one by one from a tissue, ultimately leading to cell death within a short time (Takashima and Hibiya in Tresnati et al., 2007). According to Wikiandy et al. (2013), histological necrosis is characterized by unclear cell and nucleus boundaries or even their disappearance. Mandia (2013) states that liver structural damage in fish, such as necrosis, is caused by toxic compounds suspected to originate from organic waste, leading to the emergence of *A. hydrophila* bacteria.

CONCLUSION

The histological organ response of haruan snakehead fish liver with the addition of kelakai extract effectively influences the improvement of histological liver structure post-infection with *A. hydrophila* bacteria, as evidenced by the increased number of melanomacrophages.

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