Potential Use of Tembelekan Leaf Plant Extract (Lantana camara linn) on Hematological and Histopathological Profiles of Goldfish (Cyprinus carpio) infected with Edwardsiella tarda Bacteria

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Abstract

The purpose of this study was to analyze the hematological and histopathological features of carp (C. carpio) gills treated with the active compound of Tembelekan leaf extract (L. camara Linn). The method used in this research is Completely Randomized Design (CRD). The treatment used in this study was treatment A (50 mg/L), B (100 mg/L), C (150 mg/L), D (200 mg/L), E (250 mg/L), negative control (healthy fish), and positive control (Tetracycline antibiotics). The results of haematological and histopathological research on the gills of carp (C. carpio) after being infected with E. tarda bacteria experienced stress, this was seen from the decreased total erythrocytes, increased total leukocytes, and there was considerable damage to the gill organs such as edema, fusion, and necrosis. The goldfish returned to their normal condition after being treated with tembelekan leaf extract. The best dose obtained after treatment was treatment D with a dose of 200 mg/L. The results of the clinical symptom study showed a change from an abnormal condition to a normal condition in carp after being treated. Tembelekan leaf extract contains predominant compounds such as flavonoids and alkaloids which can be useful as medicine for goldfish infected with E. tarda bacteria.

Keywords: Cyprinus carpio, Edwardsiella tarda, Erythrocytes, Leukocytes, Lantana camara linn

Introduction

Goldfish (Cyprinus carpio) is a freshwater consumption fish whose cultivation is quite developed, has high economic value and has the potential to be developed more widely in Indonesia. The demand for fresh goldfish products is quite large and has become one of the favorite fish of the Indonesian people. According to KKP (2019), the target for carp production is 785,800 tons. However, this production target is not accompanied by achieving maximum production from year to year. Goldfish has several advantages, namely relatively fast growth, fecundity or the number of eggs produced is relatively high. Goldfish farming is generally inseparable from biological risks, especially those caused by disease (Purwaningsih et al., 2015).

Various efforts have been made to increase the production of goldfish farming to meet the demand for goldfish consumption. This has led to a shift from traditional carp cultivation systems to intensive cultivation systems. Intensive ponds are an improvement from non-intensive pond patterns, intensive systems provide continuity of production and business for a longer period of time and feed that is relied upon not only natural feed such as non-intensive ponds. Stech et al. (2022), states that an intensive system is a system by applying stocking densities and high feed doses. So that it has an impact on reducing water quality and providing opportunities for infection with pathogenic microorganisms. This can have a negative impact so that it is likely to cause disease in the fish being cultivated.

Diseases that attack goldfish are generally caused by bacterial infections. One of these species of bacteria is Edwardsiella tarda (Diniarti et al., 2019). This E. tarda causes a disease called Edwardsiellosis (Kerie et al., 2019). Generally, Edwardsiellosis is a disease that can infect carp (C. carpio) aquaculture systems. The prevalence of death due to E. tarda attacks reached 100% in goldfish (Charassius auratus) and Catla catla (Loch et al., 2017). E. tarda infection shows external symptoms in the form of small wounds measuring 3-5 mm, skin necrosis occurs, then these wounds develop in the flesh and dermis, causing the skin to blister and lose its pigment color. When the wound gets worse, it will give rise to a foul odor and spread throughout the body. This severe wound causes the fish to lose its balance of motion (Maryani & Rosdiana, 2020). Fish that have been infected with bacteria need to be treated to increase production.

The knowledge of cultivators in terms of diagnosing and controlling the disease is still very limited. Therefore it is necessary to make efforts to prevent, treat and control the invasion of E.tarda. Generally the treatment is done to overcome this disease is by using antibiotics and chemicals. Some examples of chemicals and antibiotics that are widely used are tetracycline, chloromphenicol, formalin, methylene blue and gentian violet (Mariela et al., 2016). According to Nurhasnawati et al. (2016), argued that the excess use of chemicals and antibiotics is easy to obtain and the effect is faster but creates residues that can be harmful to the sustainability of the harmonization of living systems in the waters. Residues of chemicals and/or their metabolites in tissues or organs can cause resistance to antibiotics making them ineffective and relatively expensive. These problems can be overcome by optimizing the use of herbal ingredients. Herbal or natural ingredients are rarely used as an alternative drug that can control E.tarda bacteria.

Currently, research related to natural ingredients has begun to be investigated by scientists because they are considered to be an alternative for disease control in order to minimize the use of antibiotics. Recently, natural ingredients are often used as anti-bacterial and can even be used as medicine. One of the natural ingredients currently being developed is the tembelekan plant with the Latin name Lantana camara L. This plant grows wild and has various secondary metabolites, especially in the leaves, such as terpenoid compounds which include volatile compounds, flavonoids, phenols, saponins, alkaloids, steroids, tannins and quinones (Kotala et al., 2019). Princess et al. (2018), stated that secondary metabolites in tembelakan leaves have potential as antibacterial compounds.

Based on studies on the potential of Lantana camara Linn as an antibacterial from various references, many have been carried out, but its use as an antibacterial alternative to E.tarda and its application to fish treatment is not optimal, thus, it is necessary to carry out further research and a detailed study of the active compound content of Lantana camara L. which is useful as an antibacterial, as well as its application as an alternative treatment for treating goldfish (C. carpio) infected with E.tarda bacteria through haematological and histopathological observations of gills and liver in carp (C. carpio).

Based on this background, the formulation of the problem in this study is how is the hematology and histopathology of the gills of carp (C. carpio) treated with the active compound of Tembelekan leaf plant extract (Lantana camara Linn)?

The purpose of this study was to analyze the hematology and histopathology of the golden gills (C. carpio) treated with the active compound of Tembelekan leaf extract (Lantana camara Linn).

Methods

Research Materials

The material used in the extraction activity is tembelekan leaves (L. camara Linn) obtained from Herbal Materia Medica, Batu, East Java. The solvent for maceration is ethanol with pro-analytical (PA) quality based on research by Hikmawanti et al. (2021), For medicinal activities, the materials used are carp seeds (C. carpio) with a size of 7 – 10 cm.

Research Methods

The method used in this study is the experimental method. According to Hastjarjo (2019), the experimental method is a study that involves manipulating independent variables, controlling external variables and measuring the effects of independent variables on the dependent variable.

Research Design

The design in this study was Completely Randomized Design (CRD). RAL is a single factor design.

The treatment used in this study was with different doses, positive control, negative control and 3 repetitions. The implementation is as follows:

Treatment A = Tembelekan leaf extract (L. camara Linn) 50 mg/L

Treatment B = Tembelekan leaf extract (L. camara Linn) 100 mg/L

Treatment C = Tembelekan leaf extract (L. camara Linn) 150 mg/L

Treatment D = Tembelekan leaf extract (L. camara Linn) 200 mg/L

Treatment E = Tembelekan leaf extract (L. camara Linn) 250 mg/L

Treatment K (-) = Treatment without bacterial infection and without giving extracts

Treatment K (+) = Treatment of infection with administration of tetracycline

Research Procedures

E. tarda Infection and Soaking of Tembelekan Leaf Extract (L. camara linn)

The bacteria that had been prepared on the TSB media were infected directly into the goldfish live media according to the desired density until they showed signs of being infected. The treatment process for carp that has been infected with E. tarda bacteria is soaked in a container filled with tembelekan leaf extract (L. camara linn) according to the treatment dose.

Hematological Observations

Hematological observations were made on normal fish, fish that had been infected with E. tarda bacteria and after rearing for 7 days after being given the addition of tembelekan leaf extract (L. camara linn. Fish blood was taken with a 1 ml syringe which had been mixed with EDTA anticoagulant and erythrocyte count was carried out, hemoglobin, leukocytes and leukocyte differential.

Histopathological Observations of Gills

According to Andayani et al. (2018), tissue harvesting of target organs was carried out on all test fish. The tissue that has been taken is cleaned using distilled water. After that, put it in a film bottle containing 10% formalin. Then, histopathological preparations were made.

Fish Survival

The survival of carp (C. carpio) was observed after 7 days of post-treatment rearing. Where the number of dead and live fish was counted from the treatment, both the control treatment and the test treatment.

Research Supporting Parameters

Supporting parameters in this study were clinical symptoms (behaviour, appetite and visible damage to fish from the outside), namely by carrying out a series of observation activities on the object under study, in this case, goldfish to see signs/physical abnormalities that occur in fish. mas who is stricken with the disease, as well as behavioral disorders (behavior). Data collection techniques in this study were carried out by observation or direct observation of the objects studied in the field, and microscopic observations in the laboratory on sampled fish.

Results and Discussion

Hematology

Phase 2 of the study aimed to determine the best dose of tembelekan leaf extract for the treatment of carp (C. carpio) after being infected with E. tarda bacteria with a bacterial density of 2.53 x 107 cells/ml according to the LD50 test results. Observations were made for 7 days with extract doses of 50,100,150,200, and 250 mg/L.

Erythrocytes

The results of research on total erythrocytes in carp showed that treatment using tembelekan leaf extract gave significantly different results (P≤0.05) after treatment. Treatment D (200 mg/L) gave the best results compared to other treatments. This is because treatment D experienced the highest increase in total erythrocytes. The results of research on total goldfish erythrocytes are presented in Table 1 below:

Table 1. Total Goldfish Erythrocytes During the Study ($x10^6$)

Treatment (mg/L)	Healthy	Infected	After Treatment
K+	1,41±0,98	0,92±0,15	1,07±0,57b
A	1,40±0,10	0,92±0,10	1,08±0,55a
B	1,39±0,01	0,80±0,17	1,11±0,60a
C	1,37±0,01	0,92±0,77	1,26±0,12a
D	1,38±0,10	0,93±0,10	1,22±0,11b
And	1,36±0,10	0,88±0,12	1,15±0,17from
K-	1,39±0,99	1,34±0,85	1,36±0,37a

Observation of the total erythrocytes of carp (C. carpio) was carried out 3 times during the study, namely before infection, after bacterial infection, and after treatment using the extract. Factors that affect the value of fish erythrocytes include sex, age, environment and nutritional status as well as hypoxic conditions or lack of oxygen (Abdelhamid et al., 2019). Based on the data in Table 10, the total erythrocytes of carp before infection ranged from 1.36-1.41×106 sel.mm-3. This value indicates that the total erythrocytes are still in normal condition. This is in accordance with research by Hartika et al., (2014); Maryani & Rosdiana, (2020), that the normal total number of goldfish erythrocytes ranges from 20,000-3,000,000 cells/mm3.

The results of observing the total erythrocytes of carp after infection with E. tarda bacteria decreased the number of erythrocytes. The decrease in the number of erythrocytes was caused by bleeding due to infection with the bacterium E. tarda. The decreased number of erythrocytes can also be caused by other factors, including a lack of nutrients that enter the fish's body. These nutrients play a role in the formation of red blood cells in the body. E. tarda bacteria produce extracellular hemolysin enzymes. Hemolysin has the ability to lyse red blood cells so that the number of red blood cells decreases (Sarkar et al., 2021).

Erythrocyte values after treatment showed an increase. The highest increase in erythrocyte values was in treatment D (200 mg/L), which was 1.26 x 106 cells/mm3. An increase in erythrocyte levels is a sign of homeostatic efforts in the fish body (pathogen infection) where the body produces more blood cells to replace erythrocytes that experience lysis due to infection (Prasetio et al., 2017).

Increased levels of erythrocytes in the blood of this carp is the effect of giving tembelekan leaf extract (L. camara linn). Compounds contained in tembelekan leaf extract, one of which is flavonoids, can increase the number of erythrocytes. Maharani et al. (2021), flavonoids are polyphenolic active compounds that act as antioxidants, which can increase erythropoietin. Erythropoietin is a glycoprotein hormone found in the blood, the hormone erythropoietin circulating in the blood vessels stimulates the bone marrow to increase the formation of red blood cells or erythropoiesis (Amudi & Stella, 2021).

Leukocytes

The results of the study on total leukocytes in goldfish showed that treatment using tembelekan leaf extract did not give significantly different results (P≤0.05) after treatment, but treatment D (200 mg/L) gave the best results compared to other treatments. This is because treatment D experienced the highest decrease in total leukocytes. The results of the research on total carp leukocytes are presented in Table 2 below:

Table 2. Total Goldfish Leukocytes During the Study

Treatment (mg/L)	**Healthy**	**Infected**	**After Treatment**
K+	9,26±0,40	12,57±0,21	9,79±0,10
A	9,66±0,14	12,76±0,93	11,73±0,33
B	9,36±0,11	12,61±0,76	11,63±0,26
C	9,51±0,11	12,46±0,76	11,19±0,66
D	9,41±0,14	12,46±0,26	9,83±0,11
And	9,36±0,15	12,59±0,21	10,11±0,49
K-	9,31±0,28	9,42±0,21	9,46±0,21

Goldfish after being infected with E. tarda bacteria showed a difference in the total number of leukocytes. This can be seen from healthy fish and after being infected. The increase in total leukocytes is thought to be caused by a response to the presence of pathogenic microorganisms that enter the body of the fish. Viernanda et al. (2018), leukocytes play a role in the immune system of fish. The increase in leukocyte levels is because leukocytes are protecting against foreign bodies, one of which is by invading pathogens through an immune response system against bacteria. Leukocytes work through the process of phagocytosis and synthesize antibodies. Sumsanto et al. (2019), leukocytes are blood components that act as specific body defenses that are able to neutralize and fight pathogens by phagocytosis.

Observation of the total leukocytes of goldfish after treatment using tembelekan leaf extract decreased. Seen in treatment D with a dose of 200 mg/L which is equal to 9.83 x 104 cells/mm3. This treatment experienced a lower decrease compared to other treatments. This is due to the activity of tembelekan leaf extract which plays a role in the process of treating carp after being infected with E. tarda bacteria, so that the number of leukocytes in the fish's blood returns to normal.

Extract concentrations that are too high are not necessarily good for fish, concentrations that are too high will actually have a toxic effect on fish or even death. Active compounds in high amounts that cross the line will be toxic to fish (Zebua et al., 2019).

Hemoglobin

The results of the study on hemoglobin in carp showed that treatment using tembelekan leaf extract did not give significantly different results (P≤0.05) post-treatment, but treatment D with a dose of 200 gave the best results compared to other treatments. This is because treatment D experienced the highest increase. The results of the goldfish hemoglobin study are presented in Table 3 below:

Table 3. Goldfish hemoglobin levels during the study

Treatment (mg/L)	Healthy	Infected	After Treatment
K+	7,12±0,79	4,73±0,60	7,10±0,20
A	7,18±0,58	4,64±0,39	5,99±0,76
B	7,11±0,96	4,50±0,59	6,03±0,17
C	7,21±0,79	4,70±0,50	6,30±0,20
D	7,12±0,69	4,74±0,51	7,12±0,59
And	7,15±0,43	4,51±0,37	6,76±0,21
K-	7,12±0,79	7,40±0,84	7,27±0,23

Based on Table 16 above, it shows that the average after being infected with bacteria has decreased, but increased after treatment. The highest increase in hemoglobin levels of carp was in treatment D (dose of 200 mg/L) which was 7.12 g/dL and the lowest hemoglobin level was in treatment A (dose of 50 mg/L) which was 5.99 g/dL.

Hemoglobin levels after infection decreased. Decreased hemoglobin indicates stress conditions that increase metabolism in the fish's body which results in a lack of oxygen (hypoxia) (Matofani et al., 2013). According to Salosso et al. (2020), sick/infected fish will have a low hemoglobin count. Low hemoglobin levels indicate the occurrence of anemia in the body of the fish. Fish suffering from anemia have low hemoglobin concentrations due to a decrease in the number of erythrocytes (Novita et al., 2020). This is also caused by the influence of exotoxins from E. tarda bacteria which, according to Fallah et al. (2014), exotoxin from E. tarda causes damage to the blood vessels, as a result blood will come out of the blood vessels.

The value of hemoglobin levels after treatment shows that it is still in normal condition. This is in accordance with the statement of Kusrini et al. (2019), normal hemoglobin levels in goldfish range from 5.05-8.33 g/dL normal hemoglobin levels because they are supported by high erythrocytes. This statement is supported by Sudirman et al. (2021), which states that increased levels of erythrocytes in the blood indicate that hemoglobin levels also increase and indicate that stress on fish is reduced.

Histopathology

Gills

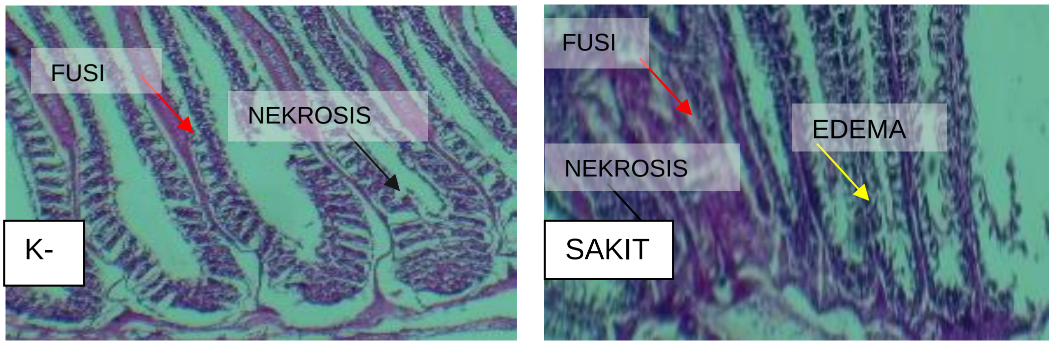
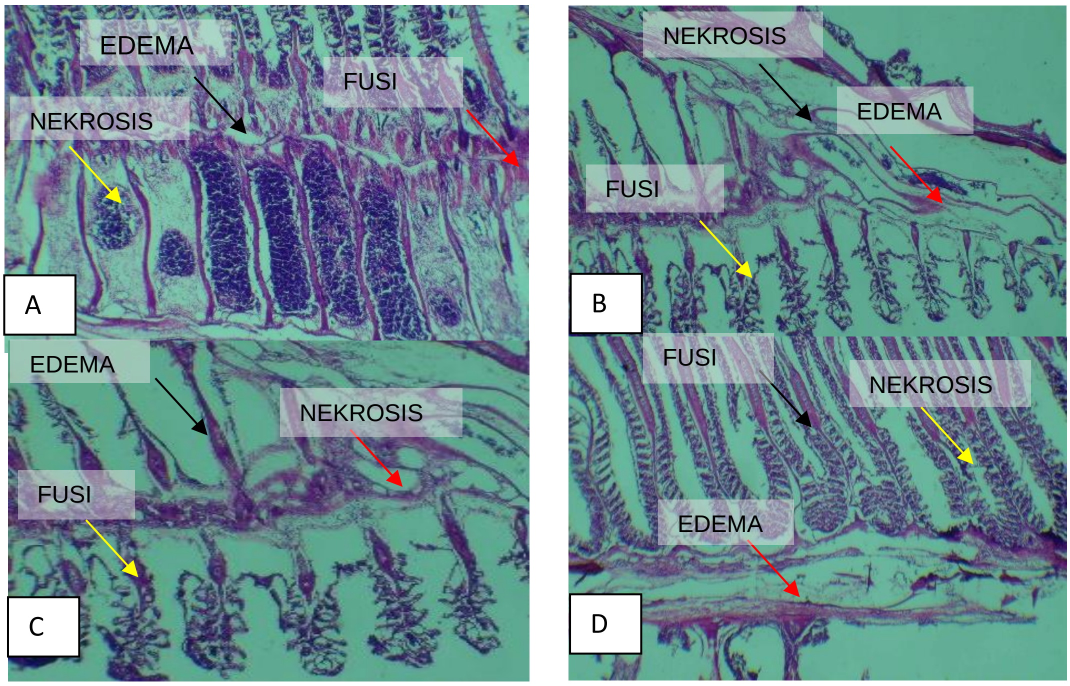


Figure 1. Histopathology of Goldfish Gills (C. *carpio*) (A) Normal and (B) Sick Fish with 400x microscope magnification (Research Documentation, 2023)

Based on Figure 1, shows the condition of a healthy carp (A) showing a little tissue that has Fusion and Necrosis, but the secondary lamellae are clearly visible and regular. The surface of the lamellae is covered with epithelial cells. Meanwhile, the gills infected with E. tarda (B) showed a lot of damage, such as fusion, edema and necrosis. Description of gills infected with bacteria after treatment using tembelekan leaf extract (L. camara linn) as presented in Figure 2.



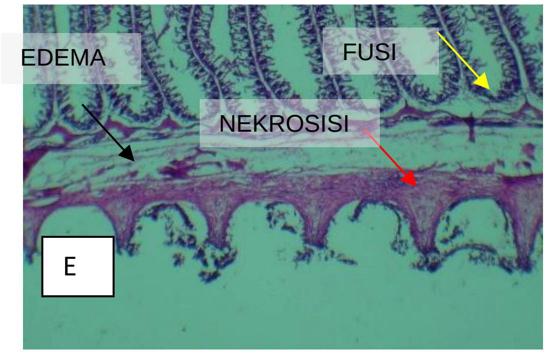


Figure 2. Post-Treatment Goldfish (C. *carpio*) Gill Histopathology with Microscopic Magnification 400x (A) dose 50 mg/L (B) dose 100 mg/L (C) dose 150 mg/L (D) dose 200 mg/L (E) dose 250 mg/L (Research Documentation, 2023).

Analysis of damage to gill organs infected with bacteria E. *Tarda* post-treatment using obtained damage as follows:

Table 4. Post-Treatment Analysis of Gill Organ Damage Infected with E. *tarda* Bacteria

Treatment (mg/L)	Merged	Edema	Necrosis
K+	2.20±0.23d	2.07±0.11d	1,67±0,23c
50	1,60±0,20c	2,27±0,11f	1,67±0,23c
100	1,40±0,20bc	2.20±0.00e	1,40±0,20bc
150	1,33±0,30bc	2,00±0,00d	1,47±0,23bc
200	1,07±0,11b	1,33±0,11b	1,20±0,11b
250	1,27±0,0,11bc	1,53±0,11c	1,40±0,11bc
K-	0,33±0,0,11a	0,53±0,11a	0,20±0,11a

Merged

Based on the results of the study, it was found that the damage that occurred to gill tissue, namely fusion, showed significant results (P<0.05). The results of the study found that the administration of tembelekan leaf extract was able to repair the level of histopathological damage to the gills of goldfish infected with *E. tarda bacteria* with the lowest value obtained in treatment D (dose 200 mg / L) which is 1.07.

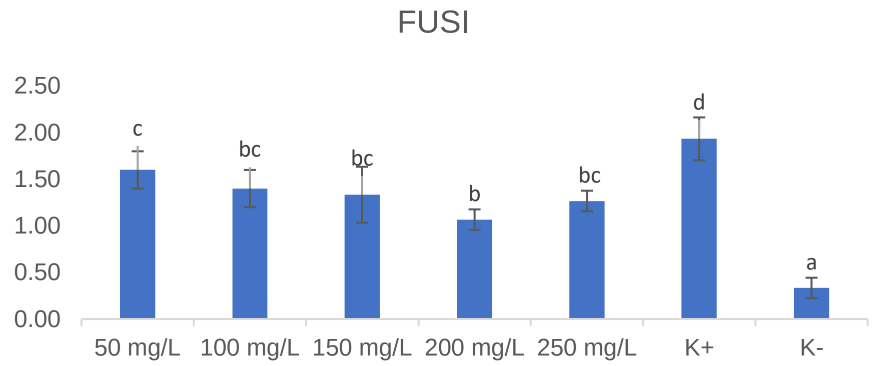


Figure 3. Goldfish (C. *carpio*) gill fusion scoring results

The lowest fusion damage was found at an extract dose of 200 mg/L, while the average fusion damage was highest at an extract concentration of 50 mg/L. It can be seen that treatment E (250 mg/L) showed higher fusion damage than treatment D. This indicated that the extract had reached its optimum point in the treatment of carp infected with E. tarda. A high dose of the optimum dose will cause high tissue damage as well. High secondary metabolite compounds will be toxic to fish organisms. This is supported by the statement of Castilhos et al. (2017), that secondary metabolite compounds will be toxic because they are part of a self-defense mechanism. The results showed that tembelekan leaf extract had a significant effect on fusion on goldfish gill damage.

Oedema

The results of the study after treatment using tembelekan leaf extract found that the damage to the gill tissue, namely edema, showed significant results (P <0.05) in carp. The results showed that the administration of tembelekan leaf extract was able to improve the level of histopathological damage to the gills of goldfish infected with E. tarda bacteria.

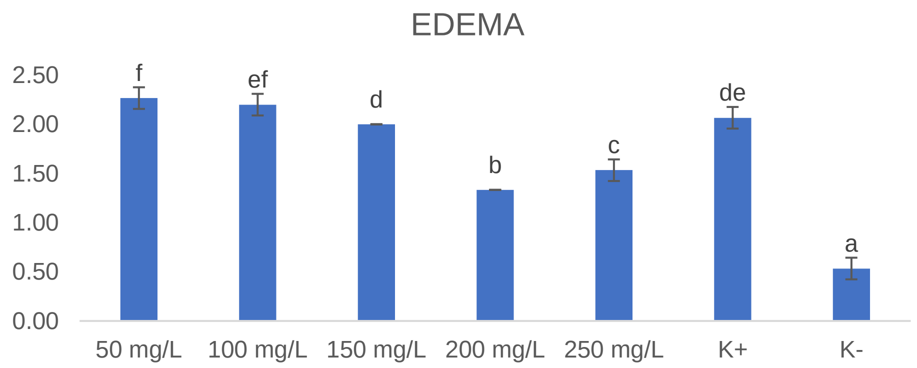


Figure 4. Goldfish (*C. carpio*) gill edema scoring results

The lowest average damage scoring result was in treatment D with an extract concentration of 200 mg/L with an average scoring value of 1.33. The concentration of treatment with moringa leaf extract showed different results on goldfish gill edema damage and was the closest to the damage scoring value in negative control fish. So that the best dose of 200 mg/L in treatment can help fish in efforts to repair tissues back to normal conditions. This is due to the ability of bioactive compounds contained in tembelekan leaf extract (L. camara linn.) such as flavonoids to stimulate the organism's defense system. (Supriyatna et al., 2015).

Necrosis

The results of the study after treatment using tembelekan leaf extract found that the damage to the gill tissue, namely necrosis, showed significant results ($P<0.05$) in goldfish. The results showed that the administration of tembelekan leaf extract was able to improve the level of histopathological damage to the gills of carp infected with E. tarda bacteria.

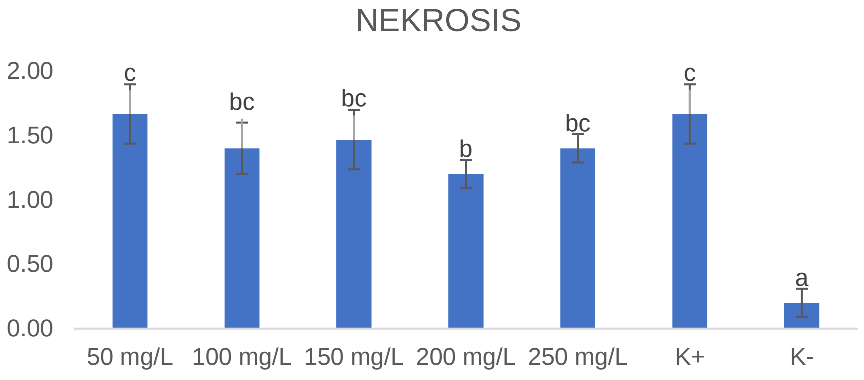
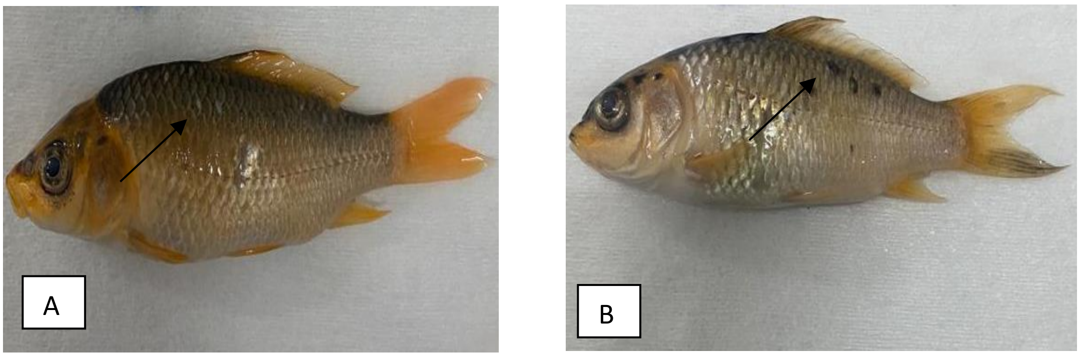


Figure 5. Gill Necrosis Scoring Results of Goldfish (*C. carpio*)

The lowest average damage scoring result was in treatment D with an extract concentration of 200 mg/L with an average scoring value of 1.20 and close to the negative control. Goldfish gill necrosis was seen to increase the average number of scores at 250 mg/L treatment. This is because the extract given has reached its optimum point in the treatment of carp after being infected with E. tarda. So that the dose that is higher than the optimal dose will reduce the effectiveness of the function of the compound in the treatment process which can cause more severe damage to the gill tissue (Rand et al., 2015).

Analysis of Clinical Symptoms

Clinical symptoms in goldfish after infection with E. tarda bacteria were characterized by changes in behavior 24 hours after infection. Changes in behavior are characterized by abnormal swimming goldfish, silent fish at the bottom of the aquarium, swimming close to aeration and decreased appetite. This is in accordance with the statement of A'yunin et al. (2019), carp infected with E. tarda bacteria causes clinical symptoms of abnormalities in swimming patterns and decreased appetite. In addition to clinical symptoms of behavior, there were also changes in the external organs of all treatments that appeared 48 hours after infection in the form of bleeding on the fins and skin. Other symptoms of fish that have been infected by bacteria include loss of pigmentation or skin color that becomes whitish, exophthalmia or swollen eyes, and swelling of the abdomen. Observations on the clinical symptoms of goldfish infected with E. tarda bacteria are presented in Figure 6.



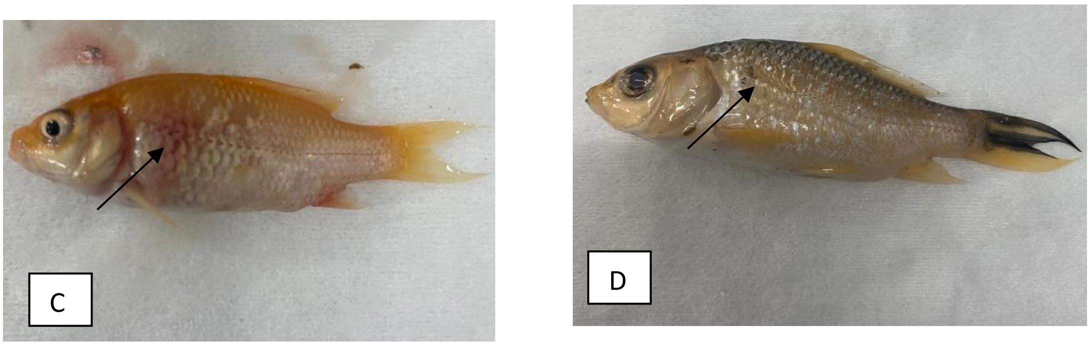


Figure 6. Clinical Symptoms of Goldfish (C. carpio) Infected with *E. tarda* Bacteria (A) Bulging Stomach (B) Thinning Scales (C) Bleeding (D) Peeling Scales (Research Documentation, 2023).

The emergence of clinical symptoms in wounds and bleeding on the body of goldfish is caused by toxins caused by E. tarda, one of which is hemolysin toxin. A'yunin et al. (2019), stated that the hemolysin toxin plays a role in breaking down red blood cells, causing the cells to come out of the blood vessels and causing a reddish color on the skin surface. The second day after infection the goldfish were soaked using tembelekan leaf extract. Treatment with the immersion system is the most applicable method because it can simplify the treatment process, especially for small fish on a large scale (Latifah et al., 2018). The clinical symptoms of fish after immersion in tembelekan leaf extract are presented in Figure 7.

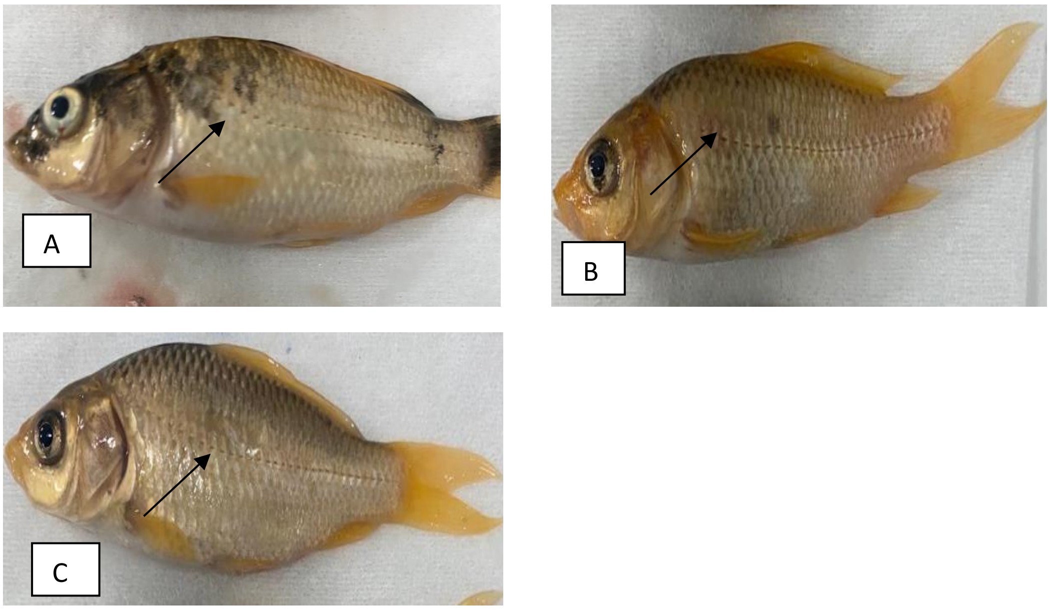


Figure 7. Changes in carp morphology after treatment of tembelekan leaf extract. Description: a. Body color returns to normal; b. Bleeding begins to decrease; c. the wound begins to close

The condition of tilapia improved after soaking with tembelekan leaf extract in treatments D (200 mg/L) and E (250 mg/L) the wound recovered faster than treatment A (50 mg/L), B (100 mg/L), and C (150 mg/L). Day 5, the wounds in treatments D and E had begun to close and body color had begun to return to normal, whereas, in treatments A, B and C, the average bleeding and body color began to improve on the 7th day post-immersion, longer than the the other two treatments. The lack of bleeding in this fish is thought to be due to the presence of flavonoids which function as anti-inflammatory so that the bleeding does not get worse. According to Maleki et al. (2019), the mechanism of action of flavonoids is anti-inflammatory related to proteins through hydrogen bonds, resulting in damaged protein structures, disrupted the stability of the cell wall and plasma membrane, then the bacteria experience lysis.

Water Quality

Measurements of water quality were observed in each treatment including temperature, pH and DO. Observation of water quality is intended to determine changes in water conditions which will affect the health conditions of cultivated organisms. Water quality measurements were carried out twice in the morning and evening. The results of water quality measurements during the study are presented in Table 5.

Table 5. Water Quality Observation Data

Parameter	Average Water Quality	Mold (SNI, 2000)
Temperature	26,5 - 28°C	25 - 30°C
pH	6,5 - 7,4	6,5 - 8
Dissolved Oxygen (DO)	5.5 - 7 mg/L	>4 mg/L

Based on the results of water quality measurements during the study, it was found that the temperature range was between 26.5 – 28°C. The DO value is 5.5 - 7 mg/L, the dissolved oxygen content during the research is still within tolerance limits. This was reinforced by Franklin (2014), explaining that the dissolved oxygen content during fish rearing was >3 mg/L. The pH value during the study ranged from 6.5 to 7.4. The pH value is still suitable for goldfish life.

Table 5 shows that temperature, DO, and pH are within the optimal range for goldfish survival. The water quality values of several parameters, namely temperature, DO, and pH in each treatment showed that the water quality for carp maintenance was within a reasonable range. This indicates that the emergence of disease during the study was not caused by water quality, but due to E. tarda bacterial infection. Schreck & Carl (2016), reported that if the water quality is proper, the physiological functions of the fish's body run smoothly. Fish rearing media must be maintained properly so as not to trigger stress in fish so that fish are more susceptible to disease (Dontriska et al., 2014).

Conclusions and Suggestions

Conclusion

The results of the research that has been done can be concluded that the administration of tembelekan leaf extract can have a significant effect on hematology and histopathology, so that this extract can be used as medicine in carp infected with E. tarda bacteria. The best dose value of tembelekan leaf extract is at a dose of 200 mg/L.

Suggestions

Suggestions that can be given for further research is to carry out tests to find out which bacteria really infect fish.

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