

The Effect of *Anredera cordifolia* Extract towards Kidney Histopathological Features on *Cyprinus carpio* Infected by *Aeromonas hydrophila*

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Article info: Submitted: 2023-05-24, Accepted: 2023-11-29, Publish: 2024-06-01

ABSTRACT: Favoured by Indonesian citizens, *Cyprinus carpio* production decreased over time due to diseases caused by *Aeromonas hydrophila* in the aquaculture process. Antibiotics are often used to manage the disease on media, but they could cause resistance to the bacteria. Herbs were used as the alternative for these antibiotics. This experiment aimed to find the effect of *Anredera cordifolia* extract on the kidney histopathology of *C. carpio* infected by *A. hydrophila*. The dosage used in this experiment was 200ppm, 400ppm, and 600ppm for each treatment with three repetitions. There was also negative and positive control. The bacteria density used for the positive control was 107 cells/ml. After infection, kidney tissue was taken three days post-recovery. Descriptive analysis was used to analyse the kidney histopathology, while to determine the level of the damage carried out by the bacteria, we used the scoring level. This research showed that treating *A. cordifolia* affects the kidney histopathology of *C. carpio* infected by *Aeromonas*. The damaged tissue found was necrosis, edema, and congestion. The best dose used to improve the damaged kidney tissue on *C. carpio* was treatment B (400ppm), indicated by the scoring results for minor damage; the structure showed the tissue figure looked similar to the normal tissue.

Keywords: *C. carpio*, *A. hydrophila*, *A. cordifolia*, Kidney histopathology

Reference to this paper should be made as follows:

Noor, H.F., C. W. Jati, and R. B. Putriani. 2024. The Effect of *Anredera cordifolia* Extract towards Kidney Histopathological Features on *Cyprinus carpio* Infected by *Aeromonas hydrophila*. Agritropica. J. Agr. Sci. 7 (1): 86-92. Doi: <https://doi.org/10.31186/J.agritropica.7.1.86-92>.

INTRODUCTION

According to Alex (2012), the economic potential of ornamental fish culture is more tempting than fish consumption. With a pattern of maintenance and feeding almost the same as consumable fish, ornamental fish farming can generate more significant income because the price of ornamental fish is more expensive.

The increase in koi fish farming production is influenced by several factors that often arise. One obstacle that needs essential attention is the attack of disease. One of them is a disease caused by *Aeromonas* sp.

According to Suhirman and Winarti (2010), efforts have been made to treat this disease using antibiotics that cause pathogenic bacterial resistance and antibiotic residues in fish and

humans. Therefore, it is necessary to develop alternative antibacterial materials from medicinal plants. Binahong leaves (*Anredera cordifolia*) contain flavonoids that can inhibit nucleic acid synthesis, cytoplasmic membrane function, and cell energy metabolism.

Whether the administration of crude extract of binahong leaves (*Anredera cordifolia*) by soaking affects the histopathology of the kidneys of koi fish (*C. carpio*) infected with *A. Hydrophila* bacteria.

The purpose of this study was to obtain the yield of crude binahong extract (*Anredera cordifolia*) and obtain the dose of crude extract of *A. cordifolia*, which can provide the effect of reducing the level of clinical symptoms and histopathological damage to the kidneys of koi carp (*Cyprinus carpio*).



MATERIALS AND METHODS

The tools used in this study were droppers, trays, photo microscopes, tissue, base mould, thermometer, DO meter, pH meter, one glass, cover glass, rotary evaporator, section set, microtome, tissue processor, and cassette embedding. The materials used in this study were koi fish (*C. carpio*), binahong leaves (*Anredera cordifolia*), pure culture of bacteria *A. hydrophila* density 10^7 cells/ml, xylene, formalin 10%, liquid paraffin, acetic acid, ethanol 95%, ethanol 70%, hematoxylin-eosin, Stellan, and tissue.

The research method used is an experimental method. The design used was a Complete Randomized Design (CRD) consisting of 3 treatments with three repetitions and as a comparison, namely Kotrol positive (fish infected with *A. hydrophila* bacteria and negative control (fish not infected with *A. hydrophila* bacteria and not given crude extract of binahong leaves). The treatment is as follows:

Treatment A = Dose 200 ppm

Treatment B = Dose 400 ppm

Treatment C = Dose 600 ppm

The main parameter in this study is the renal histopathology of koi carp (*C. carpio*). In contrast, the supporting parameters in this study are clinical symptoms of fish and water quality, which include pH, temperature, and DO.

The test fish used in this study were koi fish (*C. carpio*), measuring 7-10 cm. As many as 120 fish were obtained from Blitar, East Java. Before the study, test fish were kept for one week for acclimatization. The preparation of binahong extract begins with maceration (soaking), which uses yaki 55 gr binahong powder macerated with ethanol 965 with a ratio of 1 5, where 55 gr is

binahong powder and 5 litres of 96% ethanol— then macerated for 2 x 24 hours at room temperature. The solution obtained is filtered with filter paper and then evaporated with a *rotary vacuum evaporator*, and a crude extract of binahong is obtained. Fish are put in an aquarium containing crude binahong extract with a predetermined dose and soaked for 50 minutes with two repetitions after 48 hours. Bacterial infection is carried out by soaking fish in water media that has been given *A. hydrophila* bacteria with a density of 10^7 cells/ml and a long infection time of 24 hours. The first kidney histopathology preparation involves cutting koi carp kidney tissue and fixing it with 10% formalin. *Tissue preparation, tissue fixation, processing, tissue embedding, sectioning, staining, and observation* are carried out.

Data Analysis

To determine the effect of each treatment on the parameters tested, fingerprint analysis is used, and if different results are obtained, natural or very real, then proceed with the test (BNT).

RESULTS AND DISCUSSION

Observation of Histopathology and Histopathology of Koi Fish Kidneys

Histopathological examination in fish can give an image of the changes in infected tissue pathogen and detect the presence of infectious pathogenic components through micro-observation of abnormal changes in the tissue level. The network that can be used as an observation indicator is the kidneys. Fish kidneys secrete nitrogen waste like ammonia, which protein metabolism generates.

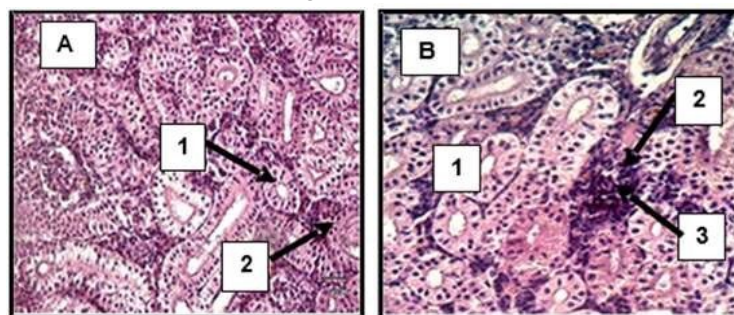


Figure 1. (A) Normal renal histology. Arrow No. 1. Distal tubule; 2. Hematopoietic tissue. Arrows and Kidneys infected with bacteria. Arrow No. 1. Necrosis; 2. Edma; 3. Congestion. Light microscope 400x magnification.

Fish kidneys also play a role in the body's balance of water and electrolytes. In the Figure, see pictures of kidney tissue of healthy fish showing the absence of damage. Cross-section of renal tissue in the distal tubule and hematopoietic tissue under normal conditions. While the kidney tissue of fish infected with bacteria is damaged a

lot. Damage that occurs includes necrosis, edema and congestion.

Features of Histopathological Changes in Koi Fish Kidney Tissue

Histopathological observations showed pathological changes in koi fish soaked in a crude extract of binahong leaves (*A. cordifolia*); different doses and infected bakery *A. hydrophila*.

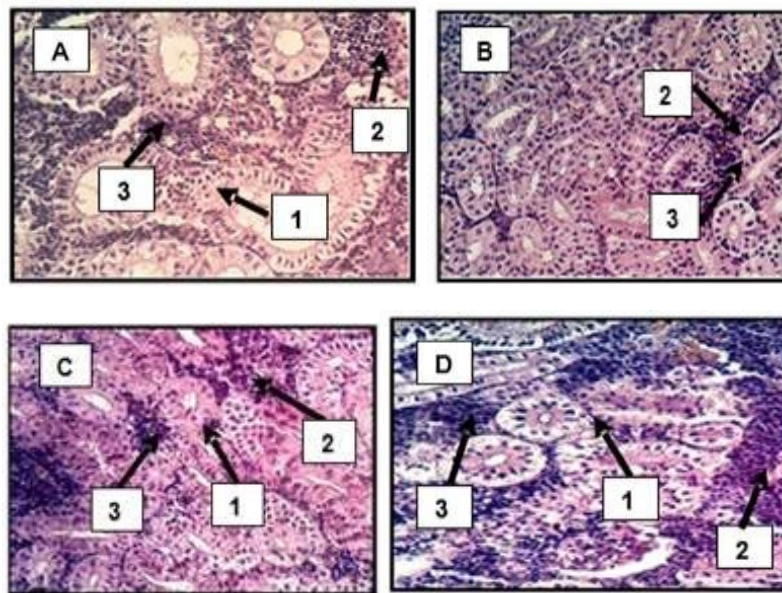


Figure 2. Histopathology of the kidneys, (A). Fish with soaking crude extract of binahong leaves 200 ppm (B). Fish with soaking of crude extract of binahong leaves 400 ppm, (C) Fish with soaking of crude leaf extract binahong 600 ppm, (D) Fish with soaking crude extract of binahong leaves 0 ppm. Arrow No. 1. Necrosis; 2. Edema; 3. Congestion. 400x magnification light microscope.

The histopathological results showed that administering a crude extract of binahong leaves (*A. cordifolia*) as an immunostimulant with different doses affected the different levels of damage in the koi carp's kidney tissue.

Adding crude extract of binahong leaves (*A. cordifolia*) with different concentrations as immunostimulants in koi fish influences the damage to fish kidney tissue. This can be known from the scoring value of kidney tissue damage results. The one-way analysis of diversity data (*one-way ANOVA*) showed that damage to kidney tissue (necrosis, edema, and congestion) had different results. Results of damage data analysis on *hydrophilic* and soaking of crude extract of binahong leaves (*A. cordifolia*) as follows:

Necrosis damage to the kidney tissue of koi carps

According to Ersa (2008), necrosis is the death of cells or tissues accompanying cell degeneration in animal life and is the final stage of irreversible degeneration. Characteristics of necrotic tissue include a paler colour than standard colour, loss of range power (tissue becomes brittle and easily torn), and poor or pale consistency.

The scoring value of renal tissue damage is moderate. This is known from the average scoring value of kidney tissue damage, which is 2.2. The administration of crude extract of binahong leaves with a concentration of 400 ppm (Treatment B) showed a mild tissue damage scoring value. This is known from the average scoring value of kidney tissue damage, which is 1.6. The administration of crude extract of

binahong leaves with a concentration of 600 ppm (Treatment C) showed a scoring value of almost severe tissue damage. This is known from the average scoring value of kidney tissue damage, 2.6. In the K + treatment, namely infection of *A. hydrophila* bacteria without giving crude extract of binahong leaves (*A. cordifolia*) showed the heaviest level of tissue damage, which was 3

The fingerprint table shows that the value of the F result is calculated > F5% and F1%, so it can be said that the administration of *A. cordifolia* extract has a genuine effect on necrosis damage in the kidney tissue of koi fish infected with bacteria

A. hydrophila. Mutdjiutami et al. (2005) stated that an immunostimulant is a substance included in prevention that can increase the body's resistance to infection. The use of immunostimulants in fish farming is new for fish health and disease prevention.

So, a BNT (Smallest Real Difference) test was carried out to determine the difference between the treatments. The results of the BNT test scoring necrosis damage in koi fish kidney tissue infected with *A. hydrophila* bacteria are presented in Table 1.

Table 1. The results of the BNT test scoring necrosis damage in koi fish kidney tissue infected with *A. hydrophila* bacteria

Average	D=1.4	C=1.73	B=2.73	A=3.47	Note
B	-	-	-	-	a
A	0.53**	-	-	-	b
C	0.93**	0.40*	-	-	c
K	1.26**	1.53**	0.33*	-	d

Note:

ns: non-significant

** : very significant

* : significant

The BNT Test calculation results showed different results. It is suspected that binahong leaves contain flavonoids that can function as antibacterials. As found by Wahjuningrum, et al, (2008). Flavonoids are natural response makers, as shown by the results of several studies that show the ability of flavonoids to change the body's reaction to allergens, viruses, and cancer-causing causes. The substance also has anti-allergic, anti-inflammatory, antimicrobial, and anticancer activity.

Edema damage to the kidney tissue of koi carp

It is known from the average scoring value of kidney tissue damage, which is close to 1.8. Giving binahong leaf extract with a concentration of 600 ppm (Treat C) showed a scoring value of almost severe tissue damage. This is known from the average scoring value of

kidney tissue damage, 2.6. In the K + treatment, namely infection of *A. hydrophila* bacteria without giving crude extract, binahong leaves showed the heaviest level of tissue damage, which was 2.9.

The fingerprint table shows that the F value is calculated >F5% and F1%, so it can be done that the administration of crude extract of binahong leaves has a genuine effect on oedema damage in the kidney tissue of koi fish infected with *A. Hydrophila* bacteria. According to Andreson (1992) and Alifuddin (2002), immunostimulants are chemical compounds, drugs or other materials that can improve the mechanism of fish immune response.

To find out the difference between each BNT test treatment. BNT test calculation results score oedema damage in koi fish kidney tissue infected with bacteria *A. hydrophila* is presented in Table 2.

Table 2. The results of the BNT test scoring oedema damage in koi fish kidney tissue infected with *A. hydrophila* bacteria

Average	D=1.4	C=1.73	B=2.73	A=3.47	Note
B	-	-	-	-	a
A	0.53**	-	-	-	b
C	0.80**	0.27*	-	-	c
K	1.07**	0.53**	0.26*	-	d

Note:

ns: non-significant

** : very significant

* : significant

The BNT Test calculation results showed different results. It is suspected that the dose for immunostimulants is not good if it is low because it is feared that it will have no effect, and if it is too large, it will be toxic to fish. According to Jawetz et al. (1996) and Sukmaningtyas (2012), immunostimulant doses that are too small cause effects on the immune system and can also turn into toxins.

Congestive damage to the kidney tissue of koi carp

Congestion is characterised by dilated blood capillaries that are redder in colour and wider in size than normal capillaries. According to Lukostyowati and Kurniasih (2011), congestion is an increase in the amount of blood in blood vessels, which microscopically shows that blood capillaries appear dilated and filled with erythrocytes.

At the average scoring value, the lowest congestion damage at a dose of 400 ppm with a value of 1.86 and the highest congestion damage

value at a dose of 600 ppm with a value of 2.73. The fingerprint table of various congestion damage shows that the value of the result F is calculated >F5% and F1%, so it can be said that the administration of *A. cordifolia* extract has a significant effect on congestion damage in the kidney tissue of koi fish infected with *A. hydrophila* bacteria. According to Chariningsih (2002), in previous studies, flavonoids were Polyphenolic compounds that have shown antimicrobial effects and boost the immune system. According to Jawetz et al. (1996) and Sukmaningtyas (2012), immunostimulant doses that are too small cause effects on the immune system; they can also turn into toxins.

So, to determine each treatment, a BNT (Smallest Real Difference) test was carried out. The results of the BNT test calculation of congestion damage scoring in koi fish kidney tissue infected with bacteria *A. Hydrophila* are presented in Table 3.

Table 3. The results of the BNT test scoring congestion damage in koi fish kidney tissue infected with *A. hydrophila* bacteria

Average	D=1.4	C=1.73	B=2.73	A=3.47	Note
B	-	-	-	-	a
A	0.6**	-	-	-	b
C	0.8**	0.2 ^{ns}	-	-	c
K	1.2**	0.6**	0.4*	-	d

Note:

ns: non-significant

** : very significant

* : significant

The BNT Test calculation results showed different results. In the regression graph above, it can be said that the dose of crude leaf binahong extract affects the percentage of congestion kidney damage; it can also be shown by the equation $y = -6.15x + 3.1367$ which has a coefficient of determination (R^2), namely as much as close to 1 which means that the dose of crude extract of binahong leaves affects kidney congestion damage. This may be because the administration of crude extract of binahong leaves as an immunostimulant affects koi fish after being given an increase. Still, under normal circumstances, binahong leaves are suspected to contain substances Active in the form of flavonoids that can inhibit and kill bacteria. By Wahjuningrum's statement. D, N. Ashry, and S. Nuryati (2008). Flavonoids are natural response changes, as shown by the results of several studies that show flavonoids change the body's reaction to allergens, viruses, and cancer-causing causes. The substance also has anti-allergic, anti-inflammatory, antimicrobial, and anticancer activity.

Clinical symptoms

Clinical symptom measurement was performed during infection, and 3 days after the test, fish were infected with *A. hydrophila* bacteria. There is passive movement, peeling scales and no bright colours. The clinical symptoms are shown in Figure 6 below:

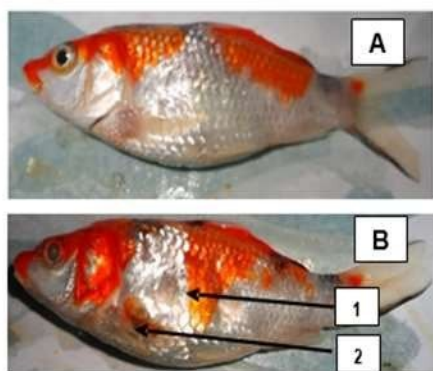


Figure 3. (A) Normal koi carp.; (B) Koi fish infected with bacteria. Arrow No. 1. Scales peeling off; 2. Mucus and wounds.

Water Quality

During the study, water quality measurements were carried out, namely temperature, pH, and DO, where the results of

water quality measurements can be seen in Table 4. Mercury is a poison that accumulates systemically, primarily in the liver, the kidneys, and the spleen. It happens through faeces and urine. Still, it can also occur through sweat, milk, or saliva. Total parameter coliform and that clean water are safe to drink qualified physics, chemical and microbiology, one of the requirements for clean water is not found the total amount of coliform and escherichia coli per 100 ml sample. Afif, Early and Endrinaldi, (2015). Based on field test results on the pH's chemical parameters, The pH value in this study ranges between 7,63 - and 7.69. The range of that value still matches the default water quality standard because it hasn't crossed the threshold value.

Table 4. Water Quality Measurement Results

Variable	Results	Literature
Temperature (°C)	25-25	25-30
pH	7.63-7.69	6.75-8.2
DO (mg/l)	5.72-6.29	>5

CONCLUSION

Based on the study's results, the conclusions are as follows. The yield of crude extract of *A. cordifolia* is 0.0555%. Then, Immunostimulant administration of crude extract of binahong *A. cordifolia* leaves with different concentrations can provide an effect on reducing clinical symptoms and the level of histopathological damage to kidney tissue of koi carp (*Cyprinus carpio*) with an optimal dose of 400 ppm.

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