Pengaruh Ekstrak Sambiloto (*Andrographis paniculata*) terhadap Perubahan Histopathology Mucosa Usus Ikan Gurami (*Osphronemus gouramy*) yang Diinfeksi *Aeromonas hydrophila*

Effect of Sambiloto (*Andrographis paniculata*) Extract Based on Histopathological Changes of Gouramy (*Osphronemus gouramy*) Villi Intestine Infected *Aeromonas hydrophila*

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Abstract

The aim of this study was to know the effect of extract sambiloto leaf (*Andrographis paniculata* to histopathological changes of Gouramy (*Osphronemus gouramy*) intestine which was infected by *Aeromonas hydrophila*. Twenty five gouramy with weight of 100 gram and 8-12 cm length were divided into five (n=5) groups, that were P0+, P1, P2, P3, and P4. Except P0+, the groups that infected with 10^6CFU/mL of *Aeromonas hydrophila* in 5 L water, were dipped with 100 ppm sambiloto extract in the time of 30, 60, 90, and 120 minutes respectively. The data of histopathological changes of intestinum were analyzed with Kruskal wallis test, then continued with Mann Whitney. This researched showed there were significant different ($p<0.05$) among groups based on histopathological changes of intestine. It was indicated that P4 (infected with *Aeromonas hydrophila* and dipping with sambiloto (*Andrographis paniculata*) in 120 minutes) was most effective dosage if be seen from histological changes was mildest.

Keywords: Sambiloto (*Andrographis paniculata*) leaf extract, *Aeromonas hydrophila*, intestine, gouramy (*Osphronemus gouramy*), dipping.

Introduction

Gouramy fish is very familiar for human. This gouramy could be divided into two type for consumption or ornamental fish**.** The examples for ornamental fish are *Osphronemus laticlavius*, *Blue Gouramy*, *Moonlight Gouramy*, *Honey Gouramy*, *Pearl Gouramy*, *Kissing Gouramy*, *Neon Blue Dwarf Gouramy* and so all, and for consumption is *Giant Gouramy* with species *Osprhonemus Gouramy*. As consumption this fish is best choice cause the great taste, savory and not fishy. Gouramy (*Osprhronemus Gouramy*) is one of the freshwater fish for consumption that have high economic price (Sendjaja and Riski, 2002).

Now, treatment method for fish disease still used antibiotic treatment or chemical treatment. Antibiotic and chemical using for therapy in aquaculture got critic from many division (FAO, 2005). Farmers usually use antibiotic like oxytetracycline, inroflaxic and malachite green (Jangkaru, 2007) but using high dose of antibiotic cause bacteria resistant. So, in spite of this problem need alternative for therapy in aquaculture like herbal plant as traditional drug which is have antiparasitic, antibacterial, antifungal and antiviral. One of traditional drug from herbal plant is sambiloto (*Andrographis paniculata*). Sambiloto leaf contains of material active like saponin, tannin, flavonoid dan lakton which have andrographolide (Dalimartha, 2005). Niranjan *et.al* (2010) research sambiloto extract have antibacterial activity, antiinflamation, antioxidant, antidiabetik, anticarsinogen, antipiretik, hepatoprotektif, antitoxic and treatment for gastrointestinal.

The aim of this study is to know effect of extract sambiloto leaf (*Andrographis paniculata*) with 100 ppm dosages with 30, 60, 90 and 120 minutes of dipping to histopathology changes of gouramy *(Osprhonemus gouramy)* intestine which is infected with *Aeromonas hydrophila.*

Outcome of this study is to know the effect of extract sambiloto leaf as treatment for *Aeromonas hydrophila* infection in gouramy *(Osprhonemus gouramy)* and then it could be used for the fisheries to improve their management.

Materials and Method

These researches will be done at Veterinary Pathology Laboratory, Veterinary Medicine Faculty, Universitas Airlangga SurabayaMuscle smear and intestine histopathology inspection of gouramy (*Osphronemus gouramy*) will be done at Histology Laboratory, Biology Department of Sains and Technology Faculty , Universitas Airlangga Surabaya.

Twenty five gouramy (*Osphronemus gouramy*) with weigh of 100 gram and 8-12 cm in length, comprising both sexes, were collected from fish culture ponds at Ikan Gunung Sari market Surabaya city.

Feed standard fish in pellet shape (Takari® PT. Central Proteina Prima (CPP) production code PCP401), water, sambiloto extract, *Aeromonas hydrophila* 10^6 CFU/ml concentration of bacteria take from Veterinary Medicine Universitas Airlangga Surabaya, PZ fluid, Muller Hilton Broad (MHB), TSA (Tripticase Soya Agar), 10% formalin, 70%, 80%, 96% alcohol, absolute alcohol, paraffin, xylol, glycerin, albumin and coloring substance Hematoxylin Eosin (HE).

Fish were reared in five aquaria (50 x 30 x 30 cm, 15 L volume) with five fish in each aquarium, five aerator machine, five aerator tube, zeolit rocks, measurement milligram digital and small dragnet fish.

Sterile scalpel, scissors, tweezers, sonde, ointment pot and stereoform table use for exploring and collecting sample.

Equipments used for preparation histopathology is object glass, cover glass, oven, hot plate, iron plate, microtome, oil emersion and light microscope.

After one week adaptation, all experiment group from animal laboratory infected with *Aeromonas hydrophila*, except group P0-. After infection period, fish will get experimental like experimental group: Group 1 (P0+) positive control, infected fish with *Aeromonas hydrophila*, without sambiloto extract. Group 2 (P1) infected fish with *Aeromonas hydrophila*, dipping with 100 ppm dosage of sambiloto extract in 30 minutes/day. Group 3 (P2) infected fish with *Aeromonas hydrophila*, dipping with100 ppm dosage of sambiloto extract in 60 minutes/day. Group 4 (P3) infected fish with *Aeromonas hydrophila*, dipping with100 ppm dosage of sambiloto extract in 90 minutes/day. Group 5 (P4) infected fish with *Aeromonas hydrophila*, dipping with100 ppm dosage of sambiloto extract in 120 minutes/day

Based on the others research, histopathlogical scoring from number of vili intestine damage (Mahasri, 2007). Number damage of vlili intestine in one preparation view could be determined by; Score 0: negative, no erosion of intestine villi in microscopic field, Score 1: Positive, one per third erosion of upper intestine villi in microscopic field. Score 2: Positive, one-third until two per third upper erosion of intestine villi in microscopic field. Score 3: Positive, two per third upper until all part erosion of intestine villi in microscopic field. Microscopically examine view in intestine histology preparation used microscope with 100x and 400x magnification.

Experimental Design and Data Analysis

Formulation sample number t (n-1) ≥ 15, t is number of experiment and n for repeating number (Kusriningrum, 1989). From this formulation repeating number for this research is four fish in each experiment. To avoid reflect added one fish in each experiment. So, in this research used five fish in each experiment.

Variable in this research is histopathology view of gouramy (*Osphronemus gouramy*) intestine. Experimental trial in this research used Complete Experimental Trial, because repeating and experiment have same number. Each experiment has repeating five times (Kusriningrum, 2010).

The obtained sequence data, translate and edited manually using table form then analysis with SPSS 16 for windows software. To determine number damage of intestine vili in pars anterior infected by Aeromonas hydrophila with sambiloto extract, statistic non parametric test uses Kruskal wallis when there is 0,05% number of false then with Mann Whitney U test.

Result and Discussion

Through under microscopic examination, scoring result depend on erosion of villi intestine in gouramy (*Osphronemus gouramy*) infected with *Aeromonas hydrophila*. From statistical analysis using Kruskal Wallis

test, it was known that there were significant different of erosion villi intestine among treatments. Detail result of histopathologycal examination of gouramy (*Osphronemus gouramy*) intestine which got erosion villi showed P0+ 2.9600±0.08944, P1 2.9200±0.10954 , P2 2.3600±0.26077, P3 1.4800±0.22804, P4 0.64±0.16733 and then, used Mann Whitney U test with 5% significantly were found group P0+ and P1 not have significant different with p>0.05.

According to results of histopathologycal changes of gouramy (*Osphronemus gouramy*) vlili intestine, that could be describe into this chart:

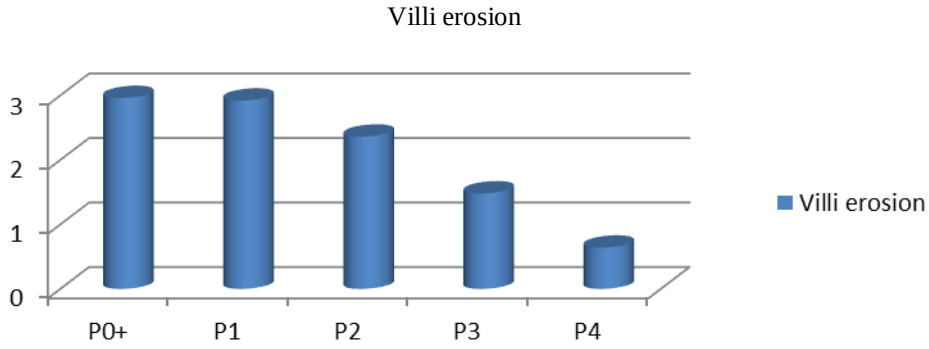

Figure 1. Mean (m) chart of level erosion villi intestine

Histopathologycal changed in gouramy (*Osphronemus gouramy*) intestine infected with *Aeromonas hydrophila* and dipping treatment with sambiloto (*Andrographis paniculata*) in 30, 60, 90 and 120 minutes showed in this figure.

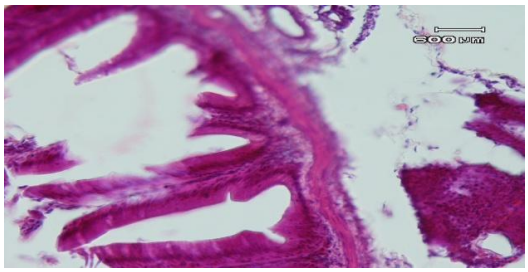

Figure 2. P0+ and P1. Score 3 positive, two per third of upper until all part of intestine villi was erosion in microscopic field (HE staining; 400x magnificent; Olympus microscope CX-400)

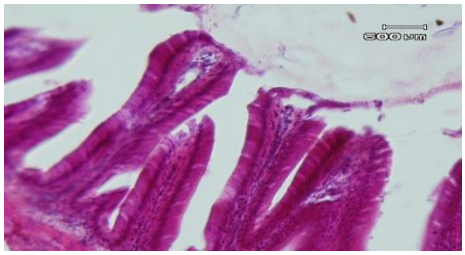

Figure 3. P3. Score 1 positive, one per third of upper part of intestine villi was erosion in microscopic field (HE staining; 400x magnificent; Olympus microscope CX-400)

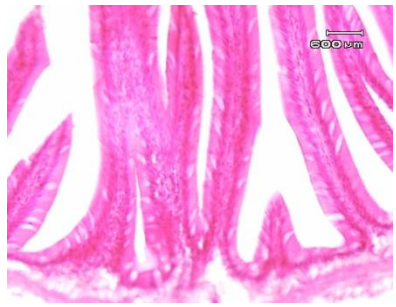

Figure 4. P4. Score 0 negative, no erosion of intestine villi was seen in microscopic field (HE staining; 400x magnificent; Olympus microscope CX-400)

The results of this study explained that fish which was infected with *Aeromonas hydrophila* showed that erosion of villi intestine were different in score. The histological changes results showed heavy infection occur in P0+ which was less significant different with P1 and have significant different with P2, P3 and P4. Heavy infection in P0+ because of infected *Aeromonas hydrophila* not followed with dipping sambiloto (*Andrographis paniculata*) extract as antibacterial (Xu *et al*., 2006). Infection processed could be happened because multiplication of *Aeromanas hydrophila* in

intestinal mucosa, produced toxin and enzyme which were attacked on intestinal epithelium. Intestinal pathogen toxin and enzyme released by *Aeromonas hydrophila* were haemolicin and protease (Holm, 1999).

Chitins enzyme produced by *Aeromonas hydrophila* has function as chitin degradation in gouramy scale, which is blocked bacterial invasion. In fish internal body, *Aeromonas hydrophila* produced lecithin enzyme that helped *Aeromonas hydrophila* enter to blood vessel (Nasran., 2003). *Aeromonas hydrophila* has ability to used protease enzyme that make damage of blood vessel. The damage of blood vessel induces blood come out from vessel and caused hemorrhages in body surface and disturbed blood flow into epithelial cell. These cases know as edema. Edema cause intestinal epithelial get out and in heavy condition will be continued to descuamasi and epithelial rupture (Susanto, 2008). Exotoxin effect caused necrosis and permanent deadly of tissue (Kamalludin, 2011).

Based on the results conclude that dipped time in 30 minutes of sambiloto extract not effective for healing processed. But P2, P3, and P4 seen significant different with P0+ and P1. Among P2, P3 and P4 best effect found in P4. Under microscopic examination for P4 found the score was 1 to 0.

Score 3 (heavily necrosis showed with erosion all villi) found in P1 which was less significant different with P0+ and P2. Dipping with sambiloto extract in 30 minutes has not been able to blocked *Aeromonas hydrophila* activity, because some bacteria still able to produced exotoxin and enzyme to damage intestine.

Statistic analysis showed erosion of vili intestine group P2 has significant different with P0+. It was able to conclude that dipping with sambiloto extract in 60 minutes better to neutralize *Aeromonas hydrophila* than dipping in 30 minutes. More highly dosage of extract sambiloto gave highly antibacterial activity. This result supported with Widyawati (2007) mentioned that highly concentration of antibacterial agent give highly effect of bacteriostatic or bacteriosid. Group P3 has significant different with P0+, P1 and P2. Erosion level villi intestine in group P3 lower than P1 and P2.

Group P4 has best effect than the other experiment. This group has significant different with P0+ and P1. Almost all sample of P4 has score 1 to 0. These conditions accelerate healing processed in intestinal which have abnormal signs. The damage of intestinal epithelial will heal in two until three days (Mc.Gavin and Zachary, 2007).

Sambiloto leaf extract contains of andrographolide, saponin, flavonoid, and tanin which have antibacterial effect (Mahendra, 2006). Tanin and falvonoid antibacterial has effect to damage cell wall and cell membrane cause precipitate protein and abnormality of membrane permeability. That lead to inhibit growth of bacteria. Saponin as antibacterial blocked cell wall synthesis and permeability membrane cell Gram negative bacteria, which was cause damage of membrane cell and make protein, nucleic acid and nucleotide get out from cell (Jaya, 2010). Saponin have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda *et al*., 2000). The effects of saponins at the intestinal level may also need attention, given its presence in some common dietary ingredients. Also several important systemic infections gain access to the body via the intestinal route (World Health Organization, 1996).

Conclusion

Based on the results, dipping sambiloto (*Andrographis paniculata*) extract 100 ppm in 120 minutes gave best effect to decrease erosion of vili intestine gouramy (*Osphronemus gouramy*) which was infected with *Aeromonas hydrophila*.

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