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Kepada: incofims@fpk.unair.ac.id

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The effect of noni *morinda citrifolia* l. fruit extract on gill histopathological changes of Nile tilapia *Oreochromis niloticus*

A T Mukti[1*], E Dewi[2], W H Satyantini[1], L Sulmartiwi[3], Sudarno[1] and M Hassan[4]

[1] Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Unair Jl. Mulyorejo Surabaya 60115, Indonesia
[2] Graduate, Study Program of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Unair Jl. Mulyorejo Surabaya 60115, Indonesia
[3] Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Unair Jl. Mulyorejo Surabaya 60115, Indonesia
[4] Institut of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

[*] Corresponding Author: atm_mlg@yahoo.com

Abstract. Noni *Morinda citrifolia* L. fruit has bioactive compound which is potentially used as a candidate for immunostimulatory ingredient to fish. So that the toxicity of noni fruit extract is important to know further. This study was aimed to know effects of noni fruit extract on gill histopathological changes of Nile tilapia. The method that used was experimental using completely random design with the six treatments of noni fruit extract concentrations and four replicates, respectively. The fish was immersed in noni fruits extract solution for 24 and 96 hours. Then, gill tissue were collected and stored in BNF solution at room temperature for histology. This study was showed that concentration of 18 and 20% (5.2 and 6.0 g/l, respectively) were caused severe damage to the gill tissue where necrosis were found in this tissue. Concentration of less than 18% (5.2 g/l) was indicated a decrease in damage of gill tissue after immersion of 96 hours than immersion of 24 hours. Increased concentration of noni fruit extract has caused more damage in either tissue or organ.

Keywords: noni fruit, aquos extract method, immersion, gill histopathological change, toxicity

1. Introduction

Noni fruit *Morinda citrifolia* L. contained some of the main active substances, including scopoletin, polyphenols, carotenoids, flavonoids, tannins, anthocyanins, ascorbic acid, nitrate, oxalate, phytate, saponins, and free amino acids (Singh *et al*., 2016). Noni fruit has known to contain several active compounds that are capable of affecting the imunomodulator, which it has activated the immune body responses after pathogen infection (Nayak and Sushma, 2009), besides antibacterial, antiviral, antifungal, and antiparasitic (Earle *et al*., 2001).

Tilapia is an organism used for toxicity test (Muhammad, 2002), it had a widespread population and was euryhaline (Agah *et al*., 2009). Tilapia inhabited a variety of habitats, including freshwater shallow waterways, ponds, rivers, and lakes. Toxicity tests using fish tilapia organisms provided an important impact on the development of aquaculture management (Lee *et al*., 2005).

The presence of a foreign substance in the form of noni fruit extract received by fish from the environment will affect the structure of cells or tissues. Changes of the gills on fish is one of the most common responses, which it had unrecognizable against foreign substances in the environment (Au, 2004). High toxic substance concentration would decrease the ability of the liver to eliminate toxic substances, as this organ is particularly vulnerable to the influence of the chemical substances, making this organ has frequently damaged and having abnormalities structure.

Toxicity test of noni fruit aqueous extract using immersion method has conducted to observe the impact of accidental poisoning from noni fruit extract on fish. Deng *et al*. (2012) reported that the toxicity of the noni fruit extract using Brine Shrimp Lethality Test (BLST) obtained 76.13% of LC_{50}. LD_{50} test results of noni fruit extract for 24 hours was not toxic for fish tilapia on the concentration of 1.42 g/L (Muharrama *et al*., 2015). Antimicrobial active compounds at high concentrations could

poison fish seed due to high antimicrobial compounds which are toxic compounds, namely saponins (Ezraneti and Fajri, 2013).

The role of noni fruit extract on tilapia had not much been known. This study was aimed to observe the immersion influence of noni fruit extract on gill histopathological changes of Nile tilapia fish.

2. Material and Methods

This study was conducted at Laboratory of Faculty of Fisheries and Marine, Universitas Airlangga and Balai Karantina Ikan Kelas I Juanda, Surabaya, East Java, Indonesia.

2.1 *Test Organisms*

In this study, fish that used were Nile tilapia of 7-9 cm size and 11 g of average weight originated from Instalation of Freshwater Aquaculture Umbulan, Pasuruan, East Java, Indonesia.

2.2 *Noni Fruit Extract*

Noni fruit that characterized white yellowish color and slightly soft meat was used as the extraction object according to Kusuma *et al*. (2017). Noni fruit was washed and cutted into small pieces. Aqueous extract method of noni fruit was conducted according to Berkovich *et al*. (2013), i.e.100 g/l of noni fruit pieces were mixed aquadest using a blender and filtered using the filter cloth. Then, extract solution was used directly to treatment.

2.3 *Fish Rearing*

Tilapias were reared in 10 L-volumed aquaria at density of 1 fish/l for each treatment. Fish was acclimated during 2 days before treatment was done.

2.4 *Treatments*

This study was used experimental method using complete randomized desigen with 6 treatments of noni fruit extract concentration and 4 replications, respectively. The concentrations of noni fruit extract were based on the preliminary trials that had been done to determine LC_{50} during 96 hours resulted of 5.4 g/l. Treatments were done through immersion of tilapia into noni extract solution (0.0 g/l as control, 3.6, 4.2, 4.8, 5.4, and 6.0 g/l) for 96 hours. Sublethal effects on fish swimming and movement were measured by counting of tachiventilation for 24 and 96 hours, respectively.

2.5 *Histological Preparation*

Sampling of fish gill tissue was done after 24- and 96-hours-immersed fish. Gill tissue from each treatment of noni fruit extract concentration was separated on different sample bottles and fixated using buffer neutral formaline (BNF) solution, respectively according to Junqueira and Carneiro (2005). Histological method was conducted by McCann (2015) using hematoxylin and eosin staining (Genten *et al*., 2009).

2.6 *Histopathological Observation*

Histology preparates were observed under 100 × and 400 × magnifications using a Olympus microscope (Olympus Optical Ltd. Tokyo, Japan), which was equipped with a monitor. Histopathological was evaluated using the histopathological scoring standard according to Crawford (2005), such as shown in Table 1.

Table 1. Histopathological scoring (Corley *et al*., 2013)

Field Area	Score of damage
Normal	0 (normal)
Abnormal < 25%	1 (mild)
Abnormal 26 - 50%	2 (moderate)
Abnormal 51 - 75%	3 (heavy)
Abnormal 76 - 100%	4 (very heavy)

2.7 *Data Analysis*

Data was analyzed using non-parametric statistical method of Kruskal-Wallis and continued using Mann-Whitney test with SPSS 16.0 computer software application.

3. Results

Based on the Kruskal Wallis test, concentration of 0.0 g/l was observed no damage with histopathological scoring of 0.00, while other concentrations were indicated that histopatological scoring of more than zero, which mean the damage occurrence of gill histopathological organ was ranged from mild to very heavy. The highest damage was observed in concentration of 6 g/l, as there was a massive loss of gill tissue on the treatment. Significant histopathological damage was observed in concentrations of 4.8 and 5.4 g/l, such as shown in Table 2. Histopathologial change of fish gill for 24 and 96 hours were shown in Figures 1 and 2, respectively.

Table 2. Histopathological scoring of tilapia gill after treatment of noni fruit extract concentration.

Concentration of noni fruit extract (g/l)	Damage score on observation time	
	24 hours	96 hours
0.0	0.00^{a}	0.00^{a}
3.6	0.75^{b}	1.00^{b}
4.2	1.00^{c}	1.25^{c}
4.8	1.75^{d}	1.50^{d}
5.4	3.50^{e}	3.75^{e}
6.0	3.75^{f}	4.00^{f}

Note: Different superscript in the same column indicate significant difference ($p<0.05$).

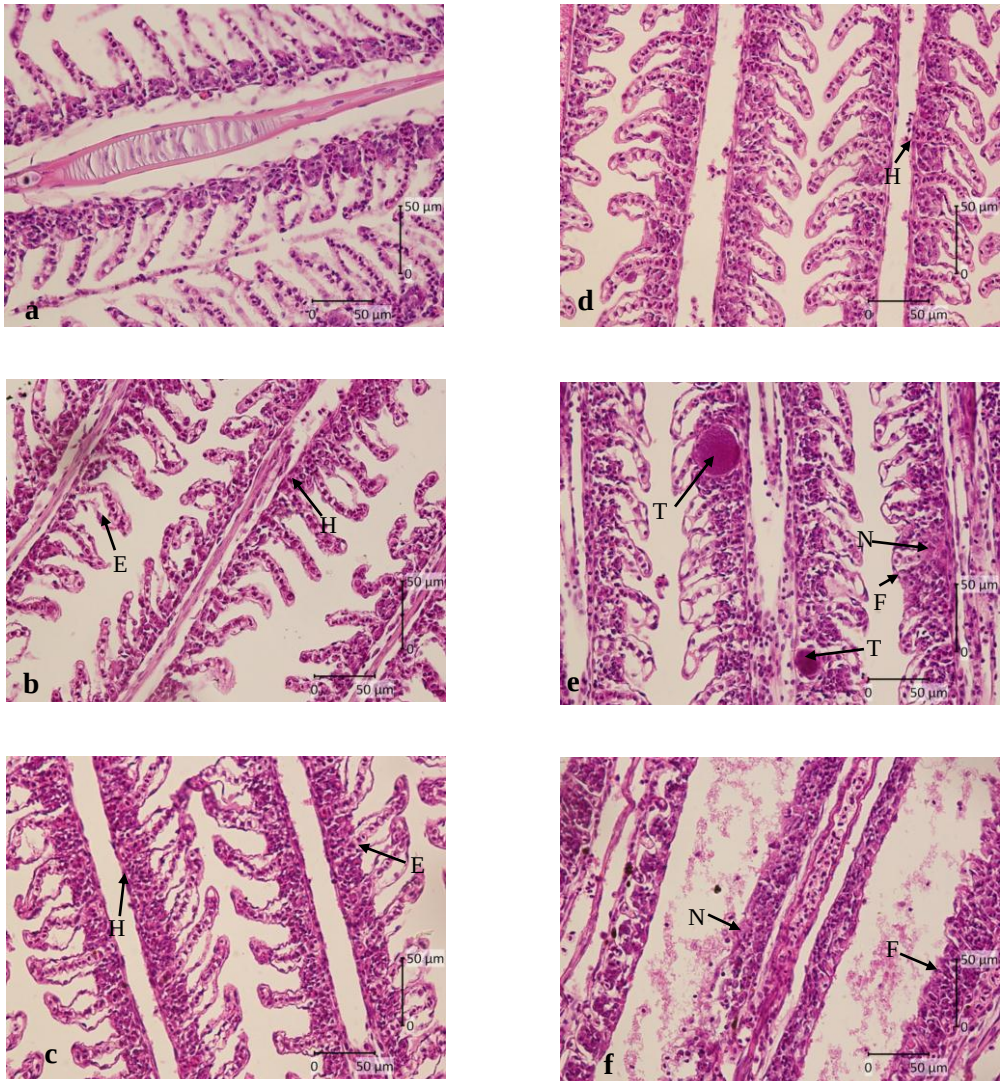

Figure 1. Gill histopathological change of tilapia after immersion of noni extract concentration for 24 hours observed at 400 × magnification. a: 0.0 g/l, b: 3.6 g/l, c: 4.2 g/l, d: 4.8 g/l, e: 5.4 g/l, and f: 6.0 g/l; E = oedema, F = lamellae fusion, H = hyperplasia, N = necrosis, and T = telangiectasis. Bar scale = 50 µm.

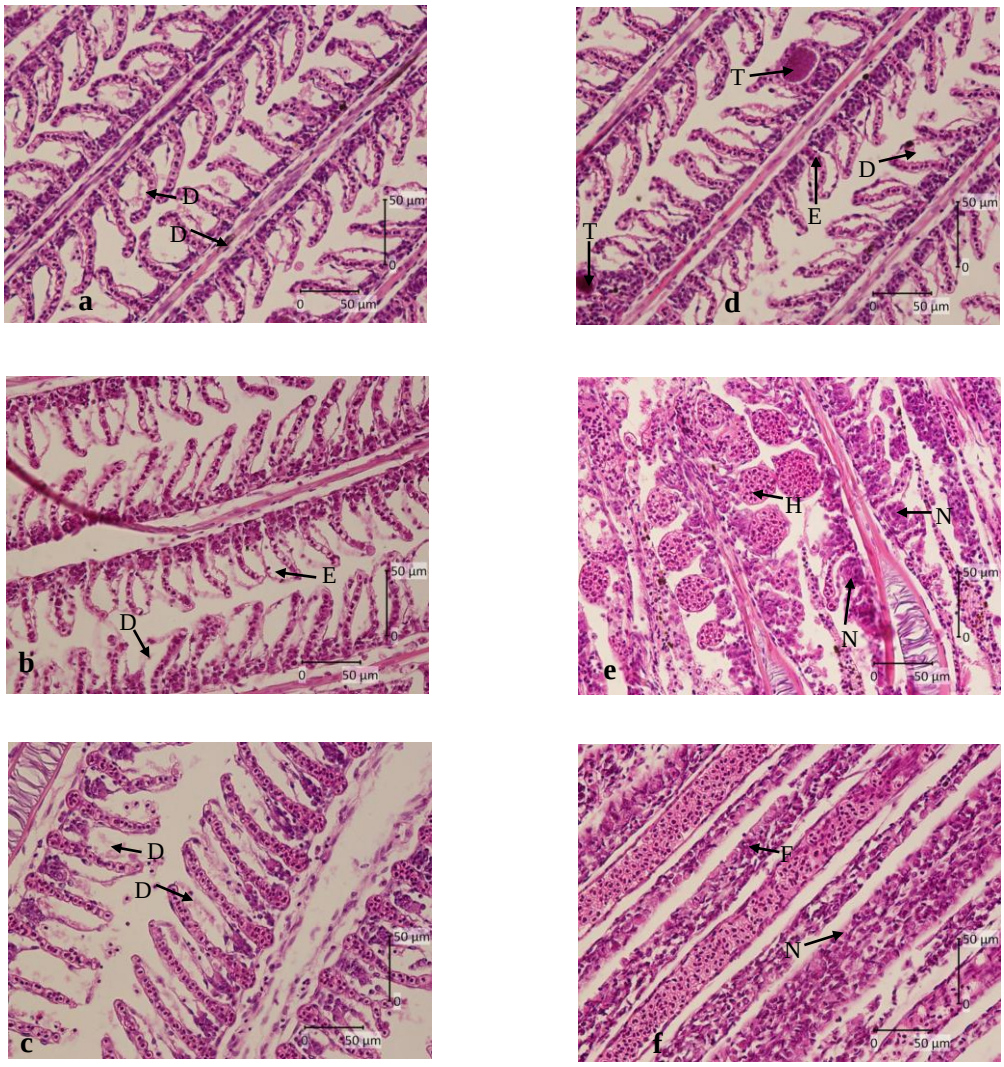

Figure 2. Gill histopathology change of tilapia after immersion of noni extract concentration for 96 hours observed at 400 × magnification. a: 0.0 g/l, b: 3.6 g/l, c: 4.2 g/l, d: 4.8 g/l, e: 5.4 g/l, and f: 6.0 g/l; D = desquamation, E = oedema, F = lamellae fusion, H = hyperplasia, N = necrosis, and T = telangiectasis. Bar scale = 50 µm.

4. Discussion

Histopathological changes occurred in the gill tissue of tilapia immersed in noni fruit extract. Early damage which occurred on the gill was seen at 24 and 96 hours after immersion. Changes of histopathological tissue were comprised oedema, hyperplasia, telangiectasis, lamellae fusion, and necrosis. A thin layer of epithel of gill was directly related to the external environment, where gill was exposed by existing pollutants in the water. The slightest damage may lead to disruption of the function of the gill as a regulator and breathing difficulties (Susanto *et al*., 2013).

The result of the scroring value with different immersion time came from the longer immersion of noni fruit extract, the greater tissue damage arose. Oedema is a condition of increased fluid amounts in the tissues (Mason, 2002). The extract contained saponin, which was foreign substance for fish from the environment. The function of the gill as the osmoregulation organ caused gill had very influential with fish environmental conditions, including the concentration difference in the environment. Oedema could cause tissue swelling and inflammation due to fluid cell accumulation, as there have an

electrolyte imbalance existed in the aquatic environment (Rennika *et al*., 2013). Oedema had usually followed by desquamation of epithelial secondary lamellae (Roberts, 2001).

Hyperplasia in the treatments happened due to a response of reducing the extract diffusion. Lin and Randall (1995) stated that mucous cell hyperplasia exposed due to the diffusin reduction of active ingredients from plant extracts through the branchial epithelium, besides the the possibility of electrolyte imbalance response caused by large permeability branch. Hyperplasia resulted an interlamellae space, making the production of mucous was clogged. Hyperplasia would also cause an epithellium thickening at the tip of the filament which showed a shape like a baseball (distal clubbing) or tissue thickening that was located near the base of lamellae (basal hyperplasia) (Ersa, 2008).

Lamellae fusion occured as hyperplasia spreaded on the basal cells of epithelium. This event resulted in the inhibition process of respiration as well as exspiration. Branchial epithelial necrosis occured due to direct cytotoxic effects (Monroy *et al*. 2005) from noni fruit extract and concurrently with the change of cellular permeability caused by surfactants/saponins (Stagg and Shuttleworth 1986). Telangiectasis happened due to blood coagulation on the secondary lamellae, making a disruption of respiration process.

Foreign substances in the form of the noni fruit extract was able to pass through blood vessels at gills and into liver of tilapia. The content of some active ingredients of noni extract characterized toxic, such as saponins, flavonoids, and poliphenol compounds. Necrosis is the low activity of cells and experienced the cell death, causing the loss of cell function in the area which suffered necrosis.

Harborne (1987) stated that saponin had active compounds like soap, which could be detected on the basis of their ability to shape the foam and haemolyze blood cells. Saponin contained in the leaves. Saponin would affect on fish, as it used in an excess concentration, causing a toxic effect (Ezraneti and Fajri, 2013). Saponin is toxic to the cold blooded organism, as it has able to haemolyze the red blood cells (Musman, 2010), the symptoms of this toxicity caused by saponin as the respiratory toxin.

5. Conclusion

The immersion of noni fruit extract affected significantly on gill histopathological changes of tilapi fish. High concentration of noni fruit extract would significantly increased the histopathological damages of gill tissue. Damages on gill tissue were oedema, desquamation, lamellae fusion, hyperplasia, telangiectasis, and necrosis. The study on effect of noni fruit extract in liver and other tissues needed further.

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