Biosaintifika 16 (2) (2024): 309-319 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Effectiveness of Tuba Root (*Derris elliptica*) on Histological Structure of Rabbit Fish Liver

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Submitted: 2024-03-07. Revised: 2024-06-05. Accepted: 2024-08-06.

Abstract. Rabbit fish *(Siganus vermiculatus)* is one of the biological resources in the sea that is widely consumed by the people in Maluku. Rabbit fish are caught during low tide with nets or by using natural resources to poison the fish, such as tuba root (*Derris elliptica*), which contains rotenone to make fish easy to catch. This study aimed to determining the concentration of tuba root extract that was effective against damage to the liver tissue of rabbit fish. Rabbit fish and tuba root were collected from Oma Village, Haruku Island, Central Maluku District, Maluku Province. In the study, a laboratory experiment was conducted in which the tuba root was treated with doses of 0.1 g, 0.3 g, 0.5 g and 1 g with an exposure time of 18 to 24 hours. Data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. As a result, the lowest level of liver damage in rabbit fish was observed at a dose of 0.1 g, with a total damage of 7 ± 0.00 and 8.5 ± 0.71. Conversely, the highest level of liver damage was observed at a dose of 1 g, with a total damage of 14 ± 0.00 and 15 ± 0.00. The results of the post hoc test showed a subset value of 0.1 g dose (7.75), 0.3 g dose (9.25), 0.5 g dose (12.00), and 1 g dose (14.50). This research indicates that administering tuba root to rabbit fish can result in damage to the liver's structure. The severity of this damage is contingent upon the dose administered, whereas exposure duration does not affect the level of damage to fish organs.

Keywords: Bio-pesticide; Histopathology Alterations; Rabbit fish; Rotenone; Tuba Root

How to Cite: Sekewael, U., Leiwakabessy, F., Warella, J. C., Rahaweman, A. C., & Rumahlatu, D. (2024). Effectiveness of Tuba Root (*Derris elliptica*) on Histological Structure of Rabbit Fish Liver. *Biosaintifika: Journal of Biology & Biology Education*, *16*(2), 309-319.

DOI: http://dx.doi.org/10.15294/biosaintifika.v16i2.6754

INTRODUCTION

Fish is an abundant marine biological resource that Indonesian people widely consume (Selviani et al., 2018; Yulyana et al., 2023). Rabbit fish (*Siganus vermiculatus*) is a species of fish belonging to the Actinopteri class, Acanthuriformes order, and Siganidae family. The Siganidae family, commonly reffered to as rabbit fish, comprises 29 identified species, with the highest species diversity observed in the coral triangle (Fishbase, 2024; Ravago-Gotanco et al., 2018). *Siganus luridus* and *Siganus rivulatus* are found along the Turkish coast of the southern Aegean Sea (Cerim et al., 2020; Soykan et al., 2020). *Siganus sutor* is found in Dares Salaam Tanzania (Mziray & Kimirei, 2016). *Siganus javus* is found in Bandon Bay, southern Thailand and the coastal bays of Mindanao, Philippines (Corsini-Foka & Zava, 2022; Müller et al., 2021). In Indonesia, 19 species of Siganus have been identified, with two species (*S. magnificus* and *S. labyrinthodes*) reported by A Global Information System on Fishes and 17 other species are *S. argenteus*, *S. canaliculatus*, *S. corralinus*, *S. doliatus*, *S. fuscescens*, *S. guttatus*, *S. javus*, *S. lineatus*, *S. puellus*, *S. Puelloides*, *S. punctatissimus*, *S. punctatus*, *S. spinus*, *S. vermiculatus*, *S. virgatus*, *S. vulpinus*, *S. Sutor* (Fishbase, 2024; Setiawan et al., 2019; Suwarni et al., 2020). Two of the 17 Siganus species were found in Maluku waters: *S. lineatus* and *S. canaliculatus* (Setiawan et al., 2019).

Many studies on *Siganus* sp. have been

reported, such as the zoonotic potential of endoparasites infecting the gut of *Siganus fuscescens*, which showed an overall prevalence of *Sclerocollum rubrimaris* and *Hexangium sigani* worms of 45% with a mean intensity of 30±23.34 (N=150) (Villahermosa et al., 2022). Other researchers have shown that acanthocephalan-infected *Siganus rivulatus* reduces the harmful effects of toxic metals by decreasing cadmium (Cd) and lead (Pb) concentrations in the liver; the levels of liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT)), glucose, triglycerides, andurea in fish blood serum; and the levels of total protein and albumin (Hassanine & Al-Hasawi, 2021). In addition, studies related to feeding habitat revealed that the distribution of mucosal cells in the lining of the oral cavity and digestive tract showed a complex characteristic pattern in the intestinal mucosa of *Siganus rivulet* (Sayed et al., 2019).

Several studies have also reported that toxic metals such as aluminium (Al), cadmium (Cd), copper(Cu), iron (Fe), lead (Pb), and zinc (Zn) consumed by the *Siganus sutor* have high concentrationsin the muscle, liver, and fins, posing a risk to human health (Mziray & Kimirei, 2016). In *Siganus javus* species collected from Bojonegara waters, Banten Bay, Indonesia, the heavy metal copper (Cu) (0.348-1.530 mg) accumulated in fish flesh can be harmful to health (Tresanayaputri et al., 2021). In addition to toxic metals, toxic substances from plants used in fishing are also found in fish organs. Toxic substances from plants are usually crushed and fed to fish so that the fish beginto rise to the surface of the water. The tuba (*Derris elliptica*) is one of the plants used to poison the fish.

The Tuba (*Derris elliptica*) is one of the plants that can be found in all regions of Indonesia. Tuba (*Derris elliptica*) is a vine that twines to a height of 10 meters (Siswanto et al., 2022). Several studies have been conducted on *Derris sp.* such as its use as a source of environmentally friendly plant pesticides (Mangardi & Yulianingsih, 2023; Souto et al., 2021; Zubairi et al., 2016). *Derris* sp. contains bioactive compounds that are environmentally friendly and used as an effective insecticide. *Derris* sp. contains rotenone, which is a pyranofurochromon derivative with a basic structure derived from isoflavone. It is a stomach poison and a touch poison that plays a role in inhibiting metabolism and the nervous system. Therefore, it is used as an insecticide and is very toxic to aquatic life (Han et al., 2020; Li, Chen, et al., 2023; Li, Tian, et al., 2023; Radad et al., 2019). Several studies have reported the toxic effects of rotenone compounds in *Derris* sp. on animals, such as ticks, mites, amphibians, marine macroinvertebrates, and invasive fish (Badruzzaman et al., 2021). However, the effects of rotenone compounds on *Siganus* spp. have not been widely reported.

Therefore, research on the effects of exposure to rotenone compounds in tuba root on the liver damage in rabbit fish is important to accurately prove the effects of tuba root feeding on fish. The purpose of this study was to identify damage to the liver tissue of rabbit fish after exposure to rotenone compounds from tuba root. The benefits of this study are that it fills the field of science, especially in the field of marine biology of rabbit fish, as a food source for the people in Maluku, and in the field of ethnobotany of tuba root as a plant used by the people in Maluku to catch fish. Thus, the impact of rotenone compounds on fish and human health can be determined.

METHODS

Study Area

S. vermiculatus was collected from the marine waters of the Lease Islands, and *D. elliptica* was collected from the forest of Oma Village, Haruku Island, Central Maluku District, Maluku Province at coordinates -3.6297532050539143, 128.44010999857068. The sampling area was determined by purposive sampling, based on the habitat of rabbit fish (*S. vermiculatus)* on coral and rocky substrates.

Preparation of Tuba root (*D. elliptica*)

The tuba root (*D. elliptica*) used was blackish brown in color. The samples were collected directly and subsequently cleaned by washing with running water. The analysis was conducted according to a modified version of a previously established method (Handayani et al., 2020; Maliya et al., 2019). The tuba roots were grinded by using a mortar and pestle. The weight of the tuba roots was measured precisely, with different sizes being used namely 0.1, 0.3, 0.5, and 1 g, and then added directly into the aquarium.

Preparation of Rabbit fish (*S. vermiculatus*)

A total of 16 rabbit fish (*S. vermiculatus*) were caught by netting in the waters near Oma Village. The fish used in this study were of both sexes and were evenly divided into four treatment groups of four fish each. In the first group the average length and weight of the fish were 17.5 cm ± 2.08 and 407.75 g ± 10.14, in the second group 20.75 cm ± 2.87 and 431.25 g ± 6.84, in the third group 25.75 cm ± 3.77 and 459 g ± 11.04, in the fourth group 21 cm ± 2.98 and 430.5 g ± 8.96. The fish were then transferred to an aquarium containing seawater for acclimatization. The acclimatization process was carried out at 21ºC for 3 days. After acclimatization, each treatment group was given tuba roots at a concentration of 0.1 g in the first group, 0.3 g in the second group, 0.5 g in the third group, and 1 g in the fourth group. The behavior of the fish was observed until they did not move and was left for 18 to 24 h. The fish was then dissected and the liver organ removed and placed in a sample bottle labeled (Mandefro et al., 2024; Méndez-Tepepa et al., 2023).

Histopathological Assessment of Rabbit fish Liver

Liver organs were cut into small pieces with a thickness of 5 mm, rinsed with physiological saline solution, and immersed in a fixative solution (4% neutral-buffered formalin) for 96 h at room temperature (Ramírez et al., 2020). The liver pieces were dehydrated with increasing concentrations of ethyl alcohol 70%, 80%, 90%, 96%, and xylene for one hour, embedded in paraffin, and sectioned at a thickness of 3 mm using a microtome (Slee Mainz Cut 6062, Germany). Liver preparations were stained with hematoxylin-eosin (HE). Liver tissue damage was observed under a microscope (Olympus CX23, Japan) at 400× magnification in 1 field of view on each slide (Abusrer & Shtewi, 2023; Rohmah et al., 2020). Liver tissue damage is characterized by changes in the normal structure or nuclei of cells.

Statistical Analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) to see the description of fish liver damage and inferential statistics using two-way ANOVA test to examine the effect of tuba root administration on liver, followed byDuncan's test in the post hoc test. Data with a sig value <0.05 were further tested. Meanwhile, data with sig > 0.05. not be further tested (Sihotang et al., 2023).

RESULTS AND DISCUSSION

In this study, the extent of damage to rabbit fish liver tissue was discovered to be influenced by the amount of tuba root administered. The lowest damage rate in rabbit fish liver histology was foundat a dose of 0.1 g, while the highest damage rate in rabbit fish liver histology was found at a dose of 1 g. Based on the observation of liver damage in each of the aquariums (Table 1).

Table 1. Histopathological effect of rabbit fish liver

Treatment Concentration (g/L)	Exposure (h)	Total Tissue Damage		
		P1	P2	Average ± SD
0.1/20	18	7	7	7 ± 0.00
	24	8	9	8.5 ± 0.71
0.3/20	18	8	9	8.5 ± 0.71
	24	9	11	10 ± 1.41
0.5/20	18	12	12	12 ± 0.00
	24	12	12	12 ± 0.00
1/20	18	14	14	14 ± 0.00
	24	15	15	15 ± 0.00

The duration of exposure to tuba root did not affect liver tissue damage in rabbit fish. However, different doses of tuba root caused damage on the liver tissue of rabbit fish. The higher dose in the treatment, the greater the amount of liver damage.

Table 2. The effects of dose, time, and the interaction of dose and time on liver tissue damage in rabbit fish (analyzed using two-way ANOVA).

Source	Sig.
Doses	.000*
Exposure Time	.067
Doses and Exposure Time	.287

Note: Values followed by (*) are not significantly different from doses exposure at p<0.05

The data presented in Table 2 indicate that the administration of tuba root had a statistically significant impact on rabbit fish liver tissue damage, with a p-value of 0.00. The effect of time on rabbit fish liver tissue damage resulted in a sig value of 0.67. According to the data, rabbit fish liver tissue damage was not affected by the exposure time. The outcome of the dose-time interaction revealed a non-significant result, with a p-value of 0.287. This finding indicates that the dose of tuba root exerts an impact on the histological damage of rabbit fish liver tissue, but is not influenced by the passage of time. The post hoc test was continued using Duncan's test, as shown in Table 3.

Table 3. Average and notation from Duncan's test

Dose	Average and Notation	Subset Values
0.1 g	7.75[a]	Subset value for dose 0.1/20/g/liters
0.3 g	9.25[b]	Subset value for dose 0.3/20/g/liters
0.5 g	12.00[c]	Subset value for dose 0.5/20/g/liters
1 g	14.50[d]	Subset value for dose 1/20/g/liters

Table 3. shows that the lowest value of the subset is found at a dose of 0.1/20/g/liters, and the highest value of the subset is found at a dose of 1/20/g/liters. The administration of a 1 g dose was found to be effective in causing liver tissue damage. On the other hand, the lowest amount of damage was observed with a dose of 0.1 g. The higher the dose of tuba root, the greater the level of damage. On the other hand, the lower the dose of tuba root used, the less the liver damage.

Based on the microscopic observation of liver tissue, the histology of rabbit fish liver is shown in Figures 1 and 2. In Figure 1, the pre-treatment liver tissue appears normal, exhibiting clear hepatic histological features, including hepatocytes, liver plates, endothelial cells, erythrocytes, Kuffer cells, and the presence of slits and sinusoids. Figure 2, shows tissue damage characterized by necrosis, karyolysis, sinusoidal dilatation, karyorrhexis, pyknosis, fatty degeneration, macrovesicular, inflammation, oedema, congestion, hydropic degeneration, inflammatory cell infiltration, central venous thrombus, haemorrhage, hepatocyte clumps, and hepatocyte fading.

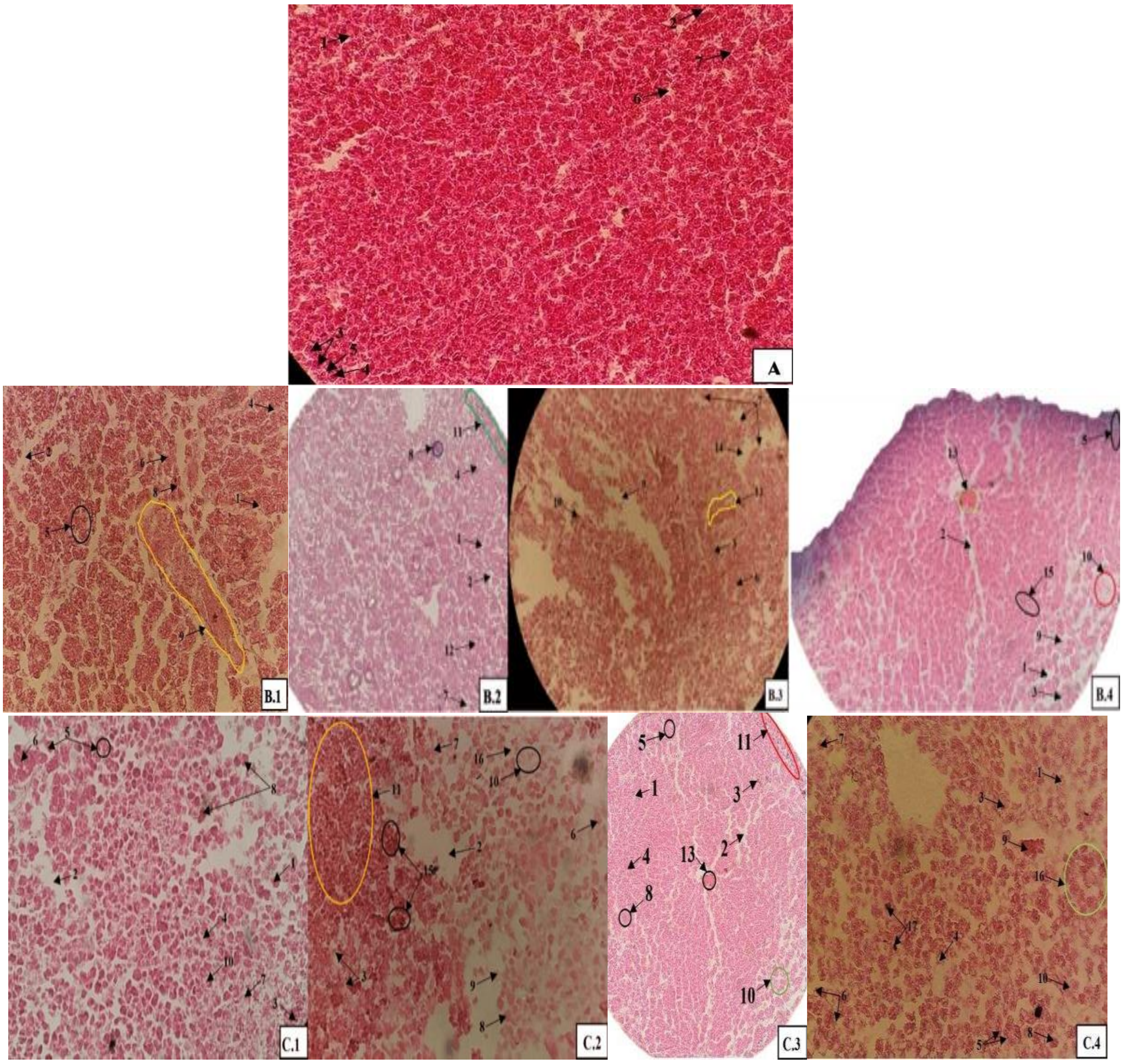

Figure 1: (A) Normal liver of *S. vermiclatus* at 400x magnification with 1. Hepatocytes, 2. Kupffer cell, 3. Hepatic lobule, 4. Endothelial cells, 5. Erythrocytes, 6. Sinusoid, 7. Space of Disse.

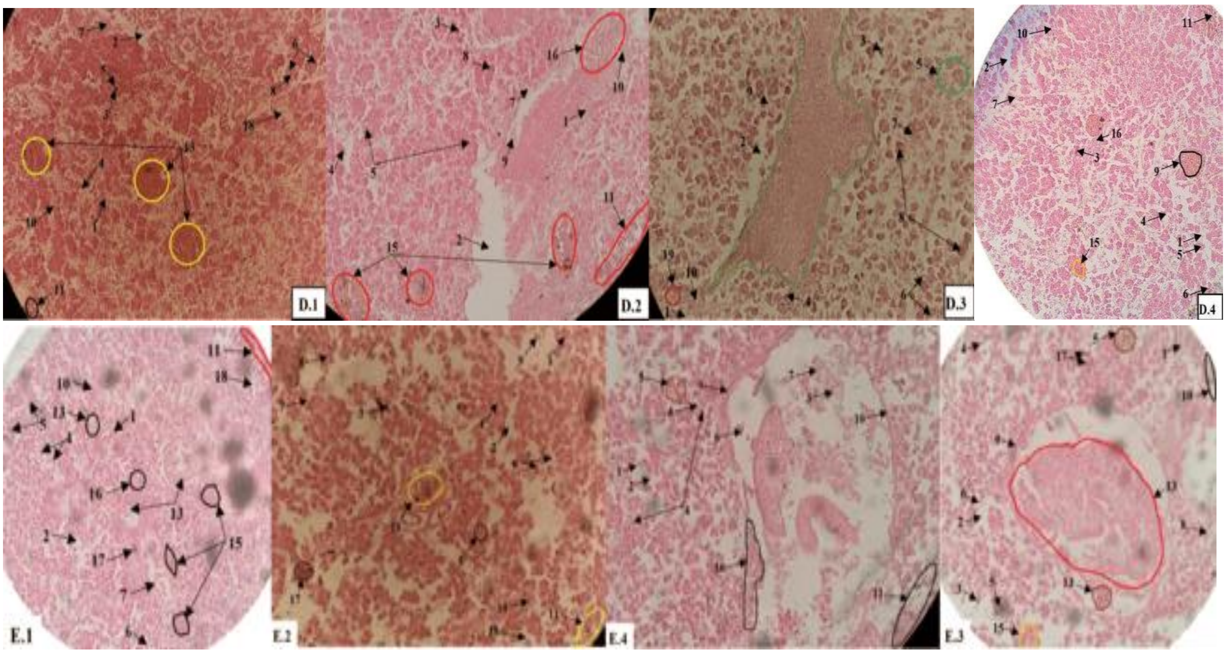

Figure 2: First aquarium (B), Second aquarium (C), Thrid aquarium (D), Fourth aquarium (E) with 1. Karyolysis, 2. Sinusoidal dilatation, 3. Karyorrhexis, 4. Pyknosis, 5. Fatty degeneration, 6. macrovesicular, 7. Inflammation, 8. Edema, 9. Kongesti, 10. Hydropic degeneration, 11. inflammatory cells infiltration, 12. Nuclei pushed to the border of cells, 13. Central vein thrombus, 14. Loss of Hepatocytes, 15. Hemoragik, 16. Necrosis, 17. Hepatocyte clumps, 18. Fading hepatocyte cells, 19. Pyknosis with karyolysis

Tuba roots contain a toxic compound called rotenone, which is a highly toxic compound classified as contact and stomach poison (Nurjayanti et al., 2022). The rotenone in tuba roots is directly transported with water through the gills during respiration. Toxic compounds interfere with electron transport and cellular respiration in the mitochondria by inhibiting NADH ubiquinone reductase and causing cell death due to excess free radicals (Santi et al., 2022; Usman et al., 2023). Additionally, rotenone compounds decrease oxygen absorption in the blood, reduce respiratory processes, and inhibit metabolism. The effect is damage to the liver and failure to detoxify toxins that enter the body (Ling, 2003; Liu et al., 2022; Topić Popović et al., 2023a). Therefore, this study proves that time does not affect the extent of liver tissue damage. Thus, regardless of the length of time used to determine the amount of liver tissue damage, it has no effect unless a dose of tuba root is added.

During respiration, rotenone compounds enter the bloodstream through the gills and enter the portal vein and hepatic artery into the liver (Dunker et al., 2020). Rotenone inhibits metabolism, resulting in decreased respiration. In addition, hepatic dysfunction in toxin detoxification results in liver damage and oxygen depletion. This supports previous research, which states that the use of high doses of tuba root causes death in fish because oxygen cannot bind to hemoglobin, resulting in slow breathing and a reduced metabolic rate (Gaspersz, 2022; Mucha et al., 2023). The response of the fish body after exposure to rotenone compounds is a change in behavior, such as irregular fish movements, shock, swimming to the surface, and pausing for a moment accompanied by another shock until the fish loses body reflexes and ends in death. Exposure to rotenone causes a loss of control over fish movement, which eliminates the function of dopaminergic neurons, resulting in locomotor dysfunction (Hua & Ekker, 2020). Furthermore, because the blood binds less oxygen and prevents the exchange of ions and gases, rotenone exposure made the gills slimy (Idzni, S et al., 2020). The central nervous system's ability to interfere with cellular respiration and bodily functions beyond the body's tolerance limit results in tissue damage and death in fish, which is the reason of this response (Mucha et al., 2023).

Microscopic observations showed that the type of toxicity influences hepatic tissue damage (Topić Popović et al., 2023b). The changes and increases in the amount of liver tissue damage in this study increased with an increase in the dose of tuba root. This is in accordance with the correlation between the concept of dose use and the amount of tissue damage. The use of low doses produces a small amount of damage, whereas the use of high doses produces a large amount of damage. In this study, liver tissue damage was different in each sample, and not all fish liver tissues were damaged. Disruption of hepatocyte function causes the liver to store large amounts of triglycerides for metabolic energy (Alves-Bezerra & Cohen, 2018; Dutta et al., 2021). In general, toxic substances that enter the body can be detoxified by hepatic microsomal enzymes in the smooth endoplasmic reticulum through oxidation, reduction, and hydrolysis. This process converts hydrophobic compounds, which are difficult to eliminate, into hydrophilic compounds. The toxic substances are then silently excreted in the bile or urine (Burton & Burton, 2017; Lall & Kaushik, 2021). Under certain conditions, the liver can still maintain its normal structure and function, even though only 0.1 g was administered in the low-dose treatment. However, the administration of higher doses, such as 1 g, results in liver failure, tissue damage, and death.

Hepatocyte damage in hydropic degeneration is the same as that in parenchymatous degeneration. However, the degree of hydropic degeneration is more severe and reversible owing to the disruption of active transport, causing the cell to be unable to pump out Na^+ ions and increase the dose of ions in the cell (Mohammadinejad et al., 2019; Ridho et al., 2020). Degeneration is the cell response to a reversible injury, but if the cause is not removed immediately, it can lead to cell death (Miller & Zachary, 2017). Necrosis is characterized by pyknosis and karyolysis. Pycnosis is characterized by shrinkage of the cell nucleus, and fading or lysis of the cell nucleus characterizes karyolysis. Inflammatory cell infiltration is the body's response to harmful stimuli, such as bacterial pathogens, which functions as a non-specific defense that localizes pathogens through phagocytosis (Lutfiyah et al., 2021; Rahayu et al., 2021; Situmorang & Ilyas, 2018; Wisudawati et al., 2023). Thus, when toxic substances damage liver cells, various changes occur. All were clearly visible in the hepatic histology and histopathology.

The use of rotenone compounds found in tuba root as natural pesticides has been used by fisheries managers in America for more than 70 years (Tat et al., 2024). However, exposure to rotenone can cause adverse health effects in humans. Exposure to rotenone occurs via inhalation, skin contact, or ingestion of poorly processed food. The mechanism of action of rotenone involves the inhibition of the mitochondrial complex, thereby reducing oxidative phosphorylation and ATP production (Kumar et al., 2023). Decreased ATP production can increase cellular glycolysis, which leads to increased lactic acid production and metabolic acidosis. In addition, inhibited mitochondria produce byproducts in the form of reactive oxygen species (ROS), which induce oxidative stress, are cytotoxic, and lead to apoptosis (Sharma et al., 2022; Tat et al., 2024; Yarmohammadi et al., 2020). The toxic effects of rotenone include nausea, vomiting, diarrhea, impaired movement function, and respiratory disorders, resulting in hypoxemia, hypercapnia, and hypotension (Gupta, 2014; Radad et al., 2019). Several studies have found that rotenone compounds can cause Parkinson's disease, which is characterized by mitochondrial dysfunction as a trigger for increased oxidative stress, dopamine loss, and neuropathological changes in the brain (Lawana & Cannon, 2020; Tanner et al., 2011). Unlike pesticides used in the fishing industry, such as organophosphates, triazines, and genotoxins, can cause cancer (Khatib et al., 2022; Srivastava et al., 2016). The rotenone compound found in tuba roots is known to not induce cancer cells. This is evidenced by research by the U.S. National Institute of Health in which rats were given rotenone compounds up to 1200 mg and found no carcinogenic activity in liver and subcutaneous cells. Therefore, rotenone is not mutagenic at concentrations below those that are acutely toxic to the cells (Ling, 2003).

In general, rotenone can be broken down by heating at high temperatures. Therefore, cooking food or fish containing rotenone before consumption may reduce the risk of human poisoning (Tat et al., 2024). In addition, rotenone is a poison that is easily broken down by sunlight and easily degraded by soil and water; therefore, the poison disappears within 2-3 days (Rosfiansyah et al., 2022). This is reinforced by research conducted by (Gaspersz, 2022), which proves that the use of a dose of tuba root of 10 mg in 20 liters of water left for four days, it turns out that the poison does not kill ten fish within 96 hours. Currently, the lethal oral dose of rotenone for tuba root is unknown. However, some researchers have estimated the oral LD50 for rats to be 60-135 mg/kg (Gupta, 2014) and for humans to be 300-500 mg/kg (Lazo et al., 2014). Although fishing with rotenone compounds is not harmful to other marine mammals, it can be harmful to fish, plankton, and marine micro-invertebrates (Dalu et al., 2015). The use of tuba root as a natural pesticide on fish is not toxic to the environment because it binds easily to organic matter and can be broken down by photolysis and thermal degradation in water; therefore, it does not last long in the environment (Dunker et al., 2020).

This study aimed to determine the tuba root concentration that is effective in damaging the liver organs of rabbit fish, a marine fish favored by people in coastal areas and fishermen. This follows up on previous studies on tuba roots that were only used in freshwater fish species (Lesmana et al., 2022; Prariska et al., 2017; Rahayaan et al., 2020; Tobigo et al., 2017). Our investigation demonstrated that the administration of tuba root to rabbit fish caused damage to the liver's structure. The amount of damage was influenced by the administered dose. The exposure time did not affect the level of damage to fish organs. The study provides critical information regarding the utilization of tuba root as a natural pesticide in fishing, with a focus on its advantages over chemicalalternatives that cause ecological damage to marine ecosystems. Scientifically, rotenone-containing tuba root are toxic to fish, plankton, and micro invertebrates, whereas other marine mammals are not toxic. In addition, the results of this study can be used as a reference for the development of herbicide products from tuba root.

CONCLUSION

The dose of tuba root (*Derris elliptica*) affects liver damage in rabbit fish (*S. vermiculatus*). The higher the dose, the greater the damage; the lower the dose used, the less the damage produced. Liver damage was independent of exposure time and the type of toxicant administered. The percentage calculation of liver damage in this study was not included, which is a limitation of thisstudy, and it can be considered for future studies. Further research is necessary, involving histological tests on other organs, including the gills, brain, and digestive system, which are directly associated with the absorption of toxins into the bloodstream. Furthermore is imperative to quantify the residual toxicity percentage in the fish tissue prior to consumption.

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