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The Effects of Pasak Bumi (*Eurycoma longifolia* Jack) Product on Liver Histology of Wader Pari (*Rasbora lateristriata* Bleeker, 1854)

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Abstract. *Eurycoma longifolia Jack (commonly known as pasak bumi) is a medicinal plant that contains various bioactive compounds such as eurycomanone, saponins, sterols, and isoprenoids, which are known to exert multiple physiological effects, including on the liver as an organ. This study aimed to examine the effects of pasak bumi root product on liver histology in yellow rasbora (Rasbora lateristriata) as an animal model and to compare the effects between continuous and cyclic (5 days ON, 2 days OFF) administration methods. The experiment lasted 2 weeks, with three treatment groups: control (no extract), continuous administration (P1), and cyclic administration (P2). Observed parameters included liver tissue structure, such as the central vein and hepatocytes arrangement, as well as histological features of hepatocytes. Data were analyzed using one-way ANOVA followed by Duncan's test. The results showed that pasak bumi administration affected the histological structure of the liver in R. lateristriata, with observable cellular damage including pyknosis, karyorrhexis, and necrosis. Continuous treatment (P2) caused more severe damage than cyclic treatment (P1), which showed tissue structures more similar to those of the control group. Therefore, cyclic administration is considered safer and tends to induce milder liver damage in fish than continuous administration*

Keywords: *continuous, cyclic, Eurycoma longifolia, liver, Rasbora lateristriata*

INTRODUCTION

Eurycoma longifolia Jack, commonly known as pasak bumi, is a medicinal plant from the Simaroubaceae family distributed across Indonesia, Malaysia, the Philippines, Vietnam, and Myanmar (GBIF, 2023). Its root contains bioactive compounds such as eurycomanone, eurycomalactone, and tannins, which are believed to have therapeutic properties, including enhancing male reproductive health, antimalarial, antibacterial, and antitumor potential (Effendy et al., 2012). Despite its wide traditional use, comprehensive toxicological evaluations remain essential to ensure the safety of this plant. Previous studies have shown that pasak bumi root extract is

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hepatotoxic in mammals. For instance, Jannah (2020) reported that administration of 52 mg/kg BW in mice led to hepatocyte swelling, vacuolization, and glycogen infiltration. The liver is a vital organ involved in metabolism, detoxification, and excretion. Hepatocytes play a central role in biotransforming hydrophobic drugs and xenobiotics into hydrophilic metabolites before renal elimination (Long et al., 2022)

Fish have gained attention as alternative vertebrate models for toxicological and pharmacological studies due to their transparent embryos, rapid development, high fecundity, and well-documented genetics (Lleras-Forero et al., 2020). Although differences exist between fish and mammals, fish remain valuable bioindicators capable of exhibiting rapid physiological responses to environmental and chemical stressors. *Rasbora lateristriata*, an Indonesian endemic freshwater species from the family Cyprinidae (ITIS, 2024), is particularly suitable because of its small size, transparent embryos, tolerance to environmental fluctuation, and well-described developmental stages (Retnoaji et al., 2016; Retnoaji et al., 2023).

Fish were used as the experimental model in this study because they are recognized as sensitive indicators for detecting toxicological impacts of natural extracts, with rapid hepatic responses to xenobiotic exposure. The liver was selected as the primary organ for observation because hepatocytes directly metabolize xenobiotic compounds, and their histological alterations provide early and reliable evidence of toxicity. Thus, examining liver structure offers a clear representation of the biological impact of pasak bumi on the organism.

Although numerous studies have explored the pharmacological and toxicological profiles of *E. longifolia* in mammals, research evaluating its effects in aquatic organisms—especially native Indonesian species—remains scarce. No prior study has assessed the hepatic effects of a commercially available pasak bumi product (not an extract) incorporated into fish feed, nor investigated how different feeding regimes influence toxicity. This represents a significant research gap because dosing intervals may alter toxin accumulation, metabolic recovery, and organ-level responses. The novelty of this study lies in: (1) using *R. lateristriata* as a native bioindicator fish model, (2) applying an LC_{10} based safe concentration incorporated directly into feed to reflect ecologically relevant exposure, and (3) comparing continuous versus cyclic administration, an approach that has never been evaluated in previous toxicological assessments of *E. longifolia*.

To date, no research has examined the effects of the pasak bumi product on the liver histology of *R. lateristriata*, particularly under different feeding patterns. Therefore, this study aims to evaluate the histological alterations in the liver of *R. lateristriata* induced by the pasak bumi product under continuous and cyclic feeding regimes. The findings are expected to contribute to toxicological understanding of E. longifolia, support safer, evidence-based use of herbal products, and provide practical insights for aquaculture, environmental health, and public awareness of potential hepatic risks associated with traditional herbal consumption.

MATERIALS AND METHODS

Type and Design of Research

This study was an experimental study that used a Completely Randomized Design (CRD) to assess the effects of *Eurycoma longifolia* Jack (Pasak Bumi) root extract on hepatic histopathology in *Rasbora lateristriata*. The research involved one independent variable: the extract-administration pattern, comprising control, continuous administration, and cyclic (interval-based) administration. The dependent variables were the fish's biological and histological responses. The research parameters included water-quality conditions during the experiment, fish body length as a growth indicator, hepatic structural condition (hepatic), and detailed histological examination of hepatocyte morphology. The experiment was conducted under controlled laboratory conditions to determine the causal relationships between the treatment patterns and the observed responses.

Time and Location of Research

The experiment was conducted from January to May 2025 at the Laboratory of Histology and Animal Embryology and the aquaculture facility of the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Sample Description and Ethical Clearance

A total of 15 adult male *Rasbora lateristriata* (approximately 6 months old) were used, sourced from a controlled breeding unit at the Faculty of Biology. Each treatment group consisted of 5 individuals. Ethical approval (09/EC-FKH/int./2025) was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, University of Gadjah Mada.

Materials and Equipment

Materials: commercial fish feed (pellets), *E. longifolia* root product, 6-month-old male *Rasbora lateristriata*, distilled water, 10% NBF, Graded alcohol solution, xylol (xylene), liquid paraffin (melting point 56°C), Hematoxylin and Eosin stain, entellan. Equipment: fiber tanks, water pump, digital balance, camera, millimeter block paper, pH meter, DO meter, thermometer, scalpel, anatomical forceps, test tube or vials, tissue cassettes, paraffin oven, microtome, heat plate, glass slide, cover slips, light microscope (Leica DM300), and ImageJ software.

Water Quality Measurement

Water quality was monitored throughout the experiment to maintain stable environmental conditions during Eurycoma longifolia extract administration. The parameters measured included pH, dissolved oxygen (DO), and water temperature. These measurements were taken every two days. The pH value was measured with a calibrated digital pH meter by immersing the electrode in the culture water until the reading stabilized. Dissolved oxygen was measured with a portable DO meter following standard calibration procedures before each measurement. Water temperature was recorded with a digital thermometer by immersing the probe until a stable reading was displayed. All measurements were conducted in the morning to minimize diurnal variation, and the recorded data were used to support the interpretation of the experimental results.

Determination of Product Dose and Feed Re-Pelleting Method

The dosage of the *Eurycoma longifolia* product incorporated into the feed was determined based on the LC_{10} value obtained from embryo-mortality analysis using the Probit Bliss method. The LC_{10} value was selected as a conservative threshold representing the initial level of toxicity. A safe concentration was then calculated following Sprague (1971) using the formula:

Safe concentration = LC_{10} / safety factor, with the safety factor ranging from 1 to 10.

The resulting safe concentration was converted into the amount of *Eurycoma longifolia* product required for feed formulation. For the re-pelleting process, 100 g of commercial Primafeed pellets were ground into a fine powder and homogenized with 0.04 g of *Eurycoma longifolia* product, yielding a final concentration of approximately 406 ppm in the treated feed. The mixture was then molded into uniform pellets using a syringe and dried in an oven at 100°C to reduce moisture content. The dried pellets were stored in a sealed container under dry conditions until use.

Experimental Treatments

K (Control): Standard feed without product, 2 times daily repeated for 14 days. P1 (Cyclic): Product-mixed feed, 2 times daily for 5 days per week, followed by 2 days of standard feed, repeated for 14 days. P2 (Continuous): Product-mixed feed, 2 times daily repeated for 14 days.

Histological Preparation and Analysis

The fish were maintained for 2 weeks before liver histological structure was examined using the paraffin-embedding method (Humason, 1961). Haematoxyline and Eosin (H&E) staining was used for histological observation. The preparation began with euthanizing the fish in cold water, followed by dissection to collect liver tissues. The liver samples were fixed in NBF for approximately 48 hours. After fixation, specimens were washed in 70% ethanol for 48 hours, with the solution replaced every 6 hours. Dehydration was performed using a graded ethanol series: 70% (4 × 30 minutes), 80% (2 × 30 minutes), 90% (2 × 30 minutes), and 96% (1 × 30 minutes). The tissues were then cleared overnight in xylol and infiltrated with liquid paraffin in the oven before being embedded in paraffin blocks. The paraffin blocks were trimmed and mounted onto wooden holders for better adhesion. Sectioning was performed using a microtome with a 5 µm thickness. The tissue sections were affixed to glass slides pre-coated with Mayer's albumin and wetted with distilled water. The slides were then placed on a hot plate at 40–45°C to remove excess water, which was absorbed using a pipette or blotting paper, and allowed to dry for 24 hours prior to staining.

Hematoxylin and Eosin (H&E) Staining

Slides containing the tissue sections were deparaffinized in xylol for 15 minutes and rehydrated through a descending alcohol series (96%, 90%, 80%, 70%, 60%, 50%, 40%, and 30%), followed by distilled water. The slides were stained with Ehrlich's Hematoxylin for 10 seconds and rinsed under running water. A second round of dehydration was conducted through an ascending alcohol series (30%, 40%, 50%, 60%, and 70%) before counterstaining with Eosin for 20 seconds. The slides were then passed through 70%, 80%, 90%, and 96% ethanol, dried on blotting paper, cleared in xylol for 15 minutes, and mounted using Entellan before a cover glass was applied.

Histological Observation

Histological observations were conducted using a light microscope at 1000× magnification. The examined areas included intact hepatic parenchyma surrounding the central vein, while regions with sectioning artifacts were excluded. Each treatment group consisted of three biological replicates, and three tissue slides were prepared from each replicate. Five randomly selected fields of view (FOVs) were examined per slide, resulting in a total of 45 FOVs for each treatment group. Nuclear abnormalities were identified based on standard histopathological criteria, including pyknosis (shrunken, hyperchromatic nuclei), karyorrhexis (fragmented nuclei), and karyolysis (faded or absent nuclei).

Hepatocyte Damage Scoring

Hepatocyte damage was quantified using a semi-quantitative scoring system. Each FOV was scored according to the proportion of abnormal nuclei: 0 = absent (0–5% affected cells), 1 = mild (<25%), 2 = moderate (25–50%), and 3 = severe (>50%). Scores from the five FOVs on each slide were summed to obtain a slide score, and the three slide scores within each biological replicate were averaged. The values presented in the results represent the mean ± standard deviation of the three replicates. Higher scores indicate more extensive hepatocellular damage, as the scoring system captures both the frequency and severity of nuclear abnormalities across multiple microscopic fields.

Statistical Analysis

Data from water quality, absolute growth, body length, histological structure, and histological examination were expressed as mean ± standard deviation (SD). Differences among treatment groups were analyzed using One-Way ANOVA, followed by Duncan's multiple-range test for post hoc comparisons, using SPSS version 20 (IBM Corp., Armonk, NY, USA). A significance level of $p < 0.05$ was used to consider statistically significant.

RESULTS AND DISCUSSION

Water quality parameters were monitored throughout the experiment to ensure stable environmental conditions and to minimize confounding effects on fish health. Temperature, pH, and dissolved oxygen were selected as key indicators influencing physiological responses. The water quality data during the experimental period are presented in Table 1.

Table 1. Water quality parameters during the 2-week experiment

Treatment	Temperature (°C)	pH	Dissolved Oxygen (mg/L)
Control	$26.98^{a} \pm 0.40$	$6.35^{a} \pm 0.16$	$6.36^{a} \pm 0.20$
P1	$26.81^{a} \pm 0.38$	$6.34^{a} \pm 0.15$	$6.30^{a} \pm 0.27$
P2	$26.87^{a} \pm 0.37$	$6.34^{a} \pm 0.17$	$6.19^{a} \pm 0.23$

Table 1 shows that the temperature ranged from 26.81–26.98°C, pH values hovered around 6.34–6.35, and DO remained between 6.19–6.36 mg/L, all within physiological tolerance for tropical freshwater fish (all values with the letter (a) indicate no significant differences were found among treatments (p>0.05)). Water quality measurements across all treatments remained within acceptable limits for *R. lateristriata*, suggesting no external environmental stress.

Table 2. Absolute growth of *Rasbora lateristriata* (cm)

Treatment	Initial Length (cm)	Final Length (cm)	Absolute Grow
Control	5.25 ± 0.18	6.23 ± 0.21	$0.98^{ab} \pm 0.25$
P1	5.20 ± 0.20	6.31 ± 0.18	$1.11^{b} \pm 0.24$
P2	5.17 ± 0.20	6.03 ± 0.23	$0.85^{a} \pm 0.10$

Growth performance was evaluated to assess the physiological response of *R. lateristriata* to *Eurycoma longifolia* administration. Absolute growth based on body length was used as an indicator of overall metabolic condition. The growth parameters of each treatment group are

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shown in Table 2. As shown in Table 2, absolute growth in this study was calculated based solely on length measurements, defined as the difference between final and initial length.The cyclic treatment (P1) produced the highest increase in length, whereas continuous exposure (P2) showed the lowest growth. The control group displayed an intermediate response. The superscript letters (a, b) indicate statistical differences among treatments, where different letters show significant differences, and values sharing at least one letter are not significantly different. The final body length of *R. lateristriata* after 2 weeks of treatment is shown in Figure 1 to illustrate growth differences among treatment groups.

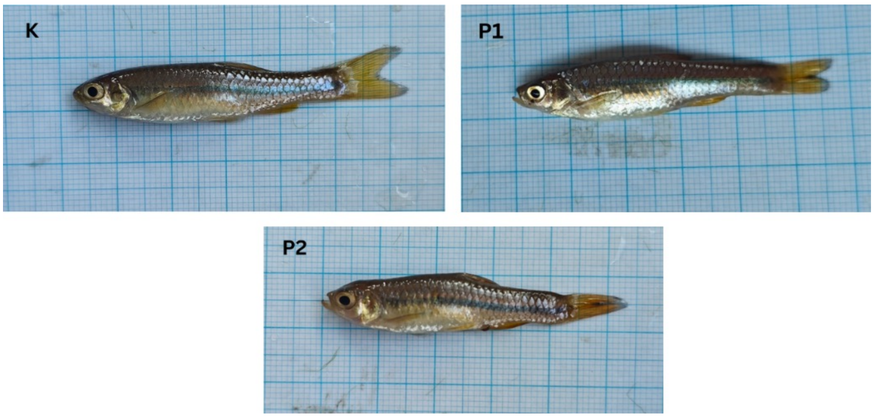

Figure 1. Final body length of 6-month-old R. lateristriata after 2 weeks of treatment. K: Control group with 100% commercial feed; P1: *R. lateristriata* fed with *Eurycoma longifolia* in a cyclic (interval) pattern; P2: *R. lateristriata* fed with *Eurycoma longifolia* continuously

Liver histology was examined to evaluate structural and cellular alterations induced by different administration patterns of Eurycoma longifolia. Representative histological features of liver tissue are shown in Figures 2 and 3.

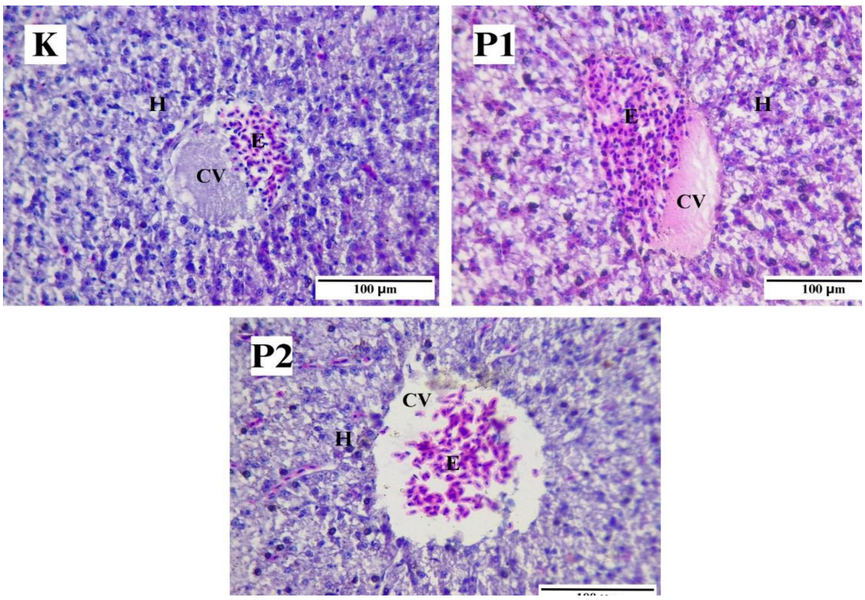

Figure 2. Histological structure of the liver in *R. lateristriata*. K: Control group fed with 100% commercial feed; P1: Treatment with *Eurycoma longifolia* in a cyclic pattern; P2: Treatment with *Eurycoma longifolia* in a continuous pattern. Description: E = Eritocytes, H = Hepatocytes, CV = Central vein. Magnification 400x, stained with Hematoxylin and Eosin (HE)

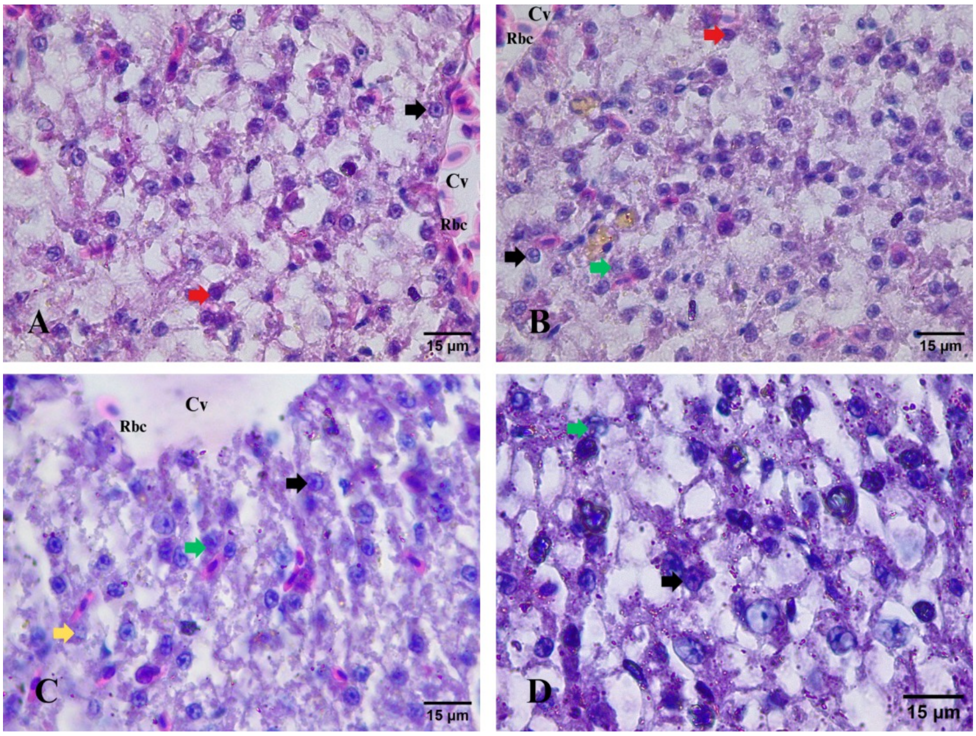

Figure 3. Histological examination of liver tissue from *R. lateristriata* fed repellet containing *Eurycoma longifolia* product at a dose of 460 ppm was performed using standard liver-tissue preparation and microscopic examination. A: Control (100% commercial feed); B: Cyclic feeding method (P1); C: Continuous feeding method (P2); D: Continuous feeding method (P2) with different pixel resolution. Scale bar A–D: 15 µm. Abbreviations: Cv = Central vein, Rbc = Red blood cell. Arrow indicators: black = Hepatocyte, red = Pyknosis, yellow=Karyorrhexis, green=Karyolysis. Magnification 1000x, stained with Hematoxylin and Eosin (HE).

A semi-quantitative scoring system was applied to assess hepatocyte nuclear damage based on pyknosis, karyorrhexis, and karyolysis. The mean damage scores for each treatment group are presented in Table 3. Microscopic cellular scoring (Table 3) revealed that continuous administration (P2) significantly increased nuclear damage (pyknosis, karyorrhexis, and karyolysis), while cyclic dosing (P1) showed moderate effects. Mean values followed by different superscript letters (a, b, c) in the same column differ significantly based on the post-hoc test at $p < 0.05$. Values sharing the same letter do not differ significantly.

Table 3. Mean score of hepatocyte nuclear damage

Treatment	Pyknosis	Karyorrhexis	Karyolisis
Control	$3.33^{a} \pm 1.53$	$3.00^{a} \pm 1.00$	$2.00^{a} \pm 1.00$
P1	$7.00^{ab} \pm 1.00$	$7.00^{a} \pm 3.00$	$7.33^{ab} \pm 1.53$
P2	$10.00^{b} \pm 4.58$	$13.33^{b} \pm 2.52$	$13.00^{b} \pm 5.00$

The findings of this study demonstrate that administration of *Eurycoma longifolia* extract significantly alters liver histology in *Rasbora lateristriata*, depending on the method of administration. Continuous exposure (P2) resulted in more pronounced hepatocellular damage compared to cyclic exposure (P1), which showed milder histological alterations, characterized by more preserved hepatic cords, fewer pyknotic and karyorrhectic nuclei, and clearer sinusoidal spaces. The features indicate a closer resemblance to the control group than to continuous (P2), which exhibited more severe lesions. These findings align with previous research in rodents, which showed that high doses of *E. longifolia* caused hepatocyte degeneration, including vacuolization and glycogen infiltration (Hamoud, 2014; Jannah, 2020).

The liver, being a major site for xenobiotic metabolism and detoxification, is highly susceptible to damage from accumulated metabolites (Chiang, 2014). In fish, although the liver lacks portal triads like in mammals, it performs similar metabolic functions (Tahang, 2018). The structural damage observed—pyknosis, karyorrhexis, and necrosis—suggests that *E. longifolia* may disrupt hepatocyte homeostasis through oxidative stress and apoptotic pathways (Yunos et al., 2023; Thu et al., 2017).

E. longifolia is known to contain several bioactive compounds, including quassinoids (eurycomanone) and β-carboline alkaloids, both of which have been reported to induce hepatocellular stress through oxidative mechanisms (Yang et al., 2021). Eurycomanone in particular has been shown to elevate reactive oxygen species (ROS) production and trigger mitochondrial dysfunction in liver cells, leading to apoptosis and necrotic cell death (Kuo et al., 2020; Rehman et al., 2016). The presence of these metabolites in the extract used in this study supports the observed increase in karyorrhexis and necrosis scores in the continuous-exposure group (P2), indicating that prolonged, uninterrupted accumulation of these compounds may have intensified oxidative injury and overwhelmed hepatocyte recovery capacity (Bhat et al., 2017; Kajahmohideen et al., 2021). Thus, the histological disruption in P2 is consistent with the known cytotoxic pathways associated with E. longifolia metabolites (Segaran et al., 2021; Lu et al., 2024).

Cyclic exposure (P1) provided a partial recovery interval (2 days OFF), facilitating detoxification and preventing extensive metabolite buildup. This mechanism is consistent with the rationale for herbal cycling, in which intermittent dosing is believed to prevent physiological tolerance and toxicity (Khan, 2021; Huberman, 2023). However, the presence of moderate damage in P1 suggests that the OFF phase may not have been sufficient to fully clear toxic compounds, especially at the tested dose.

The observation that the growth rate was highest in the P1 group further supports the potential benefits of regulated exposure. According to Zhang et al. (2021), *E. longifolia* administration in mice enhanced amino acid metabolism and reduced lipid accumulation in liver cells, which may also explain improved growth performance in fish (Chen et al., 2024; Tsai et al., 2020). However, the reduction in growth in P2 indicates that prolonged oxidative stress and liver damage may interfere with nutrient metabolism and protein synthesis, ultimately impairing somatic development.

Comparison of liver histological structure among treatments revealed notable differences in tissue integrity and cellular arrangement. The control group displayed normal hepatic architecture with well-organized hepatocyte cords, uniform nuclei, and minimal sinusoidal dilation (Palas et al., 2018). In P1, the liver structure remained relatively preserved, although mild alterations such as slight sinusoidal widening and occasional nuclear irregularities were observed, indicating an early adaptive response to extract exposure. In contrast, the P2 group showed the greatest degree of alteration, characterized by increased cytoplasmic vacuolation, less compact nuclear distribution, and more prominent sinusoidal dilation.

Moreover, the liver tissue in P2 showed a less dense nuclear distribution and empty spaces around the central vein, which may represent early signs of hepatocellular degeneration and sinusoidal dilation—features associated with toxicant exposure in fish (Sharma et al., 2020; Rohani, 2023). These findings emphasize the importance of dose, frequency, and duration in determining the safety of herbal feed additives in aquaculture. Although *E. longifolia* is widely recognized for its pharmacological benefits, including antioxidant and hepatoprotective properties at low doses (Silalahi, 2019), its bioactive compounds also exhibit dual behavior—therapeutic at moderate doses but toxic at high doses or with prolonged use (Rehman et al., 2016; Zakaria et al., 2009). The current study highlights this duality and suggests that even natural supplements should be administered with careful attention to timing and dosage.

CONCLUSION

Based on the results of this study, it can be concluded that the administration of *Eurycoma longifolia* extract affects the histological structure of the liver in *R. lateristriata*, as indicated by cellular damage including pyknosis, karyorrhexis, and karyolysis. Continuous administration resulted in more severe damage than cyclic administration, suggesting that the delivery method influences the degree of hepatotoxicity in fish liver tissue. For future research, further investigations are recommended to: (1) examine different concentration ranges to determine the threshold between safe and toxic levels; (2) analyze biochemical markers of liver function to complement histological findings; (3) evaluate long-term exposure effects, including recovery after treatment withdrawal; and (4) assess other organs or physiological systems potentially affected by *E. longifolia.* Integrating these approaches would provide a more comprehensive understanding of the safety and biological impacts of pasak bumi in aquatic organisms.

AUTHOR CONTRIBUTION

J.R.B who designed the research, collected data, analyzed, and wrote the manuscript. **B.R.** who supervised and wrote the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest.

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