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Research Article

Modulatory Effects of Fucoidan on Paracetamol-induced Hepatic Damage and Histological Alterations in *Rasbora lateristriata*

Fajar Sofyantoro, Maula Haqul Dafa, Alfian Primahesa, Shintia Oktaviana, Olvita Mayani, Eben Ezer Simanungkalit, Anthera Al Firdaus Prissandi, Ardaning Nuriliani, Bambang Retnoaji, Hendry T. S. S. G. Saragih, Zuliyati Rohmah, Slamet Widiyanto, Nastiti Wijayanti, Nur Indah Septriani*
Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Email: nurindahseptriani@ugm.ac.id

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ABSTRACT
Paracetamol overdose is a well-documented cause of hepatic injury across vertebrate species, including teleost fish. This study aimed to evaluate the hepatoprotective potential of fucoidan, a sulfated polysaccharide derived from brown algae, against paracetamol-induced liver damage in Rasbora lateristriata. Fish were divided into five experimental groups and exposed to paracetamol (3 mg/L), either alone or in combination with fucoidan at concentrations of 50, 100, or 300 µg/mL, for seven days. Histopathological evaluation of liver tissues was performed using hematoxylin–eosin staining, with semi-quantitative scoring focused on hydropic degeneration, nuclear pyknosis, and necrosis. The results demonstrated that paracetamol exposure induced moderate hepatocellular injury, characterized by cytoplasmic vacuolization, apoptotic nuclear alterations, and necrotic lesions. Co-treatment with fucoidan at 300 µg/mL was associated with reduced severity across all histopathological parameters, indicating partial hepatoprotective effects. In contrast, the 50 µg/mL fucoidan group exhibited paradoxically severe hydropic degeneration despite the absence of pyknosis and necrosis, suggesting a delayed or altered injury profile. Intermediate outcomes were observed at 100 µg/mL. Overall, fucoidan exhibited dose-dependent but inconsistent hepatoprotective effects. The observed histological variability across concentrations suggests that protection may be influenced by factors such as bioavailability, cellular uptake, or interactions with intracellular stress pathways. These findings highlight the need for further mechanistic investigations before fucoidan can be considered a reliable hepatoprotective agent in aquatic toxicology.

Keywords: *Antioxidants; Apoptosis; Fucoidan; Hepatotoxicity; Paracetamol.*

Introduction

Paracetamol is a widely used antipyretic and analgesic agent that is generally considered safe when administered at therapeutic doses [1], [2]. However, overdose or prolonged use of paracetamol is associated with severe hepatotoxicity and represents one of the leading causes of drug-induced liver injury worldwide [3]. Although the formation of reactive metabolites, such as N-acetyl-p-benzoquinone imine (NAPQI), and the subsequent induction of oxidative stress have been extensively characterized in

mammalian systems [4–8], these mechanisms have been far less explored in teleost fish, in which species-specific metabolic pathways may result in differential sensitivity to paracetamol-induced toxicity.

Fish models have emerged as valuable alternatives for toxicological evaluation due to their high sensitivity to waterborne toxicants, ease of maintenance, and physiological relevance to aquatic environments [1], [9], [10]. Among freshwater teleosts, *Rasbora lateristriata* has gained increasing recognition as a promising model organism in aquatic toxicology [11], [12]. Compared with other small fish species, *R. lateristriata* offers several advantages, including rapid embryonic development, well-defined histopathological endpoints, and established protocols for assessing hepatic injury and recovery [11–13]. Previous toxicological studies have demonstrated its pronounced physiological sensitivity to a wide range of chemical exposures, including food additives, analgesics, and environmental contaminants, thereby supporting its suitability for investigating hepatotoxic mechanisms and evaluating potential protective agents [4], [13].

Recent advances in hepatoprotection research have highlighted the therapeutic potential of marine-derived polysaccharides [14], [15]. Fucoidan, a fucose-rich sulfated polysaccharide extracted from brown algae such as Fucus vesiculosus and Undaria pinnatifida, has been reported to exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, anticoagulant, immunomodulatory, and hepatoprotective effects [16], [17]. Administration of fucoidan has been associated with reductions in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, preservation of hepatocellular integrity, and attenuation of oxidative stress–induced damage [18], [19]. The hepatoprotective mechanisms of fucoidan are proposed to involve suppression of reactive oxygen species (ROS) generation, stabilization of mitochondrial membranes, and enhancement of endogenous antioxidant enzyme activities [20]. Importantly, studies using zebrafish models have demonstrated that fucoidan administration can reduce hepatic vacuolation, enhance antioxidant enzyme activities, and attenuate apoptosis following exposure to paracetamol and other hepatotoxic agents [21–23]. These findings suggest that fucoidan exerts protective effects in aquatic vertebrates; however, comparative investigations in non-zebrafish species remain limited.

Although the hepatoprotective effects of fucoidan have been well documented in mammalian systems, its applicability in other fish models, such as *R. lateristriata*, has not been systematically evaluated. Given the growing concern over environmental pollutants and pharmaceutical residues in freshwater ecosystems, the use of fucoidan as a protective agent in fish represents a novel and ecologically relevant research direction. The freshwater habitat of *R. lateristriata* and its physiological sensitivity to hepatotoxins make this species a suitable model for assessing the effects of waterborne paracetamol exposure and the potential of fucoidan in mitigating hepatic injury. Accordingly, the present study was designed to evaluate the ameliorative effects of fucoidan on hepatic histological alterations induced by high-dose paracetamol exposure in *R. lateristriata*. Histopathological indicators, including hydropic degeneration, cellular pyknosis, and necrosis, were examined to assess the extent of hepatic damage. In addition, this study aimed to identify the fucoidan concentration that provides optimal hepatoprotection. Through this approach, the study contributes to a deeper understanding of natural compound–based hepatoprotection in aquatic models and supports the potential application of fucoidan in mitigating pharmaceutical-induced hepatic dysfunction.

Materials and Methods

Experimental Design and Animal Maintenance

This study employed *R. lateristriata* as an experimental model to evaluate the protective effects of fucoidan against paracetamol-induced hepatotoxicity. Twenty-five healthy fish were randomly assigned to five experimental groups (n = 5 per group): (1) control (water only), (2) paracetamol (3 mg/L), (3) paracetamol + fucoidan (50 µg/mL), (4) paracetamol + fucoidan (100 µg/mL), and (5) paracetamol + fucoidan (300 µg/mL). All fish were maintained in 5-L aquaria under continuous aeration at a controlled temperature of 25 ± 1°C. The fish were acclimatized to laboratory conditions and fed commercial pellets (Takari, Indonesia) twice daily.

All experimental procedures involving fish were conducted in accordance with established ethical guidelines and were approved by the Laboratory of Research and Integrated Testing (Laboratorium Penelitian dan Pengujian Terpadu), Universitas Gadjah Mada, Indonesia (Ethical Clearance Certificate No. 00034/04/LPPT/2023).

Preparation of Treatment Solutions

Paracetamol (Errita Pharma, Indonesia) was administered at a concentration of 3 mg/L by dissolving a 500 mg tablet in dechlorinated water [24], [25]. Fucoidan (Pharos Indonesia, Indonesia) was administered at three concentrations (50, 100, and 300 µg/mL) [21], [25]. The respective treatment solutions were prepared by dissolving fucoidan tablets in dechlorinated water. All treatment solutions were freshly prepared and renewed daily. Fish were exposed to the treatment conditions for 96 h. It should be noted that the fucoidan preparation (Pharos Indonesia, Indonesia) was supplied without detailed information regarding molecular weight or purity, which may influence its bioactivity and should be considered when interpreting the results.

Tissue Sampling and Histological Procedures

At the end of the treatment period, fish were euthanized and dissected for liver collection. The excised livers were fixed in 10% neutral buffered formalin (NBF) for 24 h, then rinsed and stored in 70% ethanol for 48 h. Tissue dehydration was performed using a graded ethanol series, followed by clearing in toluene for 12 h. The samples were subsequently infiltrated through a series of toluene–paraffin mixtures (3:1, 1:1, and 1:3), followed by two successive immersions in pure paraffin. Paraffin-embedded tissues were sectioned at a thickness of 4 µm using a rotary microtome. The sections were deparaffinized in toluene and stained with hematoxylin and eosin (H&E) [4], [26].

Histopathological Evaluation

Histological evaluation of liver and kidney tissues was conducted using a light microscope (Leica ICC50, Germany) at 400× magnification. Five randomly selected fields of view were analyzed per slide. The pathological features assessed included hydropic degeneration, pyknosis, and necrosis. A semi-quantitative scoring system was applied to evaluate the severity of tissue damage as follows: score 0 (no damage), score 1 (mild/focal damage affecting 11–30% of the tissue), score 2 (moderate/multifocal damage affecting 31–60%), and score 3 (severe/diffuse damage affecting 61–100%) [25], [26].

Statistical Analysis

All quantitative data obtained from histopathological scoring were expressed as the mean ± standard deviation (SD). Statistical comparisons among treatment groups were performed using one-way analysis of variance (ANOVA) to evaluate the effects of treatment. When significant differences were detected ($P < 0.05$), Duncan's multiple range test was applied as a post hoc analysis to identify specific group differences. All statistical analyses were conducted using IBM SPSS Statistics,

and a significance level of $P < 0.05$ was adopted for all tests.

Results and Discussion

To evaluate the hepatoprotective potential of fucoidan against paracetamol-induced liver injury, histological analyses were performed on liver tissues of R. lateristriata subjected to different treatment regimens. The evaluation focused on key cellular alterations, including hydropic degeneration, pyknosis, and necrosis, which represent distinct pathological manifestations of hepatic damage. A semi-quantitative scoring system was employed to assess the severity of tissue alterations, allowing for comparative analysis among the treatment groups. The results are presented below, highlighting the extent of histological protection and recovery conferred by fucoidan at different concentrations.

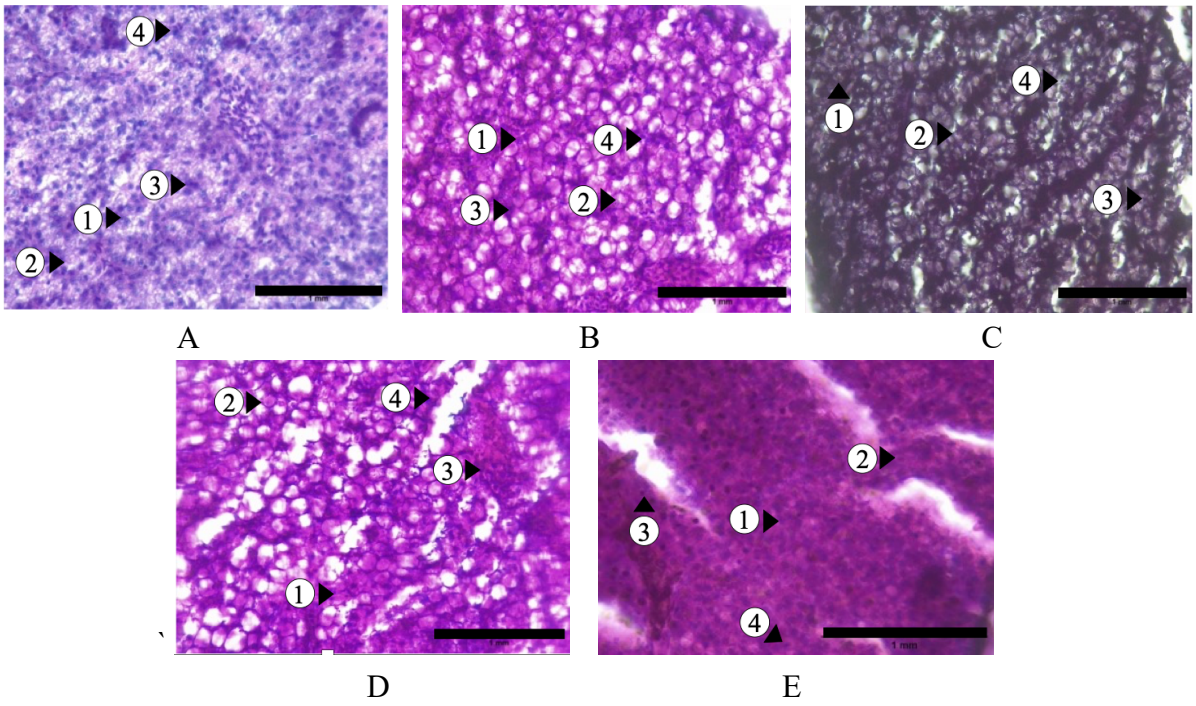

Figure 1. Representative histological images of liver tissue from *Rasbora lateristriata* following exposure to paracetamol (3 mg/L) and co-treatment with fucoidan at various concentrations; (A) Control (Water); (B) Paracetamol; (C) Paracetamol + Fucoidan 50 µg/mL; (D) Paracetamol + Fucoidan 100 µg/mL; and (E) Paracetamol + Fucoidan 300 µg/mL. Histopathological alterations are labeled as follows: (1) Normal hepatocytes; (2) Hydropic degeneration; (3) Nuclear pyknosis; and (4) Necrosis. Liver tissues were stained with Hematoxylin and Eosin (HE). Scale bar: 1 mm; Magnification: 400X.

Histological Features of Hepatocellular Alterations Following Paracetamol and Fucoidan Exposure

Histological examination revealed distinct patterns of hepatic tissue alterations in *R. lateristriata* subjected to paracetamol and fucoidan treatments (Figure 1). The control group (Figure 1A) exhibited normal hepatic architecture, characterized by well-organized hepatocyte cords, clearly defined sinusoidal spaces, and uniform nuclei, indicating physiological homeostasis. In contrast, liver sections from the paracetamol-only group (Figure 1B) showed marked morphological disruption. Prominent pathological features included hydropic degeneration, as evidenced by cytoplasmic vacuolization; nuclear pyknosis, reflecting apoptotic progression; and extensive necrosis, indicated by loss of cellular boundaries and increased eosinophilia of the cytoplasm. These

histopathological alterations are consistent with previous reports in Rhamdia quelen and Danio rerio, in which paracetamol exposure induced sinusoidal dilatation, cytoplasmic vacuolization, and nuclear abnormalities associated with oxidative stress mediated hepatocellular degeneration [27], [28].

Co-treatment with fucoidan produced concentration-dependent effects on hepatic histology (Figure 1C–E). At 50 µg/mL (Figure 1C), histological damage remained evident, characterized by extensive areas of degeneration and limited restoration of cellular integrity. Although pyknosis and necrosis were not detected in this group based on semi-quantitative scoring, cytoplasmic vacuolation and hepatocellular disorganization were still apparent, suggesting that fucoidan at this concentration did not provide sufficient structural protection. At 100 µg/mL (Figure 1D), partial histological improvement was observed, with several hepatocytes exhibiting more preserved nuclear morphology and reduced cytoplasmic swelling, indicative of attenuation of cellular stress. However, moderate degeneration persisted, and tissue integrity remained compromised in certain regions. The 300 µg/mL group (Figure 1E) displayed comparatively better-preserved hepatic architecture, with fewer degenerative features overall. Nevertheless, variability in histological appearance among samples suggests that protection at this concentration was incomplete or inconsistent.

Similar variability in the response to fucoidan has been reported in zebrafish models, in which post-exposure treatment with fucoidan at concentrations of 100–500 µg/mL reduced hepatocellular vacuolization and improved cellular alignment, albeit not consistently across all individuals [25]. Likewise, rodent studies employing high-dose antioxidant interventions, such as Chlorella vulgaris or coenzyme Q10, have demonstrated partial restoration of hepatic histoarchitecture, supporting the notion that antioxidant-mediated hepatoprotection is highly dose- and context-dependent [29], [30].

These findings do not fully support a strictly dose-dependent hepatoprotective effect of fucoidan. Although higher concentrations were associated with milder histopathological alterations, the protective effects were not uniform across samples. Moreover, the apparent absence of pyknosis and necrosis at lower fucoidan concentrations may reflect altered injury dynamics rather than complete tissue recovery. This variability underscores the complex interplay between fucoidan dosage, cellular stress responses, and tissue-level outcomes. Overall, while fucoidan appears to modulate the severity of paracetamol-induced liver injury, the histological evidence supports a more nuanced interpretation of its hepatoprotective role. The extent of hepatic restoration varied among treatment concentrations, and further studies are warranted to determine whether these effects represent true cytoprotection, delayed progression of injury, or modulation of cell death pathways.

Hydropic Degeneration and Hepatocellular Response to Paracetamol and Fucoidan

Hydropic degeneration scores among the five experimental groups reflected varying degrees of hepatocellular swelling in response to paracetamol exposure and fucoidan co-treatment (Figure 2). The control group exhibited a low mean score, indicating largely preserved hepatic architecture under physiological conditions. A slight elevation in the paracetamol-only group suggested the presence of early, sublethal cellular stress, possibly arising from the formation of reactive metabolites and mild disruption of osmotic regulation. Unexpectedly, the highest mean degeneration score was observed in the group co-treated with paracetamol and 50 µg/mL fucoidan, indicating severe and diffuse hydropic

changes. This finding contradicts the anticipated hepatoprotective effect and suggests a concentration-specific interaction, whereby fucoidan at this dose may have failed to provide sufficient antioxidant buffering or may have adversely modulated intracellular stress pathways. Such a paradoxical increase in hydropic degeneration may reflect a hormetic response, in which low doses of certain bioactive compounds elicit adaptive stress signaling or mild pro-oxidant effects rather than cytoprotection [31], [32]. Alternatively, suboptimal fucoidan concentrations may transiently exacerbate osmotic imbalance or mitochondrial dysfunction before antioxidant defenses are fully activated. Delayed injury progression should also be considered, as the absence of nuclear damage in this group may indicate an early stage of hepatocellular stress preceding apoptosis or necrosis. A moderate reduction in hydropic degeneration was observed at 100 µg/mL fucoidan, although histopathological alterations remained substantial. At 300 µg/mL, degeneration scores decreased and were statistically comparable to those of the control group, suggesting partial restoration of osmotic and mitochondrial homeostasis. Nevertheless, inter-individual variability at this concentration indicates that the hepatoprotective effect was not uniformly expressed across all samples.

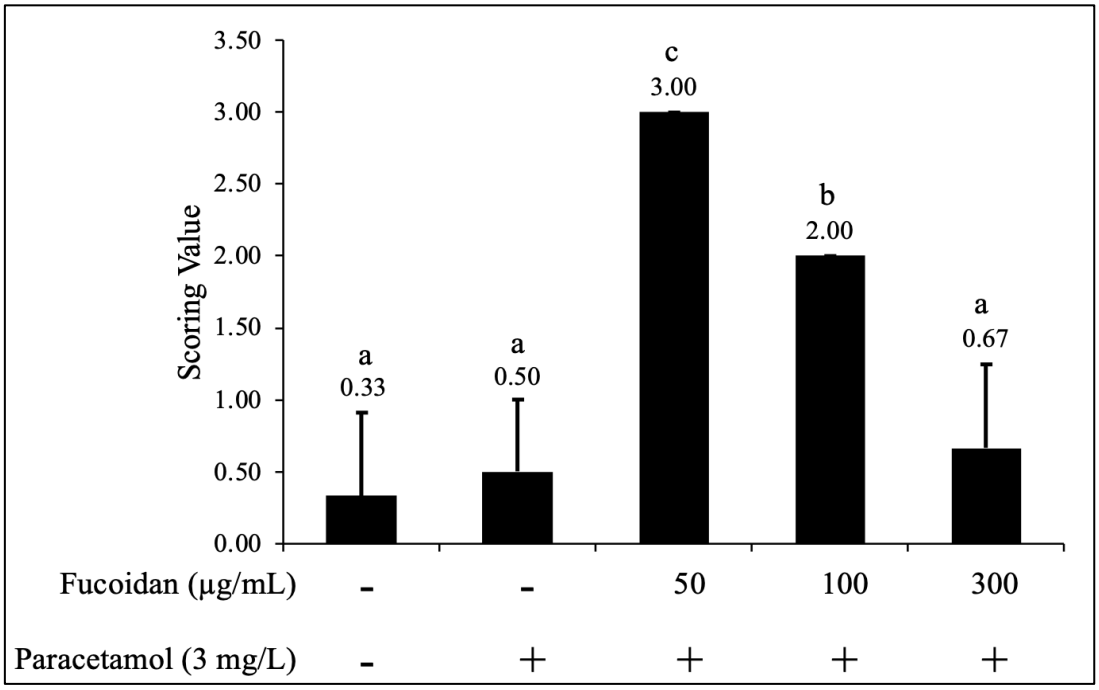

Figure 2. Hydropic degeneration in hepatocytes of *Rasbora lateristriata* following exposure to high-dose paracetamol and co-treatment with varying concentrations of fucoidan. Data represent mean ± standard deviation (n=3). Scoring was based on the extent of histopathological changes: 0= no damage (0–10%), 1= mild/focal (11–30%), 2= moderate/multifocal (31–60%), and 3= severe/diffuse (61–100%). Different letters above the bars indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA followed by post-hoc test).

From a physiological standpoint, hydropic degeneration represents a reversible form of cellular injury characterized by cytoplasmic vacuolation resulting from impaired Na^+/K^+-ATPase activity, which is commonly triggered by oxidative stress and ATP depletion [33]. In the context of paracetamol-induced hepatotoxicity, the formation of N-acetyl-p-benzoquinone imine (NAPQI) leads to glutathione depletion, mitochondrial dysfunction, and subsequent water influx

into hepatocytes [33]. Accordingly, the presence or absence of hydropic degeneration serves as an indicator of the liver's capacity to maintain ionic homeostasis under conditions of metabolic stress. Comparable patterns of paracetamol-induced vacuolar degeneration have been reported in Rhamdia quelen, where histopathological severity correlated with glutathione depletion and mitochondrial injury [27]. Similarly, studies in zebrafish have shown that fucoidan at a concentration of 500 µg/mL effectively reversed paracetamol-induced vacuolation and improved histological scores, whereas responses at lower concentrations were inconsistent [25]. Collectively, these findings suggest that the modulatory effects of fucoidan on hydropic degeneration are concentration dependent and may require threshold levels to effectively restore ionic gradients and stabilize cellular membranes.

The present findings indicate that fucoidan, at lower concentrations, does not confer hepatoprotection and may even be associated with enhanced cellular injury. Although the highest concentration tested (300 µg/mL) was linked to reduced degeneration scores, the observed inter-individual variability limits definitive conclusions regarding its efficacy. These results underscore that the effects of fucoidan on liver physiology are dose dependent and may be further influenced by factors such as bioavailability, metabolic conversion, and localized tissue responses. Further investigations are warranted to elucidate the mechanisms by which fucoidan at different concentrations modulates hepatocellular stress pathways and to determine whether its application in aquatic models can provide consistent therapeutic benefits.

Pyknosis Indicates Altered Apoptotic Activity in Response to Paracetamol and Fucoidan

Semi-quantitative scoring of pyknosis revealed variable degrees of nuclear condensation across the treatment groups (Figure 3). The paracetamol-only group exhibited the highest mean pyknosis score, indicating extensive apoptotic activity following toxic exposure. In contrast, the control group showed a low but non-zero pyknosis score, which may reflect normal physiological cell turnover or baseline apoptotic processes. Notably, no evidence of pyknosis was detected in the group co-treated with paracetamol and fucoidan at 50 µg/mL. Groups receiving fucoidan at 100 µg/mL and 300 µg/mL exhibited moderate reductions in pyknosis scores, suggesting a dose-related attenuation of nuclear condensation.

Pyknosis, characterized by chromatin condensation and nuclear shrinkage, is a hallmark of apoptosis—a programmed cell death pathway frequently activated in hepatocytes under conditions of oxidative or mitochondrial stress [34]. In paracetamol-induced hepatotoxicity, excessive formation of N-acetyl-p-benzoquinone imine (NAPQI) disrupts mitochondrial membrane integrity, leading to cytochrome c release and subsequent caspase activation, which ultimately drives nuclear fragmentation and pyknosis [34].

The present findings are consistent with previous studies reporting apoptosis-associated nuclear condensation in zebrafish and mammalian hepatocytes exposed to paracetamol [28]. Gene expression analyses have demonstrated upregulation of key apoptotic markers, including BAX, CASP3, and CASP8, in zebrafish livers following paracetamol challenge, which coincided with the presence of pyknosis and histological deterioration [28]. Conversely, attenuation of nuclear damage following antioxidant-based interventions, including fucoidan, artichoke leaf extract, and coenzyme Q10, has been reported in both aquatic and rodent models [30], [35].

The reduction in pyknosis observed in fucoidan-treated groups may reflect the compound's antioxidant and mitochondrial-stabilizing properties, which can attenuate apoptotic cascades. However,

these findings also raise important physiological considerations. The complete absence of pyknosis in the 50 µg/mL fucoidan group, together with the previously observed high hydropic degeneration scores at this concentration, may indicate that hepatocytes were experiencing non-apoptotic or pre-apoptotic stress rather than true cellular recovery. In contrast, the moderate levels of pyknosis observed at 100 and 300 µg/mL may represent a regulated apoptotic response associated with tissue remodeling and detoxification processes. Thus, although fucoidan appears to modulate apoptotic progression, its effects are nuanced and likely dose- and context-dependent. Complete suppression of apoptosis is not inherently beneficial, particularly if it interferes with normal cellular turnover or predisposes tissues to necrotic injury. Collectively, these results suggest that the modulatory effects of fucoidan on apoptotic pathways are complex and warrant further mechanistic investigation to clarify their physiological relevance.

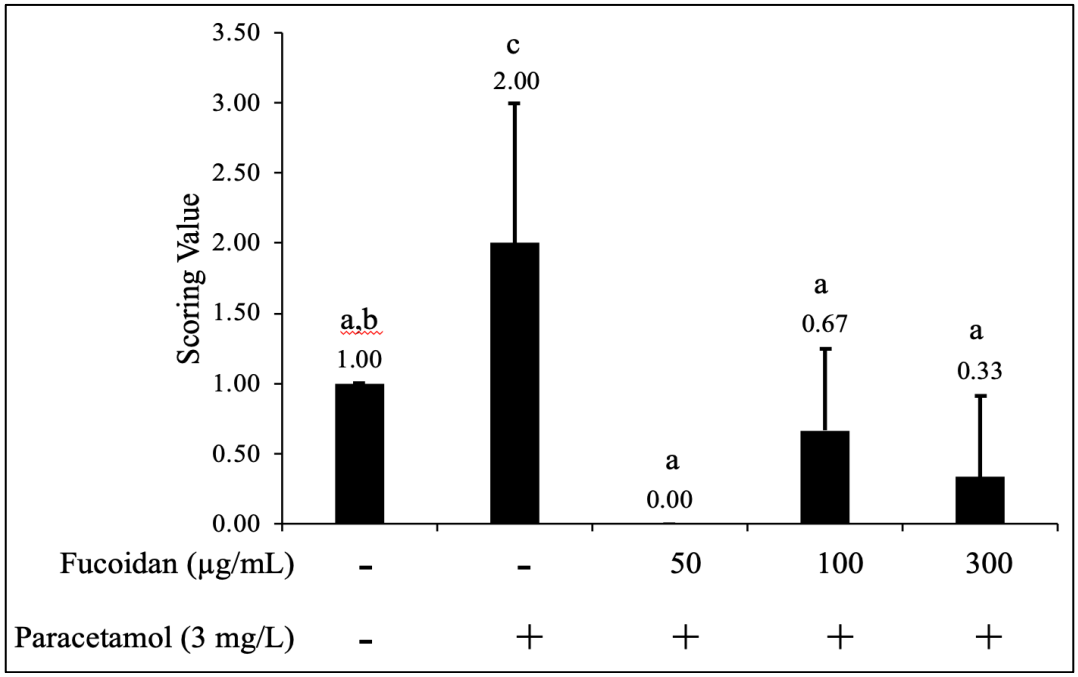

Figure 3. Hepatocellular pyknosis in *Rasbora lateristriata* following exposure to high-dose paracetamol and fucoidan treatment. Data are expressed as mean ± standard deviation (n= 3). Pyknosis was scored based on a semi-quantitative system: score 0 (no damage, 0–10% affected), score 1 (mild/focal, 11–30%), score 2 (moderate/multifocal, 31–60%), and score 3 (severe/diffuse, 61–100%). Different letters above the bars indicate statistically significant differences between treatment groups ($p<0.05$, one-way ANOVA followed by post-hoc test).

Necrosis as an Indicator of Irreversible Hepatic Injury in Response to Paracetamol and Fucoidan

Semi-quantitative scoring of hepatocellular necrosis revealed moderate tissue damage in the paracetamol-only group, indicating that exposure to 3 mg/L paracetamol induced consistent and irreversible hepatic injury (Figure 4). Interestingly, the control group also exhibited mild spontaneous necrotic activity, which may reflect baseline physiological cell turnover or low-level environmental micro-stressors inherent to aquatic experimental systems. An apparent absence of necrosis was observed in the groups co-treated with fucoidan at 50 and 100 µg/mL. At first glance, this finding suggests a pronounced protective effect. However, when interpreted alongside the

severe hydropic degeneration previously observed at 50 µg/mL, the lack of necrosis does not necessarily indicate effective tissue recovery. Rather, it may reflect an early or alternative injury trajectory in which hepatocytes remain in a reversible stress state or have not yet progressed to irreversible cell death.

The group treated with 300 µg/mL fucoidan exhibited a low necrosis score, suggesting partial hepatoprotection; however, inter-individual variability indicates that the protective response was not uniform across samples. Necrosis is characterized by irreversible loss of plasma membrane integrity, cytoplasmic swelling, and eventual cell lysis [36]. It reflects the failure of mitochondrial energy production, often triggered by overwhelming oxidative stress and ATP depletion. In the context of paracetamol hepatotoxicity, the toxic metabolite NAPQI compromises mitochondrial membranes and initiates the mitochondrial permeability transition (MPT), leading to hepatocellular necrosis [36]. Thus, necrosis represents not merely a structural endpoint but a physiological indicator of severe metabolic collapse. Previous studies in rodent models have demonstrated that antioxidants such as coenzyme Q10 and plant-derived extracts, including Artocarpus altilis, can significantly reduce necrosis scores and restore hepatocyte integrity following paracetamol exposure [30], [37]. Comparable trends have also been reported in aquatic models, where high-dose fucoidan (≥300 µg/mL) improved hepatocellular integrity and reduced necrotic lesions in zebrafish, although histological variability among individuals remained evident [25].

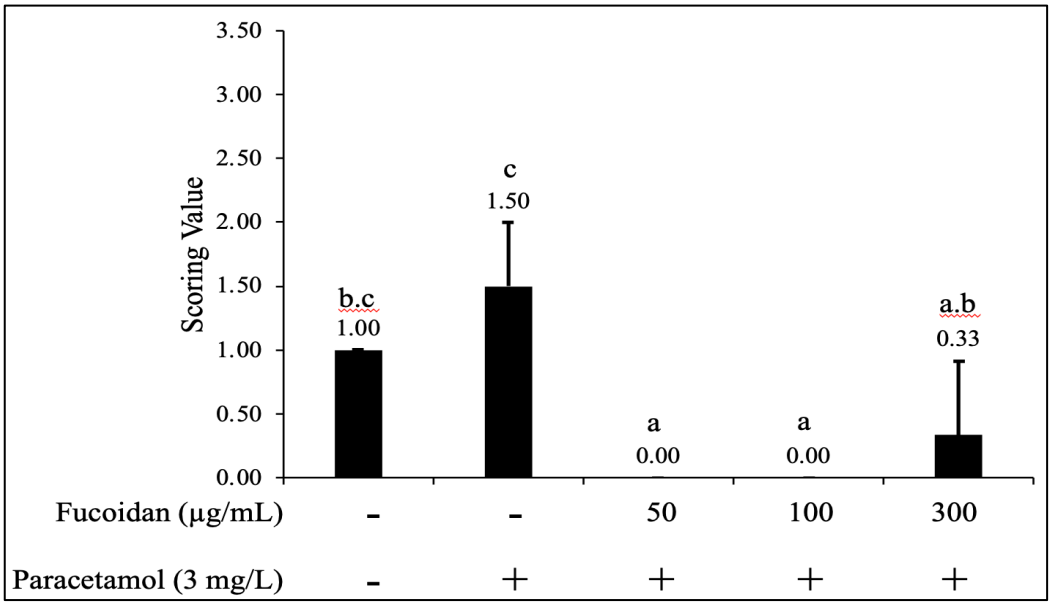

Figure 4. Hepatocellular necrosis in *Rasbora lateristriata* following exposure to high-dose paracetamol and fucoidan treatment. Data are presented as mean ± standard deviation (n= 3). Necrosis was assessed using a semi-quantitative scoring system: score 0= no damage (0–10% of cells affected), score 1= mild/focal damage (11–30%), score 2= moderate/multifocal (31–60%), and score 3= severe/diffuse (61–100%). Different letters above bars indicate statistically significant differences among treatment groups ($p<0.05$, one-way ANOVA followed by post-hoc test).

The observed reduction or absence of necrosis in fucoidan-treated groups may be attributed to the polysaccharide's proposed antioxidant and membrane-stabilizing properties, which help preserve mitochondrial function and prevent catastrophic cellular energy failure. However, as indicated by the hydropic

degeneration and pyknosis analyses, this protective pattern is not consistent across all doses, and its interpretation must be considered within the broader histological context.

The presence of necrosis in the control group further underscores the need for cautious interpretation of this endpoint. Low-level necrosis may occur naturally in fish because of hepatocyte turnover, minor fluctuations in water quality, or routine immune responses. This observation highlights a limitation of relying on necrosis alone as a definitive indicator of toxic insult in the absence of complementary biochemical or molecular evidence. Assessment of serum liver enzyme activities, oxidative stress markers, and gene expression profiles such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and nuclear factor erythroid 2–related factor 2 (Nrf2) would provide a more comprehensive understanding of the mechanisms underlying necrotic modulation [28].

Histopathological evaluation of *R. lateristriata* liver tissue demonstrated that exposure to high-dose paracetamol induced characteristic cellular damage, including hydropic degeneration, pyknosis, and necrosis, which represent distinct stages of hepatocellular stress and injury. Co-treatment with fucoidan resulted in variable histological outcomes, with the highest concentration tested (300 µg/mL) generally associated with lower damage scores across all assessed parameters. Nevertheless, these protective effects were not consistently observed across concentrations or types of histological damage. Notably, severe hydropic degeneration was paradoxically most pronounced at 50 µg/mL fucoidan, and the complete absence of pyknosis and necrosis at this concentration was not accompanied by improved cellular morphology, suggesting altered injury progression rather than true recovery. Although reductions in pyknosis and necrosis at 100 and 300 µg/mL indicate potential modulation of apoptotic and necrotic pathways, these effects were inconsistent and frequently accompanied by substantial inter-individual variability.

These inconsistencies may reflect differences in bioavailability, uptake efficiency, or metabolic responses to fucoidan, which have also been reported in other aquatic models [25]. In addition, evidence from rodent studies indicates that the hepatoprotective effects of natural antioxidants, including fucoidan, are influenced by multiple factors, such as dose, duration of exposure, and the baseline oxidative status of the liver [29], [30]. The occurrence of baseline necrosis in the control group further emphasizes the need for cautious interpretation of pathological endpoints in aquatic models, in which spontaneous lesions may arise from normal hepatic turnover or environmental fluctuations [27]. Taken together, these findings suggest that fucoidan can modulate hepatic injury outcomes following paracetamol exposure; however, its efficacy appears to be concentration dependent, context specific, and influenced by additional physiological variables.

Several limitations of this study should be acknowledged. First, the small sample size used for histopathological scoring (n = 3 per group) limits statistical power and the generalizability of the findings. Second, the absence of a fucoidan-only control group restricts the ability to distinguish the intrinsic effects of fucoidan from its interaction with paracetamol. Third, biochemical assessments of liver function and oxidative stress, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione (GSH), and reactive oxygen species (ROS), were not performed to support the histological observations. In addition, the lack of molecular validation through gene expression analyses constrains mechanistic interpretation of apoptotic and antioxidant pathways. Finally, fucoidan bioaccumulation in hepatic tissues was not evaluated, leaving its absorption and tissue distribution

uncharacterized. Addressing these limitations in future studies will be essential to strengthen and extend the current observations.

Conclusion

This study demonstrated that paracetamol exposure induces characteristic hepatocellular damage in *R. lateristriata*, including hydropic degeneration, pyknosis, and necrosis, reflecting underlying oxidative stress and mitochondrial dysfunction. Co-treatment with fucoidan modulated these outcomes in a dose-dependent but inconsistent manner. Although the highest concentration tested (300 µg/mL) was associated with reduced histopathological damage, severe hydropic degeneration was unexpectedly observed at the lowest concentration (50 µg/mL), despite the absence of nuclear and necrotic alterations. This pattern suggests that low-dose fucoidan may exert hormetic or biphasic effects, whereby suboptimal concentrations transiently exacerbate cellular stress rather than confer protection. Collectively, these findings indicate that the hepatoprotective properties of fucoidan are governed by complex physiological interactions and cannot be generalized across all concentrations. The observed variability underscores the need for further investigation into the pharmacodynamics and molecular mechanisms underlying fucoidan's actions, particularly in aquatic models. Until such evidence is available, the application of fucoidan as a consistent hepatoprotective agent should be approached with caution.

Conflict of interest

Authors declare no conflict of interest regarding the study.

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