Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.508

Research Article

Histopathological Effects of Mangosteen (*Garcinia mangostana* L.) Peel Decoction on Betta Fish (*Betta* sp.) Liver

Wiwin Ariesti[1], Siti Aeniah[1], Shuha Ma'muriyah Halim[1], Fajar Sofyantoro[1], Nastiti Wijayanti[1], Bambang Retnoaji[1], Ardaning Nuriliani[1], Hendry T.S.S.G. Saragih[1], Zuliyati Rohmah[1], Slamet Widiyanto[1], Nur Ainun Oktavia Pusparini[1], Desi Eka Putri Empra[1], Nur Indah Septriani[1]*

[1]*Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia*

**Corresponding author: nurindahseptriani@ugm.ac.id*

Abstract

Mangosteen (*Garcinia mangostana* L.) peel contains bioactive compounds known for their health benefits, yet potential toxicity at certain doses remains a concern. This study evaluates the histopathological effects of mangosteen peel decoction on the liver of Betta fish (*Betta* sp.), a sensitive model organism. Mangosteen peel decoction was prepared and administered to Betta fish at concentrations of 5, 25, and 50 ppm, with a control group receiving no treatment. Fish were observed for changes in swimming activity and appetite over five days. Liver tissues were collected, processed, and analyzed histologically to assess tissue damage including vacuolization, pyknosis, hemorrhage, and necrosis. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. Behavioral analysis indicated a dose-dependent reduction in swimming activity and appetite in treated groups. Histopathological examination revealed significant liver damage across all treatment groups, with higher concentrations of decoction correlating with increased hemorrhage, pyknosis, and necrosis. Vacuolization was highest in the control group and lowest in the 50-ppm group. The overall hepatic damage was categorized as moderate, with the control group showing the least damage. Mangosteen peel decoction induced significant hepatic damage in Betta fish, highlighting the cytotoxic effects at higher doses. The observed behavioral and histopathological changes underscore the need for careful consideration of decoction concentrations to avoid adverse effects in aquatic organisms. This study provides crucial insights into the toxicological impacts of mangosteen peel decoction on fish liver health, emphasizing the importance of dose regulation in practical applications. Further research is recommended to explore protective measures and alternative treatments to mitigate liver damage.

Keywords: Betta Fish, Hepatotoxicity, Mangosteen Peel, Necrosis, Pyknosis, Vacuolization

1. Introduction

Mangosteen (*Garcinia mangostana* L.) peel is renowned for its rich content of bioactive compounds, including xanthones, tannins, polyphenols, and flavonoids, which exhibit various health benefits such as antioxidant, anti-inflammatory, and antimicrobial activities (Ibrahim et al., 2016; Obolskiy et al., 2009; Saraswathy et al., 2022; Yuvanatemiya et al., 2022). While these compounds have been incorporated into numerous health products, it is crucial to assess their potential toxicity to ensure their long-term safety. The active compounds in mangosteen peel may elicit side effects at certain doses, necessitating thorough toxicity evaluations (Ibrahim et al., 2016; Saraswathy et al., 2022).

Toxicity testing is a fundamental scientific method employed to ascertain the potential hazards of compounds to living organisms (Aware et al., 2022; Capela et al., 2020; Krewski et al., 2020). In this study, we utilized Betta fish (*Betta* sp.) as an animal model to investigate the toxicity of mangosteen peel extract. Betta fish were chosen due to their high sensitivity and ease of acclimatization to laboratory conditions (Palmiotti et al., 2023; Yue et al., 2022; Zhang et al., 2022). Similar to zebrafish, which is widely used as a model organism for toxin studies (Sofyantoro et al., 2024), Betta fish offer comparable sensitivity, making them suitable for assessing the impacts of various compounds, including herbal extracts.

Betta fish (*Betta* sp.) are freshwater species native to several Southeast Asian countries, including Indonesia, Thailand, Malaysia, Brunei Darussalam, Singapore, and Vietnam (Yue et al., 2022). These fish are characterized by their distinctive morphology and aggressive territorial behavior (Lichak et al., 2022; Oliveira et al., 2022). Betta fish typically reach sexual maturity and are ready to reproduce between 4 to 12 months of age. Natural spawning occurs primarily during the dry season due to gonadal maturity levels, particularly in males (Lichak et al., 2022; Yue et al., 2022). Optimal spawning temperatures range from 26-30°C, with warmer temperatures promoting spawning activity. Additionally, spawning can result in female mortality due to male aggression if the males are not adequately prepared for reproduction (Lichak et al., 2022; Oliveira et al., 2022).

The liver is a critical organ involved in drug and toxin metabolism, making it highly susceptible to toxic compounds (Hosack et al., 2023; Michalopoulos & Bhushan, 2021). The liver metabolic processes often produce reactive metabolites that can inflict cellular damage (Leung et al., 2012; Simeonova et al., 2014), emphasizing the importance of assessing the effects of mangosteen peel extract on this organ. The objective of this study is to examine the impact of mangosteen peel decoction on the histological structure of the liver in Betta fish, focusing on the extent of damage and histopathological alterations. The findings from this study will provide valuable insights into the safety of long-term mangosteen peel extract usage.

2. Material and Method

Preparation of Mangosteen (*G. mangostana* L.) Peel Decoction

Mangosteen peel symplisia (10 g) was combined with 1000 mL of distilled water and heated to 90°C. After cooling, the decoction was transferred to a container and subsequently diluted to concentrations of 5, 25, and 50 ppm as administered to *Betta* sp.

Animal Acclimatization

Male *Betta* sp., approximately 4-5 months old and weighing approximately 1.51 g of Blitar origin, were acclimatized for 3 days in an aquarium. The fish were divided into four experimental groups: control, 5 ppm, 25 ppm, and 50 ppm. *Betta* sp. were exposed to a light/dark cycle of 12 hours each and were fed once daily. Behavioral observations, including swimming activity and appetite, were categorized into four levels: very active, active, less active, and inactive.

Histological Preparation of Liver

Betta sp. specimens were captured using a net, transferred to plastic containers, and then anesthetized through cold shock by placing them in a freezer at -20°C for 10-15 minutes. The heads and tails of *Betta* sp. were removed, and the remaining body parts were prepared for histological analysis. The hepatic tissues were excised, washed with physiological saline (0.9% NaCl), and fixed in neutral buffered formalin (NBF) for 24 hours. After fixation, the tissues were washed with 70% ethanol until the yellow discoloration was removed, with solution changes every 30 minutes. Dehydration was achieved using a graded series of alcohol solutions: 70, 80, 90, 96%, and absolute. Clearing was performed with toluene, followed by infiltration in paraffin at 65°C, embedding, trimming, and sectioning. Liver histological sections were prepared at a thickness of 5 µm and stained using the Harris Hematoxylin-Eosin method.

Histopathological Examination

Histopathological sections were examined using a Leica light microscope with a 40x10 magnification. Observations were made in four different fields of view per treatment group. The observed pathological changes included hemorrhage, pyknosis, necrosis, and vacuolization. Hepatocyte cell counts were conducted using Image Raster version 3.0. The extent of liver damage was assessed according to the criteria outlined in Table 1.

Table 1. Scoring values of liver damage for histopathological observations (Gibson-Corley, Olivier, & Meyerholz, 2013)

Level of Damage	Description	Score
Normal	normal, clear cell nuclei, round shape	0
Mild	hemorrhage+, pyknosis+, necrosis+, vacuolization+	1
Moderate	hemorrhage ++, pyknosis ++, necrosis ++, vacuolization++	2
Severe	hemorrhage +++, pyknosis +++, necrosis +++, vacuolization +++	3

Description:

"–" : Normal

"+" : Cell damage reaches 25% in five fields of view

"++" : Cell damage reaches 50% in five fields of view

"+++" : Cell damage reaches 75% in five fields of view

Data Analysis

Statistical analysis was performed using the Kruskal-Wallis's test. In cases where significant differences were identified ($p < 0.05$), post hoc analysis was conducted using the Mann-Whitney test to determine significant differences between treatment groups.

3. Results and Discussion

3.1. Results

Behavior of *Betta* sp.

A reduction in both swimming activity and appetite in Betta fish following treatment with mangosteen peel decoction was observed (Table 2). The behavior of Betta fish was noticeably altered following treatment with mangosteen peel decoction. In the control group, Betta fish maintained high swimming activity and appetite throughout the observation period. Conversely, Betta fish treated with mangosteen peel decoction exhibited a dose-dependent decline in both swimming activity and appetite.

Table 2. Swimming and feeding behavior of betta fish during treatment

Treatment	Swimming activity (day)					Appetite (day)				
	1	2	3	4	5	1	2	3	4	5
Control	active	very active	active	active	very active	high	high	high	high	high
5 ppm	active	normal	active	less active	less active	high	high	high	moderate	moderate
25 ppm	active	less active	less active	less active	less active	high	moderate	moderate	low	low
50 ppm	active	less active	less active	inactive	inactive	high	moderate	moderate	low	low

Specifically, swimming activity in the control group was consistently high, with fish remaining very active or active throughout the five-day observation period. In the 5-ppm group, the fish were active initially but became less active on days 4 and 5. In the 25-ppm group, there was a reduction in activity from day 2 onwards, with fish becoming progressively less active. The 50-ppm group exhibited decreased activity from day 2, with inactivity observed on days 4 and 5. Similarly, the appetite of Betta fish followed a declining trend with increasing concentrations of the decoction. The control group consistently displayed a high appetite. The 5-ppm group maintained a high appetite initially but showed a moderate reduction on days 4 and 5. The 25-ppm group had a high appetite on day 1, which declined to medium on days 2 and 3 and further reduced to low on days 4 and 5. The 50-ppm group exhibited a high appetite on day 1, which dropped to medium on days 2 and 3 and further to low on days 4 and 5.

Histopathological Analysis of *Betta* sp. Liver

Histological examination of Betta fish liver tissue revealed varying degrees of damage across all treatment groups, including the control, 5 ppm, 25 ppm, and 50 ppm. Notable damage observed included vacuolization, pyknosis, hemorrhage, and necrosis, as detailed in Table 3 and illustrated in Figure 1.

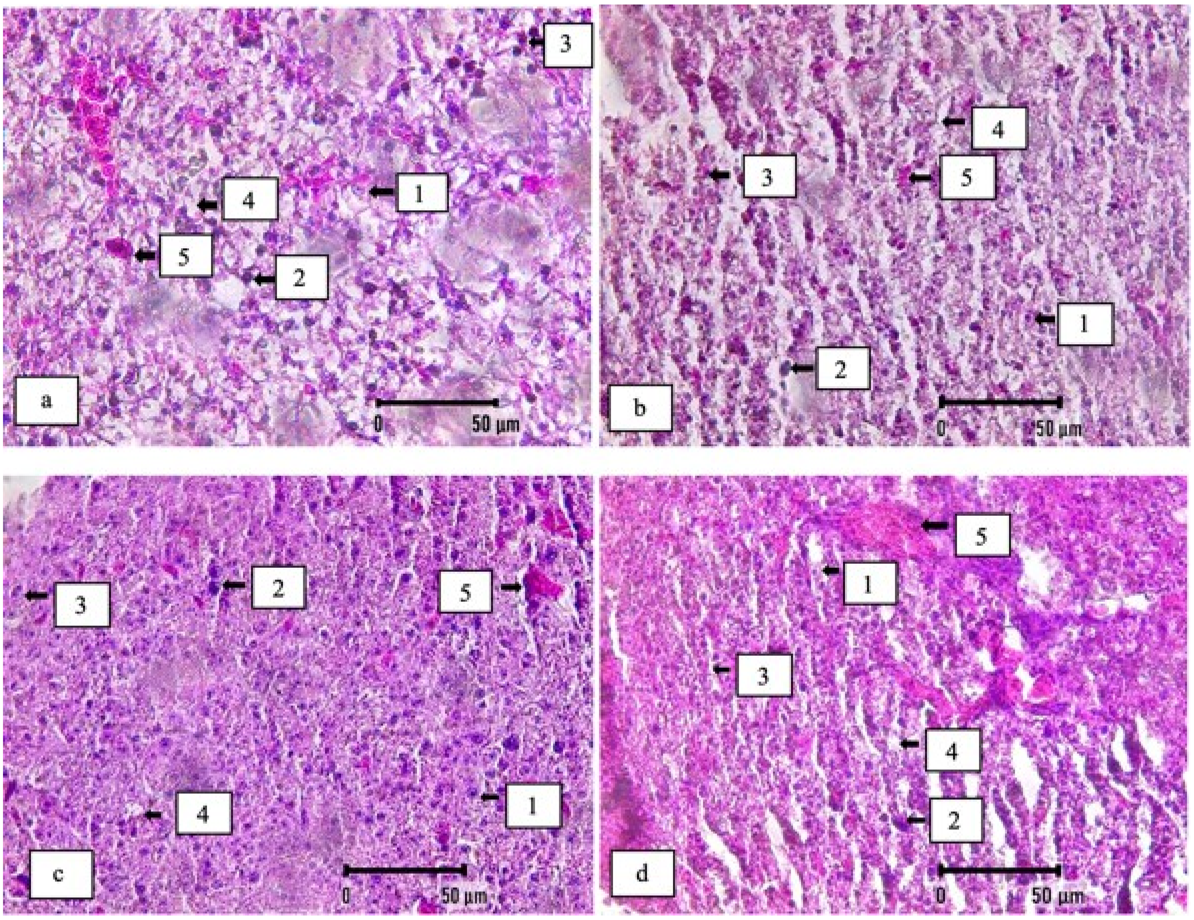

Figure 1. Histological structure of Betta fish liver of control group (a), 5 ppm (b), 25 ppm (c), 50 ppm (d). Normal hepatocytes (1), pyknosis (2), necrosis (3), vacuolization (4), and hemorrhage (5). Hematoxylin-Eosin staining. Scale bar = 50μm.

Figure 1 shows the histological structure of Betta fish liver tissue stained with Hematoxylin-Eosin. In the control group (Figure 1a), the liver tissue exhibited mostly normal hepatocytes with minimal signs of damage. Some vacuolization (4) and pyknosis (2) were observed, but the overall tissue structure remained relatively intact. In the 5-ppm treatment group (Figure 1b), the liver tissue showed increased vacuolization and moderate pyknosis, with some areas of necrosis (3) beginning to appear. Hemorrhage (5) was present but not extensive. The 25-ppm treatment group (Figure 1c) exhibited more pronounced damage. There was a noticeable increase in necrosis and pyknosis, with significant vacuolization and hemorrhage. The liver tissue structure was more disrupted compared to the control and 5 ppm groups. In the 50-ppm treatment group (Figure 1d), the liver tissue showed the most severe damage. Extensive areas of necrosis and pyknosis were observed, with considerable vacuolization and hemorrhage. The hepatocytes were significantly damaged, and the overall tissue architecture was highly compromised.

Table 3. Liver damage in Betta fish based on scoring value

Treatment	Hemorrhage	Pyknosis	Necrosis	Vacuolization	Damage level
Control	0.42 ± 0.15[a]	1.33 ± 0.14[a]	1.00 ± 0.00[a]	2.42 ± 0.15[a]	Moderate
5 ppm	0.50 ± 0.19[a]	1.50 ± 0.19[a]	1.00 ± 0.00[a]	2.12 ± 0.30[ab]	Moderate
25 ppm	0.92 ± 0.08[b]	1.50 ± 0.29[a]	1.37 ± 0.19[a]	1.67 ± 0.22[b]	Moderate
50 ppm	0.83 ± 0.11[b]	3.00 ± 0.00[b]	2.80 ± 0.08[b]	1.00 ± 0.00[c]	Moderate

Note: Identical letters indicate no significant difference between treatments.

Table 3 presents the scoring values of liver damage in Betta fish across different treatment groups. Hemorrhage, pyknosis, necrosis, and vacuolization were quantified to assess the level of hepatic damage. The analysis revealed that the mangosteen peel decoction exhibited a damaging effect on Betta fish hepatocytes. The Kruskal-Wallis's test revealed significant differences in hepatic damage across treatments, particularly in hemorrhage, pyknosis, necrosis, and vacuolization. Hemorrhage was significantly lower in the control group compared to 25-ppm and 50-ppm groups. Pyknosis was significantly higher in the 50-ppm group compared to other groups. Although no significant differences were observed in necrosis levels among control, 5-ppm, and 25-ppm groups, the 50-ppm group exhibited a higher level of necrosis. The extent of vacuolization was highest in the control group and lowest in 50-ppm group, with 5-ppm and 25-ppm not differing significantly from each other. Hepatic damage across all treatments was categorized as moderate, with the control group showing the least average hepatic damage.

3.2. Discussion

The application of mangosteen (*G. mangostana* L.) peel decoction in aquaculture, particularly in fish species, has attracted significant scientific attention due to its bioactive compounds and associated health benefits. Mangosteen peel is a rich source of xanthones and flavonoids, which possess anti-inflammatory, antibacterial, and antifungal properties (Setiawan et al., 2023; Widyarman et al., 2019). Studies on the embryological development of wader pari fish (*Rasbora lateristriata*) revealed that exposure to higher concentrations of mangosteen peel decoction negatively impacts hatching rates, survival, and heart morphology, with notable cardiac abnormalities such as edema and bending observed at 25 μg/mL (Khasanah et al., 2024). Research on *R. lateristriata* has highlighted its utility as a model organism for embryological and toxicity studies, further validating the relevance of fish models in assessing environmental and compound-related impacts (Retnoaji, Nurhidayat, et al., 2023). Further research on the influence of mangosteen peel extract on bone structure and behavior in wader fish embryos demonstrated that concentrations up to 5 μg/mL are generally safe, showing no significant morphological or skeletal abnormalities. However, higher concentrations caused developmental malformations such as yolk sac edema and collapsed swim bladder, highlighting a dose-dependent toxicity (Retnoaji, Paramita, et al., 2023). The antibacterial efficacy of mangosteen peel extract has also been evaluated against *Aeromonas hydrophila* in African catfish (*Clarias gariepinus*). Concentrations between 6.25% and 25% were found effective in inhibiting bacterial growth, establishing the extract's potential as a natural antimicrobial agent in aquaculture (Cahya et al., 2023). Similarly, in Nile tilapia (*Oreochromis niloticus*), the incorporation of mangosteen peel extract in nanoemulsion form at a concentration of 6.25 mg/g in the diet significantly improved growth performance, immune response, and resistance to *Aeromonas veronii*. The nanoemulsion formulation enhanced the bioavailability and efficacy of the extract, underscoring its potential as a sustainable aquaculture supplement (Yostawonkul et al., 2023). Additionally, dietary supplementation with mangosteen extracts in African catfish fingerlings at 0.5% inclusion improved hematological parameters such as red and white blood cell counts without negatively impacting growth. These results support the extract's role in enhancing fish health and resilience under aquaculture conditions (Soosean et al., 2010).

In this study, the behavior of Betta fish (*Betta* sp.) was significantly influenced by treatment with mangosteen peel decoction, as evidenced by observed changes in swimming activity and appetite over a five-day period (Table 2). Swimming activity and appetite are fundamental behavioral parameters commonly used to assess the physiological and health status of fish (Svendsen et al., 2021; Zhao et al., 2017). Changes in these parameters can indicate stress, toxicity, or overall well-being in response to environmental factors or treatments. Swimming activity reflects the energy level, muscular coordination, and nervous system functionality, while appetite serves as an indicator of metabolic and digestive health (Gerry & Ellerby, 2014; Nie et al., 2017). In the control group, the fish exhibited consistent and high levels of swimming activity, maintaining an active status throughout the five days, with particularly vigorous activity on days 2 and 5. This stable and active swimming behavior indicates the normal physiological condition of the Betta fish in the absence of any treatment. In contrast, the swimming activity of the treated groups (5, 25, and 50 ppm) progressively declined with increasing concentrations of mangosteen peel decoction. In 5-ppmgroup, treated with 5 ppm, the fish maintained normal swimming activity for the first three days but showed a marked decrease in activity, becoming less active on days 4 and 5. This indicates an initial resilience to the lower concentration of the decoction, with a delayed onset of activity reduction. The fish in group 25-ppm, exhibited a more pronounced decline in activity, becoming less active from day 2 onwards. By day 5, the fish remained less active, suggesting that this intermediate concentration had a quicker and more sustained impact on reducing swimming activity. The 50-ppm group, treated with the highest concentration of 50 ppm, showed the most significant reduction in activity. The fish became less active by day 2 and were completely inactive by days 4 and 5. This rapid and severe decrease in activity at the highest concentration indicates a strong dose-dependent effect of the mangosteen peel decoction on the swimming behavior of Betta fish.

Appetite levels also showed a decreasing trend across the treated groups in comparison to the control group. The control group maintained a high appetite throughout the five-day period, reflecting healthy feeding behavior under normal conditions. In 5-ppm group, the fish maintained a high appetite for the first three days, but their appetite decreased to medium levels on days 4 and 5. This suggests a gradual reduction in feeding behavior in response to the 5-ppm concentration of the decoction. The 25-ppm group exhibited a more rapid decline in appetite, with high levels on day 1, decreasing to medium by days 2 and 3, and further reducing to low by days 4 and 5. The intermediate concentration of 25 ppm clearly had a more immediate and substantial impact on feeding behavior compared to the lower concentration. The 50-ppm group showed the most drastic reduction in appetite, with high levels on day 1, dropping to medium on days 2 and 3, and reaching low levels on days 4 and 5. The 50-ppm concentration of the decoction had the strongest effect, mirroring the severe reduction in swimming activity observed in this group.

These findings suggest that mangosteen peel decoction exerts a dose-dependent impact on both the swimming activity and appetite of Betta fish. The consistent reduction in these behaviors at higher concentrations indicates potential stress or toxicity effects induced by the decoction. Further research is necessary to elucidate the underlying mechanisms of this impact, including potential physiological and biochemical changes in the fish. In addition, exploring protective measures, such as combining mangosteen peel decoction with protective agents or antioxidants, could mitigate its adverse effects. Studies focusing on identifying specific compounds responsible for the observed effects could also guide safer applications in aquaculture. Understanding these effects is crucial for assessing the safe and effective use of mangosteen peel extracts in aquaculture and other applications involving Betta fish. Overall, the observed behavioral changes underscore the importance of carefully monitoring and regulating the concentration of herbal treatments like mangosteen peel decoction to avoid adverse effects on aquatic organisms.

The histopathological analysis of *Betta* sp. liver tissue reveals significant insights into the effects of mangosteen peel decoction on hepatic health. Across all treatment groups, including the control, 5-ppm, 25-ppm, and 50-ppm, notable liver damage was observed, encompassing vacuolization, pyknosis, hemorrhage, and necrosis. These parameters are commonly used in histopathological studies to assess cellular and tissue health. Vacuolization reflects changes in intracellular organelle dynamics, often indicating cellular stress or metabolic dysfunction (Pham et al., 2016). Similar histopathological parameters, including necrosis and pyknosis, have been utilized in zebrafish exposed to paracetamol to assess hepatotoxicity and provide comparative insights into compound-specific toxicities (Dewanti et al., 2023). Pyknosis, characterized by the condensation of chromatin in the nucleus, is a hallmark of irreversible cellular injury and an early stage of apoptosis (Hou et al., 2016). Hemorrhage, which involves the extravasation of blood, signals damage to vascular integrity within the tissue (Kottke-Marchant, 1994), while necrosis is an end-stage cellular event involving uncontrolled cell death and the breakdown of tissue architecture (Golstein & Kroemer, 2007). These findings are detailed in Table 3 and visually represented in Figure 1. In terms of specific damage metrics, the Kruskal-Wallis's test confirmed significant differences among the treatment groups for all parameters of hepatic damage. Hemorrhage was significantly more pronounced in the 25-ppm and 50-ppm groups compared to the control, indicating that higher concentrations of the decoction correlate with increased hemorrhagic damage. This suggests a dose-dependent relationship where higher exposure to the decoction exacerbates vascular injury within the liver. Comparable histopathological alterations, including hemorrhage and necrosis, have been observed in *Rasbora lateristriata* exposed to paracetamol, supporting the significance of these markers in evaluating hepatic toxicity (Septriani et al., 2023).

Pyknosis, characterized by the condensation of chromatin in the nucleus, was markedly elevated in the 50-ppm group treated with the highest concentration of mangosteen peel extract. This significant increase in pyknosis in 50-ppm groups compared to other groups underscores the cytotoxic effects of the mangosteen peel decoction at higher doses. These findings suggest that hepatocyte nuclei are particularly susceptible to damage at elevated decoction concentrations, leading to impaired cellular function. Necrosis, another critical indicator of cellular damage, showed a stark increase in the 50-ppm group. While the levels of necrosis did not differ significantly between control, 5-ppm, and 25-ppm groups, the elevated necrosis in 50-ppm group highlights severe cellular injury and death at higher concentration of mangosteen peel extract. This points to the potential lethality of high concentrations of mangosteen peel decoction on Betta fish hepatocytes. Interestingly, vacuolization, the formation of vacuoles within the cytoplasm, was highest in the control group and lowest in the 50-ppm group. The reduction of vacuolization in 50-ppm compared to control, with 5-ppm and 25-ppm groups showing intermediate levels, suggests a complex interplay between decoction concentration and the cellular response. The initial hypothesis that higher decoction levels might increase vacuolization was not supported; instead, a possible explanation could be the severe cellular damage at high concentrations, leading to reduced capacity for vacuole formation or maintenance.

Overall, hepatic damage across all treatments was categorized as moderate, with the control group exhibiting the least average damage. This categorization is crucial for understanding the relative impact of the mangosteen peel decoction. Despite all groups showing medium damage, the gradation of specific types of damage indicates that higher decoction concentrations lead to more pronounced and varied forms of hepatic injury. These findings have significant implications for the use of mangosteen peel decoction in aquatic environments and its potential toxicological impacts on fish liver health. The dose-dependent increase in hepatic damage parameters, particularly hemorrhage, pyknosis, and necrosis, calls for careful consideration of the decoction concentration in practical applications. Further research is necessary to elucidate the mechanisms driving these histopathological changes and to explore potential protective measures or alternative treatments to mitigate liver damage in Betta fish and other aquatic organisms.

Comparing concentrations across studies reveals important insights into the effective and safe use of mangosteen peel extracts. In this study, the 5 ppm concentration maintained normal Betta fish swimming activity and appetite for three days, indicating a relatively safe threshold for short-term exposure. This aligns with findings in wader fish (*R. lateristriata*), where 5 µg/mL was similarly deemed safe for embryonic development (Retnoaji, Paramita, et al., 2023). However, higher concentrations (25 ppm and above) in Betta fish led to significant reductions in activity and appetite, paralleling the toxic effects reported in wader fish at 25 µg/mL and above (Khasanah et al., 2024). In Nile tilapia, a higher dietary concentration of 6.25 mg/g in nanoemulsion form yielded beneficial effects, likely due to the enhanced bioavailability offered by the nanoemulsion delivery system (Yostawonkul et al., 2023). These comparisons highlight the importance of tailoring concentrations to species-specific tolerances and delivery methods to optimize both safety and efficacy.

Conclusion

This study demonstrates that mangosteen peel decoction has a dose-dependent toxic effect on Betta fish, significantly impacting their behavior and liver health. Higher concentrations of the decoction resulted in decreased swimming activity and appetite, indicating stress or toxicity. Histopathological analysis revealed significant liver damage, including vacuolization, pyknosis, hemorrhage, and necrosis, with severity increasing at higher concentrations. The highest concentration (50 ppm) caused the most pronounced cytotoxic effects, notably in pyknosis and necrosis. These findings highlight the need for careful regulation of mangosteen peel decoction to avoid adverse effects on aquatic organisms. Further research is necessary to understand the mechanisms of these effects and to explore protective measures. This study provides important insights into the safety of mangosteen peel extracts and underscores the importance of comprehensive toxicity assessments.

Acknowledgement

The authors extend their sincere gratitude to Sefi Nur Anggaeni and Suwardi for their invaluable assistance in preparing the tools and materials used in the laboratory.

References

Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P. R., Rane, M. R., Gurav, R. G., & Jadhav, J. P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany*, *151*, 512–528. https://doi.org/10.1016/j.sajb.2022.05.028

Cahya, B., Nirmala, D., & Triantoro, B. (2023). Testing the antibacterial effectiveness of mangosteen peel extract (Garcinia mangostana L) against Aeromonas hydrophila Bacterial infection in dumbo catfish (Clarias gariepinus). *IOP Conference Series: Earth and Environmental Science*, *1273*(1), 012024. https://doi.org/10.1088/1755-1315/1273/1/012024

Capela, R., Garric, J., Castro, L. F. C., & Santos, M. M. (2020). Embryo bioassays with aquatic animals for toxicity testing and hazard assessment of emerging pollutants: A review. *Science of The Total Environment*, *705*, 135740. https://doi.org/10.1016/j.scitotenv.2019.135740

Dewanti, A. I. A., Kunjirika, T. P., Putri, R. R. R. A. D., Adaninggar, A., Raharjeng, A. R. P., Retnoaji, B., Nuriliani, A., Sofyantoro, F., Septriani, N. I., & Saragih, H. T. S. S. G. (2023). Effects of Paracetamol on the Development of Zebrafish (Danio rerio). *Pertanika Journal of Tropical Agricultural Science*, *46*(4), 1173–1188. https://doi.org/10.47836/pjtas.46.4.06

Gerry, S. P., & Ellerby, D. J. (2014). Resolving Shifting Patterns of Muscle Energy Use in Swimming Fish. *PLoS ONE*, *9*(8), e106030. https://doi.org/10.1371/journal.pone.0106030

Gibson-Corley, K. N., Olivier, A. K., & Meyerholz, D. K. (2013). Principles for Valid Histopathologic Scoring in Research. *Veterinary Pathology*, *50*(6), 1007–1015. https://doi.org/10.1177/0300985813485099

Golstein, P., & Kroemer, G. (2007). Cell death by necrosis: Towards a molecular definition. *Trends in Biochemical Sciences*, *32*(1), 37–43. https://doi.org/10.1016/j.tibs.2006.11.001

Hosack, T., Damry, D., & Biswas, S. (2023). Drug-induced liver injury: A comprehensive review. *Therapeutic Advances in Gastroenterology*, *16*, 175628482311634. https://doi.org/10.1177/17562848231163410

Hou, L., Liu, K., Li, Y., Ma, S., Ji, X., & Liu, L. (2016). Necrotic pyknosis is a morphologically and biochemically distinct event from apoptotic pyknosis. *Journal of Cell Science*, jcs.184374. https://doi.org/10.1242/jcs.184374

Ibrahim, M. Y., Hashim, N. M., Mariod, A. A., Mohan, S., Abdulla, M. A., Abdelwahab, S. I., & Arbab, I. A. (2016). α-Mangostin from Garcinia mangostana Linn: An updated review of its pharmacological properties. *Arabian Journal of Chemistry*, *9*(3), 317–329. https://doi.org/10.1016/j.arabjc.2014.02.011

Khasanah, L. U., Paramita, P., & Retnoaji, B. (2024). Mangosteen (*Garcinia mangostana* L.) Peel Decoction Effect on Embryological Development of Wader Pari Fish *Rasbora lateristriata* (Bleeker, 1854). *Journal of Tropical Biodiversity and Biotechnology*, *9*(2), 80645. https://doi.org/10.22146/jtbb.80645

Krewski, D., Andersen, M. E., Tyshenko, M. G., Krishnan, K., Hartung, T., Boekelheide, K., Wambaugh, J. F., Jones, D., Whelan, M., Thomas, R., Yauk, C., Barton-Maclaren, T., & Cote, I. (2020). Toxicity testing in the 21st century: Progress in the past decade and future perspectives. *Archives of Toxicology*, *94*(1), 1–58. https://doi.org/10.1007/s00204-019-02613-4

Leung, L., Kalgutkar, A. S., & Obach, R. S. (2012). Metabolic activation in drug-induced liver injury. *Drug Metabolism Reviews*, *44*(1), 18–33. https://doi.org/10.3109/03602532.2011.605791

Lichak, M. R., Barber, J. R., Kwon, Y. M., Francis, K. X., & Bendesky, A. (2022). Care and Use of Siamese Fighting Fish (*Betta Splendens*) for Research. *Comparative Medicine*, *72*(3), 169–180. https://doi.org/10.30802/AALAS-CM-22-000051

Michalopoulos, G. K., & Bhushan, B. (2021). Liver regeneration: Biological and pathological mechanisms and implications. *Nature Reviews Gastroenterology & Hepatology*, *18*(1), 40–55. https://doi.org/10.1038/s41575-020-0342-4

Nie, L.-J., Cao, Z.-D., & Fu, S.-J. (2017). Digesting or swimming? Integration of the postprandial metabolism, behavior and locomotion in a frequently foraging fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *204*, 205–210. https://doi.org/10.1016/j.cbpa.2016.12.007

Obolskiy, D., Pischel, I., Siriwatanametanon, N., & Heinrich, M. (2009). *Garcinia mangostana* L.: A phytochemical and pharmacological review. *Phytotherapy Research*, *23*(8), 1047–1065. https://doi.org/10.1002/ptr.2730

Oliveira, J. A., Da Silva Souza, J. G., De Jesus Paula, D. A., Carmo Rodrigues Virote, B. D., & Murgas, L. D. S. (2022). Oxytocin reduces the frequency of aggressive behaviours in male betta fish (Betta splendens). *Behavioural Processes*, *200*, 104689. https://doi.org/10.1016/j.beproc.2022.104689

Palmiotti, A., Lichak, M. R., Shih, P.-Y., Kwon, Y. M., & Bendesky, A. (2023). Genetic manipulation of betta fish. *Frontiers in Genome Editing*, *5*, 1167093. https://doi.org/10.3389/fgeed.2023.1167093

Pham, P. H., Babujee, A., Papazotos, F., & Bols, N. C. (2016). Evaluating the potential role of vacuolization in enhancing or interfering with fish virus detection and replication. *Fish & Shellfish Immunology*, *53*, 107. https://doi.org/10.1016/j.fsi.2016.04.071

Retnoaji, B., Nurhidayat, L., Pratama, S. F., Anshori, K., Hananya, A., Sofyantoro, F., & Bessho, Y. (2023). Embryonic development of Indonesian native fish yellow rasbora (Rasbora lateristriata). *Journal of King Saud University - Science*, *35*(7), 102810. https://doi.org/10.1016/j.jksus.2023.102810

Retnoaji, B., Paramita, P., & Khasanah, L. U. (2023). Mangosteen Garcinia mangostana L. simplicia effect on bone structure and behaviour of Wader Fish Rasbora lateristriata (Bleeker, 1854) Embryo. *Indonesian Journal of Pharmacy*. https://doi.org/10.22146/ijp.6348

Saraswathy, S. U. P., Lalitha, L. C. P., Rahim, S., Gopinath, C., Haleema, S., SarojiniAmma, S., & Aboul-Enein, H. Y. (2022). A Review on Synthetic and Pharmacological Potential of Compounds Isolated from Garcinia mangostana Linn. *Phytomedicine Plus*, *2*(2), 100253. https://doi.org/10.1016/j.phyplu.2022.100253

Septriani, N. I., Saribu, R. L. C. D., Apriliyani, T., Karlina, I., Pusparini, N. A. O., Zusrina, L. M., Sari, R. V. S., Allimi, H. S., Supraitno, M. E., Saeed, F., Simanungkalit, E. E., Paramita, P., Retnoaji, B., Sofyantoro, F., & Wijayanti, N. (2023). Histopathological evaluation of hepatic tissue of yellow Rasbora (Rasbora lateristriata) exposed to paracetamol. *Biological Environment and Pollution*, *3*(1), 8–14. https://doi.org/10.31763/bioenvipo.v3i1.595

Setiawan, A. A., Budiman, J., & Prasetyo, A. (2023). Anti-Inflammatory Potency of Mangosteen (Garcinia mangostana L.): A Systematic Review. *Open Access Macedonian Journal of Medical Sciences*, *11*(F), 58–66. https://doi.org/10.3889/oamjms.2023.8746

Simeonova, R., Kondeva-Burdina, M., Vitcheva, V., & Mitcheva, M. (2014). Some *In Vitro/In Vivo* Chemically-Induced Experimental Models of Liver Oxidative Stress in Rats. *BioMed Research International*, *2014*, 1–6. https://doi.org/10.1155/2014/706302

Sofyantoro, F., Septriani, N. I., Yudha, D. S., Wicaksono, E. A., Priyono, D. S., Putri, W. A., Primahesa, A., Raharjeng, A. R. P., Purwestri, Y. A., & Nuringtyas, T. R. (2024). Zebrafish as Versatile Model for Assessing Animal Venoms and Toxins: Current Applications and Future Prospects. *Zebrafish*, zeb.2023.0088. https://doi.org/10.1089/zeb.2023.0088

Soosean, C., Marimuthu, K., Sudhakaran, S., & Xavier, R. (2010). Effect of mangosteen (Garcinia mangostana L.) extracts as a feed additive on growth and hematological parameters of African catfish (Clarias gariepinus) fingerlings. *European Review for Medical and Pharmacological Sciences*, *14*(7), 605–611.

Svendsen, E., Føre, M., Økland, F., Gräns, A., Hedger, R. D., Alfredsen, J. A., Uglem, I., Rosten, C. M., Frank, K., Erikson, U., & Finstad, B. (2021). Heart rate and swimming activity as stress indicators for Atlantic salmon (Salmo salar). *Aquaculture*, *531*, 735804. https://doi.org/10.1016/j.aquaculture.2020.735804

Widyarman, A., Lay, S., Wendhita, I., Tjakra, E., Murdono, F., & Binartha, C. O. (2019). Indonesian mangosteen fruit (Garcinia mangostana L.) peel extract inhibits Streptococcus mutans and Porphyromonas gingivalis in Biofilms In vitro. *Contemporary Clinical Dentistry*, *10*(1), 123. https://doi.org/10.4103/ccd.ccd_758_18

Yostawonkul, J., Kamble, M. T., Sakuna, K., Madyod, S., Sukkarun, P., Medhe, S. V., Rodkhum, C., Pirarat, N., & Sewaka, M. (2023). Effects of Mangosteen (Garcinia mangostana) Peel Extract Loaded in Nanoemulsion on Growth Performance, Immune Response, and Disease Resistance of Nile Tilapia (Oreochromis niloticus) against Aeromonas veronii Infection. *Animals*, *13*(11), 1798. https://doi.org/10.3390/ani13111798

Yue, G. H., Wang, L., Sun, F., Yang, Z., Shen, Y., Meng, Z., & Alfiko, Y. (2022). The ornamental fighting fish is the next model organism for genetic studies. *Reviews in Aquaculture*, *14*(4), 1966–1977. https://doi.org/10.1111/raq.12681

Yuvanatemiya, V., Srean, P., Klangbud, W. K., Venkatachalam, K., Wongsa, J., Parametthanuwat, T., & Charoenphun, N. (2022). A Review of the Influence of Various Extraction Techniques and the Biological Effects of the Xanthones from Mangosteen (Garcinia mangostana L.) Pericarps. *Molecules*, *27*(24), 8775. https://doi.org/10.3390/molecules27248775

Zhang, W., Wang, H., Brandt, D. Y. C., Hu, B., Sheng, J., Wang, M., Luo, H., Li, Y., Guo, S., Sheng, B., Zeng, Q., Peng, K., Zhao, D., Jian, S., Wu, D., Wang, J., Zhao, G., Ren, J., Shi, W., … Hong, Y. (2022). The genetic architecture of phenotypic diversity in the Betta fish (*Betta splendens*). *Science Advances*, *8*(38), eabm4955. https://doi.org/10.1126/sciadv.abm4955

Zhao, J., Bao, W. J., Zhang, F. D., Ye, Z. Y., Liu, Y., Shen, M. W., & Zhu, S. M. (2017). Assessing appetite of the swimming fish based on spontaneous collective behaviors in a recirculating aquaculture system. *Aquacultural Engineering*, *78*, 196–204. https://doi.org/10.1016/j.aquaeng.2017.07.008