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Histopathological evaluation of hepatic tissue of yellow Rasbora (*Rasbora lateristriata*) exposed to paracetamol

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ABSTRACT

Excessive consumption of paracetamol may lead to hepatic necrosis, nephrotoxicity, extra-hepatic lesions, and even fatality in humans and animals. The objective of this study was to examine the histological changes in the hepatocytes of yellow rasbora (Rasbora lateristriata) exposed to paracetamol. The fish were subjected to varying concentrations of paracetamol over four days. Group I served as the control group without any paracetamol exposure. Groups II, III, and IV were exposed to 2, 3, and 4 mg/L of paracetamol, respectively. A total of six individuals were allocated to each of the four experimental groups. The histological analysis of the hepatic tissue following paracetamol exposure at concentrations of 2, 3, and 4 mg/L revealed hepatic damage characterized by picnosis, necrosis, and vacuolization. In summary, higher concentrations of paracetamol were associated with an increase in the severity of histopathological changes in the hepatic tissues of yellow rasbora.

Conflict of interest: The authors declare that they have no conflicts of interest.

Introduction

Paracetamol exhibits a high degree of bioavailability, with approximately 80% of the drug being absorbed following oral administration[1]. Within therapeutic dosage ranges, paracetamol is generally regarded as a safe medication. However, elevated doses of paracetamol can generate a toxic compound known as N-acetyl-p-benzoquinone imine (NAPQI)[2,3]. The

detoxification of NAPQI occurs through its conjugation with glutathione (GSH), resulting in the production of harmless metabolites that are excreted. However, in instances of excessive drug accumulation, the depletion of GSH levels can trigger the release of hydrogen peroxide (H_2O_2) and an upsurge in Reactive Oxygen Species (ROS)[3-5]. Moreover, diminished GSH levels can induce mitochondrial permeability transition, leading to ATP depletion, DNA fragmentation, and necrosis. Consequently, the fragmentation of DNA and damage to the cell membrane culminate in cell death and the onset of acute inflammation[1,3].

Paracetamol possesses inherent risks, as demonstrated by its potential to induce nephrotoxicity and hepatotoxicity at elevated dosages[6]. The adverse effects can also contribute to developmental abnormalities in the fetus and neonate[3,7]. Furthermore, the hepatotoxicity of paracetamol becomes evident at excessive levels, such as in cases of overdose. This hepatotoxicity manifests as diverse cellular and tissue damages within the hepar, including cell necrosis, eosinophilic degeneration, and picnosis of the cell nucleus. From a histological perspective, the toxic effects are characterized by the depletion of glycogen and vacuolization of hepatocytes, resulting in a distinct demarcation in the centrilobular region from other hepatic areas. Additionally, centrilobular hepatocytes undergo nuclear alterations, and individual-cell necrosis occurs, accompanied by picnosis changes[8].

Rasbora lateristriata, commonly known as the yellow rasbora, is significant among the local freshwater fish species in Indonesia[9-11]. This remarkable species has demonstrated high adaptability to survive in diverse and challenging environmental conditions[10,12]. The yellow rasbora has also established a wide distribution throughout Southeast Asia[13,14]. Furthermore, the abundant production of eggs by this species ensures a consistent and ample supply of samples for various scientific studies[12]. One notable area of research that benefits from the yellow rasbora is toxicity testing, particularly in embryos and larvae. The copious number of eggs this species produces allows researchers to conduct extensive studies on the impact of toxins and pollutants on early life stages[15,16]. By exposing yellow rasbora embryos and larvae to different substances, scientists can gain insights into the potential risks and adverse effects of various pollutants and drugs in freshwater ecosystems. Beyond toxicity testing, the yellow rasbora has also emerged as a valuable tool in freshwater bioindicator research. As bioindicators, these fish are indicators of environmental quality and ecological health in aquatic ecosystems[17]. The extensive use of yellow rasbora in freshwater bioindicator research reflects its significant role in monitoring and evaluating the overall health and well-being of aquatic environments. By studying the responses and conditions of this species, scientists can gain crucial insights into the ecological status of freshwater ecosystems, enabling informed conservation and management decisions.

Based on the aforementioned background, it becomes apparent that prolonged employment of paracetamol at specific doses can heighten the risk of hepatotoxicity. Therefore, the present study aimed to examine the histopathology of the hepatic tissues of yellow rasbora (*Rasbora lateristriata*) following exposure to paracetamol.

Method

Fish collection and acclimation

Yellow rasbora (*R. lateristriata*) were obtained from the Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Indonesia. The fish, which were randomly selected males and females aged 2 months with an average weight of 5 grams, were kept in an aquarium measuring 30 cm x 25 cm x 15 cm. The aquarium was housed within the laboratory, and the fish underwent a one-day acclimation period in aerated water with continuous aeration before the commencement of the experiments.

Paracetamol administration

A homogeneous cohort of fish, matched in terms of size and weight, was carefully chosen from the available stock. The fish population was then divided into four distinct groups, with each group consisting of six individuals. The experimental groups were as follows: group I (control) served as the reference group and did not receive any paracetamol exposure, while group II (2mg/L), group III (3mg/L), and group IV (4mg/L) were subjected to varying concentrations of paracetamol. The fish in each group were exposed to their respective paracetamol concentrations for a duration of four days.

Preparation of hepatic tissue

The tissues were prepared using paraffin method for histological analysis of the hepar. The Haematoxylin-Eosin staining technique was employed to analyze the hepatocytes damage. The quantification of normal hepatocyte cells, as well as those exhibiting picnosis, necrosis, and vacuolization, was conducted using Image-J software. The assessment of damage levels was performed in accordance with Table 1.

Table 1. the level of hepatic histopathological damage (Gibson-Corley et al.[18] with modifications)

Level of damage	Description	Score
Normal	Normal, clear nucleus, round shape	0
Mild	Hemorrhage +, picnosis +, necrosis +, vacuolization +	1
Moderate	Hemorrhage ++, picnosis ++, necrosis ++, vacuolization ++	2
Severe	Hemorrhage +++, picnosis +++, necrosis +++, vacuolization +++	3

Description:
- : Normal
\+ : 25% of damage in five fields of view
++ : 50% of damage in five fields of view
+++ : 75% of damage in five fields of view

Statistical analysis

Given the variability in the data, non-parametric tests were employed for analysis. The Kruskal-Wallis test was used to evaluate differences among groups, serving as an analysis of variance. For inter-group comparisons, the Mann-Whitney U test was conducted, employing the least significant value. A significance level of $p<0.05$ was deemed statistically significant.

Results and Discussion

The hepar plays a vital role in various physiological processes such as metabolism, synthesis, secretion, and storage of substances[19,20]. Consequently, the hepar is susceptible to damage resulting from the metabolic breakdown of consumed drugs[21]. Paracetamol represents the most commonly utilized antipyretic drug worldwide, serving to alleviate fever and facilitate improved rest and sleep[22,23]. While low doses of paracetamol have safe and positive effects, overdose and prolonged usage can lead to hepatotoxicity and acute hepatic failure[1,2,7]. The initial stage of hepatocyte damage involves hydropic and fatty degeneration, commonly referred to as vacuolization[24]. However, more severe, and irreversible damage manifests as necrosis, an uncontrolled cell death process induced by acute cellular injury. Necrosis commences with picnosis, characterized by darkening (hyperchromatic) and shrinkage of the cell nucleus, followed by karyorrhexis, where the nucleus breaks into fragments, and ultimately karyolitic, resulting in the complete disappearance of the cell nucleus[25-27].

The histological analysis of the hepatic tissue of yellow rasbora exposed to paracetamol at doses of 2, 3, and 4 mg/L revealed hepatic damage characterized by picnosis, necrosis, and

vacuolization. These findings align with prior studies conducted on *Phalloceruos harpagosa*[28], *Rhamdia quelen*[8], and *Labeo rohita*[29] fish, where exposure to paracetamol resulted in vacuolization of hepatocytes and congestion of red blood cells. In the control group (Fig 1A), the histology of the hepar exhibited normal hepatocyte cells, displaying a lobular arrangement with basophilic cytoplasm dispersed around the central vein area. However, the control group of yellow rasbora displayed mild damage, such as vacuolization and picnosis. This phenomenon is commonly observed in the hepar organ, as the hepatic organ undergoes damage over time while performing its function of detoxifying harmful substances.

Paracetamol hepatotoxicity is associated with extensive centrilobular necrosis, vacuolar degeneration, and inflammatory cell infiltration[4,5,25]. Additionally, paracetamol-induced hepatotoxicity results in protein accumulation, contributing to centrilobular necrosis within the hepar[1,26]. According to James et al.[30], toxic doses of paracetamol typically occur at doses exceeding 150 mg/kg body weight. In this study, the group treated with a concentration of 2 mg/L paracetamol displayed picnosis, with a significant number of hepatocytes exhibiting vacuolization and fatty degeneration (Fig 1B). Additionally, the administration of paracetamol at a concentration of 3 mg/L induced karyolitic and karyorrhexis, ultimately leading to hepatocyte necrosis (Fig 1C). These findings indicate that a dose of 3 mg/L paracetamol is sufficient to cause hepatic abnormalities. Notably, when the paracetamol concentration was increased to 4 mg/L, hepatocyte necrosis (Fig 1D) and karyorrhexis were also observed. The toxic effect of paracetamol on hepatocytes is mediated through various mechanisms, including the formation of NAPQI, depletion of glutathione, oxidative stress, mitochondrial dysfunction, and subsequent cellular damage[3,25,26]. The observed hepatocellular abnormalities in the experimental groups treated with different concentrations of paracetamol underscore the detrimental effects of this drug on hepar health.

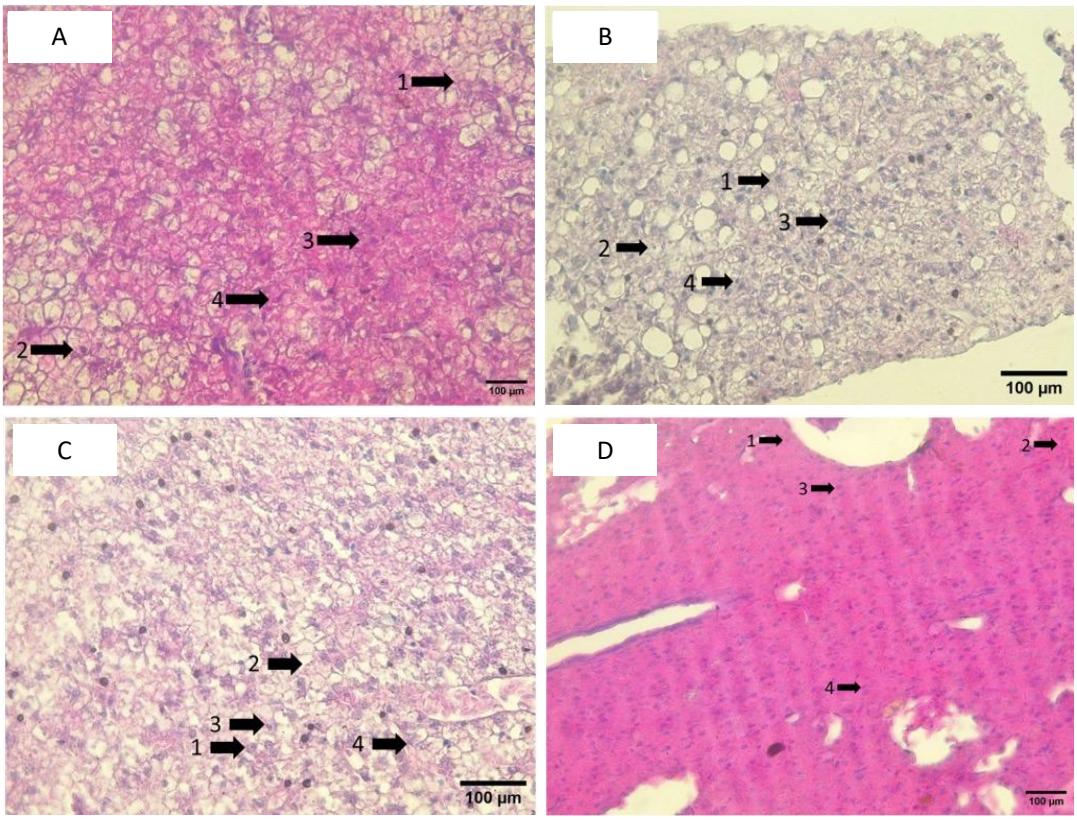

Fig 1. Histological examination of the hepatic tissue from yellow rasbora fish exposed to a high dosage of paracetamol. The following histopathological findings were observed and are labeled accordingly: 1) normal cell morphology, 2) vacuolization, 3) picnosis nuclei, and 4) necrosis. A: control; B: 2 mg/L; C: 3 mg/L; and D: 4mg/L of paracetamol

As shown in Fig 2, the Kruskal-Wallis analysis revealed significant differences in vacuolization and necrosis cell damage among the treatment groups ($p<0.05$). However, there were no significant differences in terms of picnosis cell damage. To further investigate the specific distinctions between the treatment groups, the Mann-Whitney U-test was employed. The group treated with a concentration of 4mg/L exhibited a significant decrease in vacuolization compared to the control group (Fig 2A). Vacuolization is a characteristic early stage of hepatocyte damage, which involves the accumulation of fluid-filled vacuoles and fatty degeneration. Vacuolization is commonly observed as a result of cellular stress or injury[1,25]. According to the expected outcome, a higher concentration of paracetamol would lead to an increased number of vacuolization. However, the results of this study showed the opposite. This unexpected finding could be attributed to various factors, such as the specific characteristics of the experimental model, individual variations in response to paracetamol, or potential interactions with other factors that require further investigation.

Severe and irreversible damage of hepatocyte occurs in the form of necrosis. The process of necrosis begins with picnosis, characterized by the darkening and shrinkage of the cell nucleus[25,26]. Based on Fig 2B demonstrates a significant increase in necrosis damage in the 4mg/L group compared to the control group. It is plausible that the higher concentration of paracetamol administered in this group exceeded the threshold for cellular tolerance, leading to more severe cellular injury and subsequent necrosis. However, no significant variations among the treatment groups in terms of picnosis damage (Fig 2C). The lack of significant differences suggests that the levels of picnosis damage were similar across the treatment groups. This finding might be attributed to the specific characteristics of the experimental model, or the dose range of paracetamol administered, which may not have been sufficient to induce significant variations in picnosis damage. Further research and investigation are warranted to better understand the unexpected results regarding vacuolization and the contrasting findings related to necrosis and picnosis damage in different treatment groups. Additional factors, such as the duration of exposure, genetic variations, and potential interactions with other substances, should be considered to provide a comprehensive explanation for the observed outcomes.

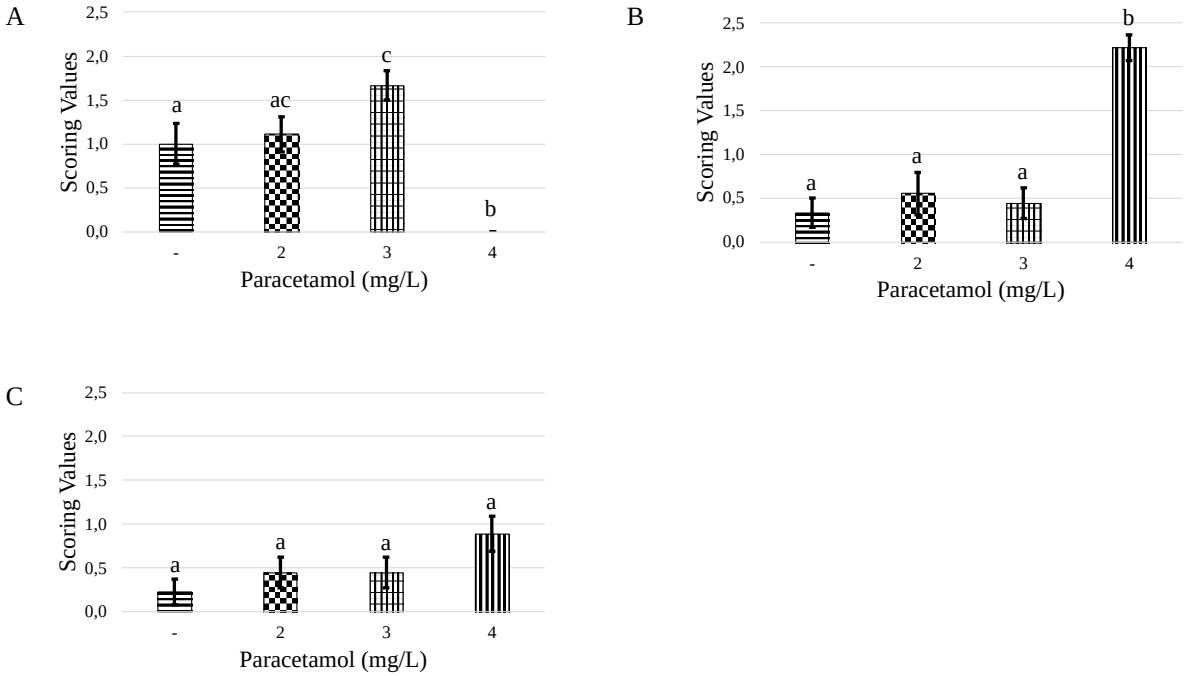

Fig 2. Effect of high-dose paracetamol on vacuolization (A), necrosis (B), and picnosis (C) of hepatocytes in yellow rasbora (*Rasbora lateristriata*). Different letters denote statistically significant differences. (–): control group

Conclusion

The histological analysis of yellow rasbora hepatic tissue exposed to various paracetamol concentrations demonstrated significant hepatic damage characterized by vacuolization, necrosis, and picnosis. The control group exhibited mild damage, indicating that even under normal conditions, the hepar may undergo some level of damage due to its detoxification function. The higher paracetamol concentrations led to increased necrosis damage, indicating a dose-dependent toxic effect.

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Author contributions

All authors contributed to the study design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by the first author and all authors commented on early versions of the manuscript. All authors read and approved the final manuscript.