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ARTICLE

HISTOPATHOLOGICAL EVALUATION OF THE HEPATOPROTECTIVE EFFECT OF PINEAPPLE PEEL EXTRACT (*ANANAS COMOSUS*) IN PARACETAMOL-INDUCED WISTAR RATS

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ABSTRACT

Paracetamol forms the poisonous metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and hepatocellular damage and is a main cause of DILI. Natural antioxidants may protect the liver. An agricultural byproduct, pineapple peel (Ananas comosus) includes antioxidant and anti-inflammatory bromelain, flavonoids, and vitamin C. This study examined pineapple peel extract's hepatoprotective efficacy against paracetamol-induced liver injury in Wistar rats. In this post-test only control group study, 30 male Wistar rats were divided into six groups (n = 5): negative control, positive control (paracetamol 2.5 g/kg body weight), and four treatment groups receiving pineapple peel extract at 250, 500, 750, and 1,000 mg/kg body weight before paracetamol induction. Histopathological examination of liver tissues used Hematoxylin–Eosin staining and Suzuki scoring. Data were analysed using Kruskal–Wallis and Mann–Whitney. Liver damage ratings differed between groups (p = 0.003). The highest efficient hepatoprotective dose was 750 mg/kg (p = 0.043). In conclusion, pineapple peel extract protects the liver from paracetamol-induced liver damage.

Keywords: *Ananas comosus*; Hepatoprotective; Paracetamol; Liver histopathology.

АБСТРАКТ

Парацетамол образует токсичный метаболит N-ацетил-п-бензохинон имин (NAPQI), который вызывает окислительный стресс и повреждение гепатоцитов и является основной причиной DILI. Природные антиоксиданты могут защищать печень. Сельскохозяйственный побочный продукт, кожура ананаса (Ananas comosus), содержит антиоксидантный и противовоспалительный бромелайн, флавоноиды и витамин С. В этом исследовании была изучена гепатопротекторная эффективность экстракта ананасовой корки против повреждения печени, вызванного парацетамолом, у крыс Вистар. В этом исследовании с контролем только после теста 30 самцов крыс Вистар были разделены на шесть групп (n = 5): негативный контроль, позитивный контроль (парацетамол 2,5 г/кг массы тела) и четыре группы лечения, получающие экстракт ананасовой корки в дозах 250, 500, 750 и 1 000 мг/кг массы тела перед индукцией парацетамолом. Гистопатологическое исследование тканей печени проводилось с использованием окраски гематоксилин-эозином и оценки по шкале Судзуки. Данные были проанализированы с использованием тестов Краскала–Уоллиса и Манна–Уитни. Оценки повреждения печени различались между группами (p = 0.003). Наиболее эффективная гепатопротекторная доза составила 750 мг/кг (p = 0.043). В заключение, экстракт ананасовой корки защищает печень от повреждений, вызванных парацетамолом.

Ключевые слова: Ананас съедобный; Гепатопротекторный; Парацетамол; Гистопатология печени.

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INTRODUCTION

Liver injury can be caused by various factors, including exposure to drugs, infections, alcohol consumption, and metabolic disorders. One of the drugs most frequently associated with hepatotoxicity is paracetamol, which is widely used as an analgesic and antipyretic. Although it is generally considered relatively safe at therapeutic doses, excessive consumption of paracetamol can lead to severe liver damage. This toxicity mechanism is primarily related to the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which, when produced in excessive amounts, depletes hepatic glutathione reserves and induces oxidative stress that ultimately results in hepatocellular injury.[1,2]

Drug-induced liver injury (DILI) remains an important clinical problem worldwide. The widespread availability of paracetamol as an over-the-counter medication further increases the risk of inappropriate use and the potential for overdose. In Indonesia, paracetamol is the most commonly used drug for self-medication, followed by other analgesics such as mefenamic acid, aspirin, and ibuprofen.[3]

Excessive metabolism of paracetamol leads to increased formation of the toxic metabolite NAPQI. Under normal conditions, NAPQI is detoxified through conjugation with glutathione. However, in cases of overdose or prolonged use, hepatic glutathione reserves may become depleted, resulting in the accumulation of NAPQI and triggering oxidative stress. This process can damage cell membranes and hepatocytes, ultimately leading to acute liver failure. Therefore, numerous studies have focused on identifying natural compounds with hepatoprotective properties to prevent or reduce paracetamol-induced liver injury.[2,4]

Pineapple (*Ananas comosus*) is a tropical fruit known to contain various bioactive compounds, including phenolic compounds, vitamin C, and the proteolytic enzyme bromelain, which have been reported to exhibit antioxidant and hepatoprotective activities. Pineapple peel, which constitutes an agricultural by-product of the fruit, has been reported to contain bromelain at a concentration of approximately 0.05–0.08% and accounts for about 28.6% of the total weight of the pineapple fruit. The presence of these bioactive compounds suggests that pineapple peel has potential as a natural source of beneficial biological agents.[5,6]

The utilization of agricultural waste such as pineapple peel as a source of bioactive compounds remains relatively limited, and studies specifically evaluating the hepatoprotective effect of pineapple peel extract against paracetamol-induced liver injury using a histopathological approach are still scarce. Therefore, this study was conducted to evaluate the hepatoprotective effect of pineapple peel extract on paracetamol-induced hepatotoxicity in male Wistar rats through histopathological examination of liver tissue following administration of the extract at doses of 250, 500, 750, and 1,000 mg/kg body weight.

Notably, although plant-derived compounds such as flavonoids and phenolics are widely recognized for their antioxidant properties, accumulating evidence suggests that at higher concentrations these compounds may exert pro-oxidant effects. This paradoxical behavior may lead to increased generation of reactive oxygen species and contribute to cellular damage rather than protection. Therefore, a comparative evaluation of histopathological severity across higher dose groups, particularly between 750 mg/kg and 1,000 mg/kg, is essential to determine whether increasing doses confer additional hepatoprotection or instead induce potential pro-oxidant effects.

MATERIAL AND METHODS

This study was a laboratory experimental study aimed at evaluating the effect of pineapple peel extract (*Ananas comosus*) on liver histopathological features in paracetamol-induced rats. A post-test only control group design was used, consisting of

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two control groups and four treatment groups receiving different extract doses. After the treatment period, liver tissues were collected and examined histopathologically to assess the degree of hepatocellular damage. Histopathological evaluation was performed independently by two anatomical pathologists.

Preparation of Pineapple Peel Extract: Pineapple peels (*Ananas comosus*) were washed, air-dried in the shade at room temperature, and ground into coarse powder. The powder was extracted by maceration using 96% ethanol for 72 hours (3 × 24 h) with occasional stirring. The mixture was then filtered, and the filtrate was concentrated using a water bath at 40–50°C to obtain a viscous extract. The extract was stored in a sealed container at 4°C until use. The extraction yield was 11.64% (w/w), calculated as the ratio of the dry extract weight to the initial dry weight of the plant material.

The volume of extract administered was calculated using the formula: Volume (mL) = (Dose (mg/kg BW) × Body weight (kg)) / Extract concentration (mg/mL)

Quantitative phytochemical analysis of the pineapple peel extract was not performed in this study. However, based on previous reports, pineapple peel is known to contain bioactive compounds such as flavonoids, bromelain, and vitamin C, which are associated with antioxidant and hepatoprotective activities. The observed hepatoprotective effects in this study were interpreted in relation to these reported bioactive constituents.

Experimental Animals: The experimental animals used in this study were male Wistar rats (*Rattus norvegicus*) obtained from Biofarma. The rats were acclimatized for seven days at the Animal Laboratory of Universitas Jenderal Achmad Yani with standard feed and drinking water provided *ad libitum*. The rats had a body weight of 200–250 g and were 8–10 weeks old. During the experiment, the animals were maintained under controlled environmental conditions with a temperature of 22–25°C, relative humidity of 50–70%, and a 12-hour light–dark cycle.

The inclusion criteria were male Wistar rats weighing 200–250 g, aged 8–10 weeks, clinically healthy, active, and without anatomical abnormalities. The exclusion criteria included rats that died during the acclimatization period or experienced a body weight loss ≥10% during the study.

Sample Size and Randomization

The sample size was determined using the Federer formula, which is commonly applied in experimental studies. Based on the six experimental groups used in this study, the minimum required sample size was four rats per group. To anticipate potential sample loss during the study, the number was increased by 20%, resulting in a total of 30 Wistar rats, with five rats in each group.

The allocation of experimental animals into treatment groups was performed randomly using a stratified simple randomization method based on body weight. Each rat was assigned an identification code, and the randomization process was carried out using the random function in Microsoft Excel to ensure a relatively balanced distribution of body weight among groups and to minimize potential research bias.

Treatment Groups

a) A total of 30 rats were divided into six groups (n = 5 per group) as follows:
b) Negative control (K1): received standard feed without treatment.
c) Positive control (K2): received standard feed and paracetamol 2.5 g/kg body weight (BW).
d) K3: pineapple peel extract 250 mg/kg BW + paracetamol 2.5 g/kg BW.
e) K4: pineapple peel extract 500 mg/kg BW + paracetamol 2.5 g/kg BW.
f) K5: pineapple peel extract 750 mg/kg BW + paracetamol 2.5 g/kg BW.
g) K6: pineapple peel extract 1,000 mg/kg BW + paracetamol 2.5 g/kg BW.

The treatments were administered for 14 days, and necropsy was performed on day 15 for liver tissue collection. This study was approved by the Research Ethics Committee of

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the Faculty of Medicine, Universitas Jenderal Achmad Yani (Approval No: 031/UH1.10/2025; Protocol No: H1.2509.031) and conducted in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement) for animal research. Liver injury was induced by oral administration of paracetamol at a dose of 2.5 g/kg body weight (BW), which is known to cause hepatic damage characterized by centrilobular necrosis, steatosis, and inflammatory cell infiltration.[7]

The negative control group (K1) received standard feed only (20–25 g/rat/day) for 14 days. The positive control group (K2) received paracetamol 2.5 g/kg BW from day 8 to day 14. The treatment groups (K3–K6) received pineapple peel extract orally at doses of 250, 500, 750, and 1,000 mg/kg BW, respectively, for 14 consecutive days, along with paracetamol 2.5 g/kg BW from day 8 to day 14. All treatments were administered using a gastric gavage.

Euthanasia and Liver Tissue Collection. At the end of the treatment period, the rats were anesthetized using carbon dioxide (CO_2) gas until the loss of pain reflexes was achieved, followed by euthanasia. The liver was removed through a necropsy procedure, washed with 0.9% physiological NaCl solution, and fixed in 10% formalin for histopathological examination.

Histopathological Preparation. Liver tissues were fixed and processed through graded alcohol dehydration, followed by clearing in xylene and embedding in paraffin. The paraffin blocks were sectioned at a thickness of 4–5 µm using a microtome and mounted on glass slides. The sections were then stained with hematoxylin–eosin (H&E) prior to microscopic examination. Histopathological Evaluation Liver tissues were examined under a light microscope at 400× magnification, with five randomly selected fields of view around the central vein (zone 3). Histological damage was assessed using the Suzuki scoring method (1977), a semi-quantitative scale evaluating sinusoidal congestion, hepatocellular necrosis, and ballooning degeneration.[7]

Each parameter was scored from 0 to 4, and the total score was calculated to represent the overall severity of liver injury. The scoring was performed independently by two observers.

Data were analyzed based on their distribution. Normally distributed data were compared using one-way ANOVA followed by the Least Significant Difference (LSD) post hoc test, while non-normally distributed data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney test. A p-value < 0.05 was considered statistically significant

RESULT

This study used 30 white rats (*Rattus norvegicus*), which were divided into six groups: a negative control group (K1), a positive control group (K2), and four treatment groups receiving pineapple peel extract at doses of 250 mg/kg BW (K3), 500 mg/kg BW (K4), 750 mg/kg BW (K5), and 1,000 mg/kg BW (K6). The study aimed to evaluate the effect of pineapple peel extract on the histopathological features of the liver in paracetamol-induced rats. The degree of liver damage was assessed using the Suzuki scoring system, which includes the parameters of hepatocyte degeneration, congestion, and hepatocellular necrosis.

Statistical Analysis of Histopathological Scores

Based on Table 1, the descriptive statistical analysis showed that each group consisted of five rats (n = 5). The negative control group (K1) exhibited the lowest mean histopathological damage score of 1.40, with a range of 1.2–2.0, whereas the positive control group (K2) showed the highest mean damage score of 3.60, with a range of 2.6–4.6. These findings indicate that administration of paracetamol at a dose of 2.5 g/kg BW was capable of inducing marked liver injury in the experimental animals.

In contrast, the treatment groups receiving pineapple peel extract (K3–K6) demonstrated lower mean damage scores compared with the positive control group, ranging from 1.72 to

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2.20. Among all treatment groups, the 750 mg/kg BW dose (K5) showed the lowest mean score (1.72), whereas the 1,000 mg/kg BW dose (K6) had a slightly higher mean score (2.20).

Table 1. Descriptive Statistical Analysis of Liver Histopathological Scores

Group	n	Mean ± SD	Minimum–Maximum
K1	5	1.40 ± 0.35	1.2-2.0
K2	5	3.60 ± 0.75	2.6-4.6
K3	5	1.88 ± 0.27	1.6-2.2
K4	5	1.80 ± 0.47	1.2-2.2
K5	5	1.72 ± 0.48	1.2-2.2
K6	5	2.20 ± 0.40	1.6-2.6

Notes:
a) K1 = Negative control
b) K2 = Positive control (paracetamol 2.5 g/kg BW)
c) K3 = Pineapple peel extract 250 mg/kg BW + paracetamol
d) K4 = Pineapple peel extract 500 mg/kg BW + paracetamol
e) K5 = Pineapple peel extract 750 mg/kg BW + paracetamol
f) K6 = Pineapple peel extract 1,000 mg/kg BW + paracetamol

The difference in the mean scores between K1 and K2 indicates that high-dose paracetamol was able to induce significant hepatocellular damage. This finding is consistent with the known mechanism of paracetamol toxicity, which involves the formation of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) that can trigger oxidative stress and subsequent liver cell injury.

In the treatment groups, the lower mean damage scores compared with K2 suggest that pineapple peel extract exerts a protective effect on liver tissue. The 750 mg/kg BW dose showed the lowest mean score, indicating descriptively that this dose may be the most effective in reducing hepatocellular damage. This effect is likely associated with the presence of bioactive compounds in pineapple peel, such as bromelain, flavonoids, and vitamin C, which possess antioxidant and anti-inflammatory activities that may help protect liver cells from oxidative stress–induced damage.[6,8]

However, increasing the dose to 1,000 mg/kg BW did not result in a greater reduction in the damage score compared with the 750 mg/kg BW dose. This finding suggests that increasing the dose does not necessarily lead to an enhanced protective effect, which is consistent with the concept that some natural compounds exhibit an optimal dose range for exerting their biological effects.

The Shapiro–Wilk normality test showed that most groups had a normal data distribution ($p > 0.05$), except for the negative control group (K1), which demonstrated a non-normal distribution ($p = 0.01$). Therefore, the analysis of differences among groups was subsequently performed using nonparametric tests. The Levene's test for homogeneity of variance showed a p-value of 0.192 ($p > 0.05$), indicating that the variances among groups were homogeneous. A Kruskal–Wallis test was then conducted to determine differences in histopathological scores among the groups. The test result showed a statistic value of 17.941 with $p = 0.003$, indicating a significant difference among the experimental groups.

Further analysis using the Mann–Whitney test revealed a significant difference between the negative control group (K1) and the positive control group (K2) with a p-value of 0.002. This result confirms that paracetamol induction at a dose of 2.5 g/kg BW was capable of producing significant liver damage.

Among the treatment groups, only the pineapple peel extract dose of 750 mg/kg BW showed a significant difference compared with the positive control group ($p = 0.043$). The other doses did not show statistically significant differences ($p > 0.05$). These findings indicate that 750 mg/kg BW was the most effective dose in reducing the degree of liver damage in this study. The absence of significant differences among the other extract

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doses suggests that increasing or decreasing the dose relative to 750 mg/kg BW did not provide additional hepatoprotective effects.

DISCUSSION

Histopathological Features of Rat Liver

In the negative control group (K1), the liver tissue appeared normal, with a regular arrangement of hepatocytes and open sinusoids without pathological alterations. In contrast, the positive control group (K2) induced by paracetamol showed parenchymatous degeneration of hepatocytes and sinusoidal congestion. The hepatocytes exhibited cellular swelling with paler cytoplasm, while the sinusoids were dilated and contained an increased number of erythrocytes. No evidence of hepatocyte necrosis was observed. These alterations are likely associated with the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes intracellular glutathione (GSH), increases oxidative stress, and induces lipid peroxidation leading to hepatocellular injury..[2,4,9]

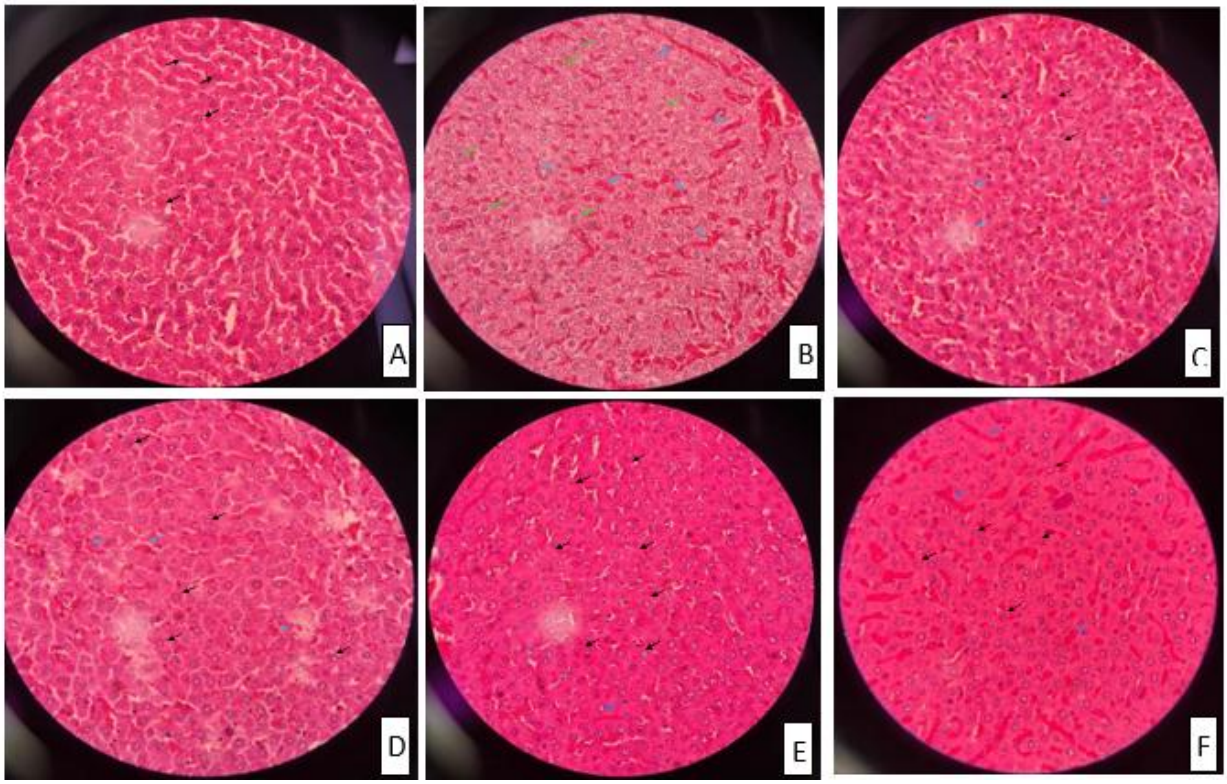

Figure 1. Histopathological features of the liver in Wistar rats across different experimental groups.

(A) Negative control; (B) positive control (paracetamol 2.5 g/kg BW); (C) pineapple peel extract 250 mg/kg BW; (D) pineapple peel extract 500 mg/kg BW; (E) pineapple peel extract 750 mg/kg BW; (F) pineapple peel extract 1,000 mg/kg BW. Black arrows indicate normal hepatocytes, blue arrows indicate parenchymatous degeneration of hepatocytes, and green arrows indicate sinusoidal congestion. (Hematoxylin–Eosin staining, Obj.40x).

The treatment groups receiving pineapple peel extract demonstrated a lower degree of hepatocellular damage compared with the positive control group. At a dose of 250 mg/kg BW (K3), parenchymatous degeneration began to decrease, although sinusoidal congestion was still observed. Increasing the dose to 500 mg/kg BW (K4) resulted in a further reduction in both hepatocellular degeneration and sinusoidal congestion The mildest degree of liver damage was observed at the 750 mg/kg BW dose (K5), in which most hepatocytes maintained a near-normal morphology with only mild sinusoidal congestion.

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These findings indicate that pineapple peel extract exerts a protective effect against paracetamol-induced hepatocellular damage.

Meanwhile, in the 1,000 mg/kg BW dose group (K6), the morphological appearance of hepatocytes did not demonstrate greater protection compared with the 750 mg/kg BW dose. This phenomenon may be related to the properties of certain flavonoid compounds, which at high concentrations can act as pro-oxidants through interactions with transition metal ions, leading to the generation of additional free radicals. This condition may explain why the hepatoprotective effect of pineapple peel extract appears to be most optimal at the dose of 750 mg/kg BW, consistent with the U-shaped dose–response concept commonly observed in natural compounds. [10, 11,12]

A comparative analysis between the 750 mg/kg BW (K5) and 1,000 mg/kg BW (K6) groups revealed a paradoxical reduction in hepatoprotective effect at the higher dose. Although both doses demonstrated lower histopathological scores compared to the positive control, the 750 mg/kg BW group showed a lower mean score (1.72) than the 1,000 mg/kg BW group (2.20), indicating superior hepatoprotection.

A non-linear, U-shaped dosage–response connection is suggested by this research. According to this relationship, increasing the dose beyond an ideal level may not improve the therapeutic impact, and it may even decrease it depending on the circumstances. At high quantities, flavonoids have a pro-oxidant action, which could be one possible reason for this phenomenon. It is possible for flavonoids to undergo redox cycling and produce reactive oxygen species (ROS) when they are in the presence of transition metal ions. This results in flavonoids instead contributing to oxidative stress rather than alleviating it.[10, 11]

Numerous research have reported on this phenomenon, which demonstrates that high doses of antioxidants produced from plants have the potential to perform dual roles as both antioxidants and pro-oxidants, depending on the quantity of the antioxidants and the environment in which they are found within the cell. As a result, the decreased efficiency that was found at the dose of 1,000 mg/kg body weight may be a reflection of the beginning of pro-oxidant effects that are directed against the hepatoprotective capabilities of the extract.
The existence of bioactive substances in pineapple peel (Ananas comosus) has been documented in earlier research to be present in pineapple peel. These chemicals include bromelain, flavonoids, and vitamin C. It is possible that the hepatoprotective action of pineapple peel extract is connected with the presence of these active compounds. The antioxidant and anti-inflammatory properties of these substances are also well-known phenomena in the scientific community. Because flavonoids are known to suppress lipid peroxidation and neutralise free radicals, they are able to protect hepatocyte cell membranes from being damaged. In addition, flavonoids have the potential to regulate the expression of genes that are involved in the oxidative stress response. This includes the upregulation of endogenous antioxidant enzymes like glutathione (GSH), superoxide dismutase (SOD), and catalase. These enzymes are essential in the process of neutralising reactive oxygen species (ROS) that are produced during the toxic metabolism of paracetamol.[13, 14]

Bromelain possesses anti-inflammatory activity that can suppress the production of inflammatory mediators such as TNF-α, IL-1β, and IL-6, as well as inhibit the activation of nuclear factor-kappa B (NF-κB) and the MAPK signaling pathway, thereby reducing damage to the hepatic parenchyma. [15,16,17,18]

Vitamin C (ascorbic acid) present in pineapple peel acts as a direct antioxidant by neutralizing reactive oxygen species (ROS) and as an indirect antioxidant by increasing glutathione (GSH) levels and enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

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These mechanisms help protect hepatocyte membranes and cellular structures from oxidative damage caused by the toxic metabolism of paracetamol, thereby supporting the protective effect against liver injury.[19,20]

Overall, the bioactive compounds present in pineapple peel provide protection to hepatocytes through several mechanisms, including direct antioxidant activity by neutralizing reactive oxygen species (ROS), indirect antioxidant effects through enhancement of endogenous defense systems such as glutathione (GSH), superoxide dismutase (SOD), and catalase, and anti-inflammatory effects by suppressing NF-κB activation and the production of pro-inflammatory cytokines. These mechanisms may contribute to the reduction in the degree of parenchymatous degeneration and sinusoidal congestion induced by paracetamol. The findings indicate that pineapple peel extract has potential as a hepatoprotective agent, with the 750 mg/kg BW dose showing the most effective protection in maintaining liver histopathological morphology.

Although biochemical parameters such as ALT, AST, and bilirubin were not evaluated in this study, histopathological assessment using the Suzuki scoring system provides direct structural evidence of hepatocellular injury. Therefore, the findings should be interpreted primarily at the morphological level.

Paracetamol-induced hepatotoxicity is known to involve the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), leading to depletion of intracellular glutathione (GSH) and increased oxidative stress. In this context, oxidative stress biomarkers such as malondialdehyde (MDA) and GSH levels would provide more comprehensive insight into the biochemical mechanisms underlying liver injury and the protective effects of pineapple peel extract.

However, since such biochemical and molecular analyses were not performed, the present study is limited to histopathological outcomes. Despite this limitation, the observed reduction in Suzuki scores in the treatment groups suggests a potential hepatoprotective effect of pineapple peel extract. Future studies incorporating serum liver enzymes (ALT, AST), oxidative stress markers (MDA and GSH), and molecular pathways are recommended to further elucidate the functional and mechanistic basis of the observed hepatoprotective activity.

Study Limitations and Futur Directions

This study has several limitations that should be acknowledged. First, phytochemical characterization of the pineapple peel extract was not performed; therefore, the specific concentrations of bioactive compounds such as flavonoids, bromelain, and vitamin C could not be determined. Although previous studies have reported their presence and associated biological activities, the absence of direct quantification limits the ability to correlate specific compounds with the observed hepatoprotective effects. In future studies, detailed phytochemical profiling and compound isolation (e.g., flavonoids such as quercetin and kaempferol) are recommended to identify the active constituents responsible for the observed activity.

Secondly, the hepatoprotective effects were evaluated exclusively based on histopathological assessment, without the measurement of biochemical liver function markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as oxidative stress biomarkers. This was done in order to determine whether or not the agents were effective. For this reason, it is recommended that future research combine biochemical and molecular investigations, such as those involving malondialdehyde (MDA), glutathione (GSH), and inflammatory signalling pathways, in order to gain a deeper understanding of the mechanisms that occur under the surface. Third, the study's statistical power may be limited due to the very small sample size taken into consideration.

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When doing future research, it is recommended that larger experimental cohorts be used in order to improve the robustness and reproducibility of the findings. Additionally, in addition to resolving these constraints, future research should also concentrate on dose optimisation. This can be accomplished by reducing the range of doses from 500 mg/kg to 1,000 mg/kg body weight in order to ascertain the precise therapeutic dose that is ideal. In addition, because the highest dose was shown to have a lower efficacy, it is required to conduct sub-chronic toxicity studies in order to examine the safety profile of pineapple peel extract, particularly when it is administered in quantities that are high. It is vital to conduct these research in order to determine if the potential toxicity of the extract itself or a pro-oxidant biological action is responsible for the diminished efficacy at a dosage of 1,000 mg/kg bodily weight.

CONCLUSION

Induction of paracetamol at a dose of 2.5 g/kg BW in Wistar rats caused liver injury characterized by increased hepatocellular parenchymatous degeneration and sinusoidal congestion. Administration of pineapple peel extract (*Ananas comosus*) at various doses demonstrated a hepatoprotective effect by reducing the degree of liver damage. The most optimal protective effect was observed at a dose of 750 mg/kg BW.

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DECLARATIONS

Author Contributions. All authors contributed to the conceptualization and design of the study, data collection, data analysis and interpretation, and the preparation and revision of the manuscript.

All authors have read and approved the final version of the manuscript.

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REFERENCES

1. Athbhaiya G, Tiwari A, Choudhary R, Samal PK. Pharmacological and toxicological effects of paracetamol: current knowledge and a review. Drug Chem Toxicol. 2026 Jan 5:1-24. Do: 10.1080/01480545.2025.2604674. Epub ahead of print. PMID: 41492720.
2. Chidiac AS, Buckley NA, Noghrehchi F, Cairns R. Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. Expert Opin Drug Metab Toxicol. 2023 Jan-Jun;19(5):297-317. DOI: https://doi.org/10.1080/17425255.2023.2223959. PMID: 37436926.
3. Larasanti G, Yurina V, Yunita EP. Korelasi antara aspek sosiodemografi dan tingkat pengetahuan orang tua terhadap rasionalitas swamedikasi parasetamol dalam pengobatan demam anak. PSCR: J Pharm Sci Clin Res. 2025;10(3):387–402. DOI: https://doi.org/10.20961/jpscr.v10i3.91407
4. Liao J, Lu Q, Li Z, Li J, Zhao Q and Li J (2023) Acetaminophen-induced liver injury: Molecular mechanism and treatments from natural products. Front. Pharmacol. 14:1122632. DOI: https://doi.org/10.3389/fphar.2023.1122632
5. Aminina, A. (2025). Utilization of Pineapple (Ananas comosus) Skin Waste as an Antioxidant for Constipation Medicine. *Jurnal EduHealth*, *16*(02), 857–863. Retrieved from https://ejournal.seaninstitute.or.id/index.php/healt/article/view/6571
6. Nadia, S., Julianty, S. M., Tambunan, I. J., & Fujiko, M. (2023). Testing the antioxidant activity of ethanol extract and pineapple skin juice (Ananas comosus (L) Merr) using the radical scavenger method. *Journal of Pharmaceutical and Sciences*, *6*(5), 422–431. https://doi.org/10.36490/journal-jps.com.v6i5-si.455
7. Islam MT, Quispe C, Islam MA, Ali ES, Saha S, Asha UH, et al. Effects of nerol on paracetamol-induced liver damage in Wistar albino rats. Biomed Pharmacother. 2021;140:111732. DOI: https://doi.org/10.1016/j.biopha.2021.111732
8. Lourenço SC, Campos DA, Gómez-García R, Pintado M, Oliveira MC, Santos DI, Corrêa-Filho LC, Moldão-Martins M, Alves VD. Optimization of Natural Antioxidants Extraction from Pineapple Peel and Their

Stabilization by Spray Drying. Foods. 2021 Jun 1;10(6):1255. DOI: https://doi.org/10.3390/foods10061255
9. Sivagurunathan N, Calivarathan L. Inflammasome Activation as a Key Driver of Acetaminophen-induced Hepatotoxicity: Mechanisms and Emerging Therapeutics. *Gene Expr*. 2025;24(3):235-249. DOI: https://doi.org/10.14218/GE.2025.00001
10. Chobot V, Hadacek F. Exploration of pro-oxidant and antioxidant activities of the flavonoid myricetin. Redox Rep. 2011;16(6):242-7. DOI: https://doi.org/10.1179/1351000211Y.0000000015 Erratum in: Redox Rep. 2012;17(4):180. PMID: 22195992; PMCID: PMC6837545.
11. Desjardins D, Cacho-Valadez B, Liu JL, Wang Y, Yee C, Bernard K, Khaki A, Breton L, Hekimi S. Antioxidants reveal an inverted U-shaped dose-response relationship between reactive oxygen species levels and the rate of aging in Caenorhabditis elegans. Aging Cell. 2017 Feb;16(1):104-112. DOI: https://doi.org/10.1111/acel.12528. Epub 2016 Sep 28. PMID: 27683245; PMCID: PMC5242296
12. Simunkova, M.; Barbierikova, Z.; Jomova, K.; Hudecova, L.; Lauro, P.; Alwasel, S.H.; Alhazza, I.; Rhodes, C.J.; Valko, M. Antioxidant vs. Prooxidant Properties of the Flavonoid, Kaempferol, in the Presence of Cu(II) Ions: A ROS-Scavenging Activity, Fenton Reaction and DNA Damage Study. *Int. J. Mol. Sci.* 2021, *22*, 1619. DOI: https://doi.org/10.3390/ijms22041619
13. Karlina, A. (2023). Review Literatur : Efektivitas Tumbuhan yang Mengandung Senyawa Flavonoid sebagai Antioksidan yang Berpotensi sebagai Hepatoprotektor. *Biocaster : Jurnal Kajian Biologi*, *3*(1), 40–45. https://doi.org/10.36312/bjkb.v3i1.157
14. Porat AE, Laut MM, Ndaong NA. Uji efektivitas ekstrak daun rumput paitan (Paspalum conjugatum) terhadap kesembuhan luka insisi pada tikus putih (Rattus norvegicus). *Jurnal Kajian Veteriner*. 2024;12(2):207–220. DOI: https://doi.org/10.35508/jkv.v12i2.17032
15. Huang JR, Wu CC, Hou RC, Jeng KC. Bromelain inhibits lipopolysaccharide-induced cytokine production in human THP-1 monocytes via the removal of CD14. Immunol Invest. 2008;37(4):263-77 DOI: https://doi.org/10.1080/08820130802083622 PMID: 18569070.
16. Nobre TA, Sousa AA, Pereira IC, Pedrosa-Santos AMC, Lopes LO, Debia N, El-Nashar HAS, El-Shazly M, Islam MT, de Castro e Sousa JM, Torres-Leal FL. Bromelain as a natural anti-inflammatory drug: a systematic review. *Nat Prod Res.* 2025;39(5):1258–1271. DOI: https://doi.org/10.1080/14786419.2024.2342553
17. Alves Nobre T, de Sousa AA, Pereira IC, Carvalho Pedrosa-Santos ÁM, Lopes LO, Debia N, El-Nashar HAS, El-Shazly M, Islam MT, Castro E Sousa JM, Torres-Leal FL. Bromelain as a natural anti-inflammatory drug: a systematic review. Nat Prod Res. 2025 Mar;39(5):1258-1271. DOI: https://doi.org/10.1080/14786419.2024.2342553. Epub 2024 Apr 27. PMID: 38676413.
18. Insuan O, Janchai P, Thongchuai B, Chaiwongsa R, Khamchun S, Saoin S, Insuan W, Pothacharoen P, Apiwatanapiwat W, Boondaeng A, Vaithanomsat P. Anti-Inflammatory Effect of Pineapple Rhizome Bromelain through Downregulation of the NF-κB- and MAPKs-Signaling Pathways in Lipopolysaccharide (LPS)-Stimulated RAW264.7 Cells. Curr Issues Mol Biol. 2021 May 7;43(1):93-106. doi: 10.3390/cimb43010008. Erratum in: Curr Issues Mol Biol. 2025 Dec 24;48(1):17. DOI: https://doi.org/10.3390/cimb48010017 . PMID: 34067064; PMCID: PMC8929103.
19. Gęgotek A, Skrzydlewska E. Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. Antioxidants (Basel). 2022 Oct 7;11(10):1993. DOI: https://doi.org/10.3390/antiox11101993 PMID: 36290716; PMCID: PMC9598715.
20. Gęgotek A, Skrzydlewska E. Ascorbic acid as antioxidant. Vitamins and Hormones. 2023 ;121:247-270. DOI: https://doi.org/10.1016/bs.vh.2022.10.008 . PMID: 36707136.