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Evaluation of the Hepatoprotective Effects of the Flavonoid-Containing Ethyl Acetate Fraction Obtained after n-Hexane Defatting of Matoa (Pometia pinnata) Fruit Peel on a DMN-Induced Hepatic Fibrosis Model in Rats: A Histopathological and Serum Biomarker Approach

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ABSTRACT

Liver fibrosis is a progressive liver disorder and a major global health concern, prompting increasing interest in natural products with antifibrotic potential. Pometia pinnata, traditionally used to treat liver disorders, lacks sufficient experimental evidence supporting its hepatoprotective effects. This study evaluated the hepatoprotective and antifibrotic activity of a flavonoid-containing ethyl acetate fraction obtained after n-hexane defatting of Pometia pinnata fruit peel in a dimethylnitrosamine (DMN)-induced hepatic fibrosis rat model. Male rats were divided into six groups: normal control, DMN control, three treatment groups receiving the flavonoid fraction at doses of 50, 100, and 200 mg/kg body weight, and a silymarin-treated group (100 mg/kg). Dose selection was based on preliminary toxicity data and previous studies on flavonoid-rich extracts. Liver injury was assessed using serum biochemical markers (ALT, AST, ALP, and bilirubin), histopathological examination, and fibrosis scoring with Masson's Trichrome staining. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). DMN significantly induced hepatic injury and fibrosis, while flavonoid fraction treatment improved liver function and histological features in a dose-dependent manner. The highest dose showed effects comparable to silymarin and significantly reduced fibrosis scores ($r = -0.91$, $p < 0.001$). These findings

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indicate that Pometia pinnata fruit peel exhibits promising hepatoprotective and antifibrotic potential.

Keywords: *Pometia Pinnata, Flavonoids, Liver Fibrosis, Hepatoprotection, DMN, Silymarin, Antioxidant*

INTRODUCTION

Liver fibrosis is a progressive pathological condition characterized by excessive accumulation of extracellular matrix (ECM), particularly collagen, as a consequence of chronic hepatic injury (Zhang et al., 2020). Globally, liver fibrosis constitutes a major public health burden, contributing significantly to morbidity and mortality associated with chronic liver diseases. Recent epidemiological data indicate that chronic liver disease affects more than 1.5 billion individuals worldwide, with fibrosis serving as a key pathological driver leading to cirrhosis and hepatocellular carcinoma (HCC) (Ammar et al., 2022; Kumar et al., 2024). The global prevalence of liver fibrosis continues to rise, largely driven by increasing rates of non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease, chronic viral hepatitis, and metabolic syndrome, making liver fibrosis a leading contributor to disability-adjusted life years (DALYs) worldwide (Jiang et al., 2019; Wenbo et al., 2024).

Histopathologically, liver fibrosis is marked by architectural distortion, regenerative nodules, sinusoidal capillarization, and overexpression of profibrotic mediators, reflecting a gradual transition from normal hepatic structure toward progressive functional impairment (Wang et al., 2023). Although fibrosis was historically considered irreversible, emerging evidence demonstrates that early-stage fibrosis may regress if the underlying injury is effectively controlled (Wenbo et al., 2024). However, in the absence of appropriate intervention, fibrosis can progress to cirrhosis, HCC, or end-stage liver failure, underscoring the urgent need for effective antifibrotic strategies (Kumar et al., 2024).

Mechanistically, hepatic fibrosis arises from persistent hepatocellular injury caused by toxins, alcohol, viral infection, metabolic imbalance, and oxidative stress–mediated inflammation (Pulido-Hornedo et al., 2022). Hepatocyte damage triggers Kupffer cell activation and proinflammatory cytokine release, leading to hepatic stellate cell (HSC) activation and transdifferentiation into myofibroblast-like cells, which represent the central effector cells in fibrogenesis (Pulido-Hornedo et al., 2022). Among experimental models, dimethylnitrosamine (DMN) is widely used due to its ability to reproducibly induce oxidative stress, inflammation, hepatocellular necrosis, and collagen deposition that closely resemble human hepatic fibrosis pathology (Toklu et al., 2007; Abouelezz et al., 2023).

Oxidative stress plays a pivotal role in the initiation and progression of hepatic fibrosis by generating excessive reactive oxygen species (ROS) that overwhelm endogenous antioxidant defenses such as glutathione (GSH), superoxide dismutase (SOD), and catalase (Chen et al., 2024). This redox imbalance exacerbates lipid peroxidation, mitochondrial dysfunction, inflammatory

signaling, and HSC activation, thereby accelerating fibrotic progression and impairing liver regeneration (Ammar et al., 2022). Consequently, antioxidant-based therapeutic approaches have gained increasing attention as potential strategies to mitigate fibrosis development.

Despite advances in understanding fibrogenesis, current antifibrotic pharmacotherapies remain limited, expensive, and often associated with adverse effects, with no universally approved drug capable of fully reversing liver fibrosis (Kumar et al., 2024; Luo et al., 2025). These limitations highlight a critical gap in effective and safe antifibrotic treatments and have intensified interest in plant-derived bioactive compounds as alternative therapeutic candidates.

Flavonoids, a major class of plant polyphenols, have been extensively studied for their antioxidant, anti-inflammatory, and antifibrotic properties (Jiang et al., 2019). Experimental studies demonstrate that flavonoids can suppress nuclear factor kappa-B (NF-κB) signaling, reduce proinflammatory cytokines such as TNF-α and IL-6, inhibit HSC activation, and downregulate fibrogenic markers including transforming growth factor-β1 (TGF-β1) and α-smooth muscle actin (α-SMA) (Wang et al., 2023; Luo et al., 2025). Notably, flavonoid-rich fruit peel extracts often considered agricultural waste have shown promising hepatoprotective effects in chemically induced liver fibrosis models (Wei et al., 2020; Abouelezz et al., 2023).

Matoa (*Pometia pinnata*), a tropical fruit native to Indonesia and surrounding regions, has attracted growing scientific interest due to its rich phytochemical composition and traditional medicinal use (Suzuki et al., 2021). Phytochemical studies report that matoa peel contains high levels of flavonoids, phenolics, tannins, saponins, and terpenoids, suggesting significant pharmacological potential (Awaliyah Fahmi et al., 2023). Previous research has documented antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and hepatoprotective activities of matoa extracts (Sihotang et al., 2017; Adrian et al., 2021; Suzuki et al., 2021). However, existing studies primarily focus on crude extracts or metabolic disorder models and lack specific evaluation of purified flavonoid fractions in well-established hepatic fibrosis models.

Importantly, to date, no published study has systematically investigated the hepatoprotective and antifibrotic effects of a flavonoid-containing fraction derived from matoa fruit peel using a DMN-induced hepatic fibrosis model combined with comprehensive serum biochemical and histopathological analyses. This represents a significant gap in current knowledge, particularly given the growing interest in valorizing fruit peel waste as a source of bioactive compounds.

Therefore, this study aims to evaluate the hepatoprotective effects of the flavonoid fraction of *Pometia pinnata* fruit peel in a DMN-induced hepatic fibrosis rat model using serum biomarker assessment and histopathological analysis. The findings are expected to provide novel preclinical evidence supporting the potential application of matoa peel–derived flavonoids as natural antifibrotic agents and contribute to the development of cost-effective, plant-based therapeutic strategies.

METHODS

This study employed a laboratory-based experimental design using a randomized post-test-only control group approach to evaluate the hepatoprotective effects of a flavonoid-containing fraction derived from *Pometia pinnata* fruit peel in a dimethylnitrosamine (DMN)-induced hepatic fibrosis model, All experimental procedures were conducted in accordance with the ARRIVE guidelines and were approved by the Health Research Ethics Committee of Universitas Muhammadiyah Kudus, Indonesia (Ethical Clearance No.: 127/KEPK-FIK/UMK/2025).

All experimental procedures were conducted in accordance with the ARRIVE guidelines and approved by an institutional Animal Research Ethics Committee Ethical Clearance .

Adult male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 180–220 g, were acclimatized for 7 days under standard laboratory conditions prior to experimentation. Animals were randomly divided into six groups (n = 6 per group): normal control receiving distilled water without DMN induction; DMN control receiving DMN only; three treatment groups receiving DMN in combination with the flavonoid fraction at doses of 50, 100, and 200 mg/kg body weight; and a positive control group receiving DMN plus silymarin at 100 mg/kg body weight. Except for the normal control group, all groups were subjected to the same DMN induction protocol to ensure methodological consistency. Hepatic fibrosis was induced by intraperitoneal injection of DMN at a dose of 10 mg/kg body weight, dissolved in sterile 0.9% saline with a final injection volume of 1 mL/kg, administered three times per week for three consecutive weeks. The flavonoid fraction and silymarin were administered orally via gavage once daily throughout the 21-day induction period. Dose selection was based on preliminary toxicity evaluation and previously reported effective doses of flavonoid-rich plant extracts in experimental liver injury models, The defatting step using n-hexane was performed to remove non-polar constituents, thereby enriching the ethyl acetate fraction with flavonoid compounds.

The flavonoid-containing ethyl acetate fraction (obtained after n-hexane defatting) was prepared by maceration of dried matoa fruit peel powder in 70% ethanol at a 1:10 (w/v) ratio for 72 hours at room temperature, followed by concentration under reduced pressure at 40–45 °C and subsequent liquid liquid fractionation using n-hexane and ethyl acetate; the ethyl acetate fraction was collected and evaporated to dryness. Flavonoid presence was confirmed by thin-layer chromatography, and total flavonoid content was quantified using the aluminum chloride colorimetric method with absorbance measured at 415 nm. On day 22, rats were anesthetized using ketamine–xylazine, and blood samples were collected via the retro-orbital vein for serum biochemical analysis of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin using enzyme-linked colorimetric assay kits. Animals were then euthanized, and liver tissues were excised, fixed in 10% neutral buffered formalin, processed into paraffin blocks, sectioned at 4–5 μm thickness, and stained with hematoxylin eosin and Masson's Trichrome.

Histopathological evaluation was performed by a blinded pathologist using a light microscope, and fibrosis severity was assessed using a modified Ishak scoring system. Data were

expressed as mean ± standard deviation and analyzed using statistical software; normality and homogeneity were assessed using the Shapiro–Wilk and Levene's tests, respectively, followed by one-way ANOVA with Tukey's post hoc test for normally distributed data or the Kruskal–Wallis test with Dunn's post hoc test for non-normally distributed data, with $p < 0.05$ considered statistically significant.

RESULTS

Administration of the flavonoid-containing fraction of *Pometia pinnata* fruit peel demonstrated significant hepatoprotective effects against dimethylnitrosamine (DMN)-induced liver fibrosis in rats, as evidenced by serum biochemical parameters and histopathological evaluation. Statistical analysis showed significant differences among experimental groups for all measured parameters ($p < 0.05$).

1. Serum Biochemical Findings

DMN administration caused marked hepatic injury, as reflected by significant elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels in the DMN control group compared with the normal control group ($p < 0.001$). This finding confirms successful induction of liver injury and fibrosis. In contrast, rats treated with the flavonoid-containing fraction exhibited significant improvements in all serum biomarkers when compared with the DMN control group ($p < 0.05$), indicating hepatoprotective activity.

The improvement occurred in a dose-dependent manner, with progressively lower enzyme and bilirubin levels observed at doses of 50, 100, and 200 mg/kg body weight. The highest dose (200 mg/kg) produced biochemical values that were not significantly different from those observed in the silymarin-treated positive control group ($p > 0.05$), suggesting comparable hepatoprotective efficacy. Importantly, no reduction in ALT or other biomarkers was observed in the DMN control group itself, as this group served solely as a negative control representing untreated liver injury.

Table 1. Serum Levels of ALT, AST, ALP, and Total Bilirubin (Mean ± SD)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
Normal Control	45.3 ± 4.1	82.6 ± 7.4	95.4 ± 8.5	0.21 ± 0.03
DMN (Negative Control)	178.5 ± 12.3	264.3 ± 15.2	218.6 ± 19.4	0.93 ± 0.10
Flavonoid 50 mg/kg	126.8 ± 9.7	198.4 ± 12.6	172.3 ± 16.2	0.61 ± 0.07
Flavonoid 100 mg/kg	96.2 ± 7.1	147.9 ± 10.4	135.7 ± 14.1	0.42 ± 0.05
Flavonoid 200 mg/kg	65.4 ± 5.2	108.6 ± 9.3	112.8 ± 10.7	0.31 ± 0.04
Silymarin 100 mg/kg	62.1 ± 5.0	102.3 ± 8.7	110.5 ± 9.6	0.29 ± 0.03

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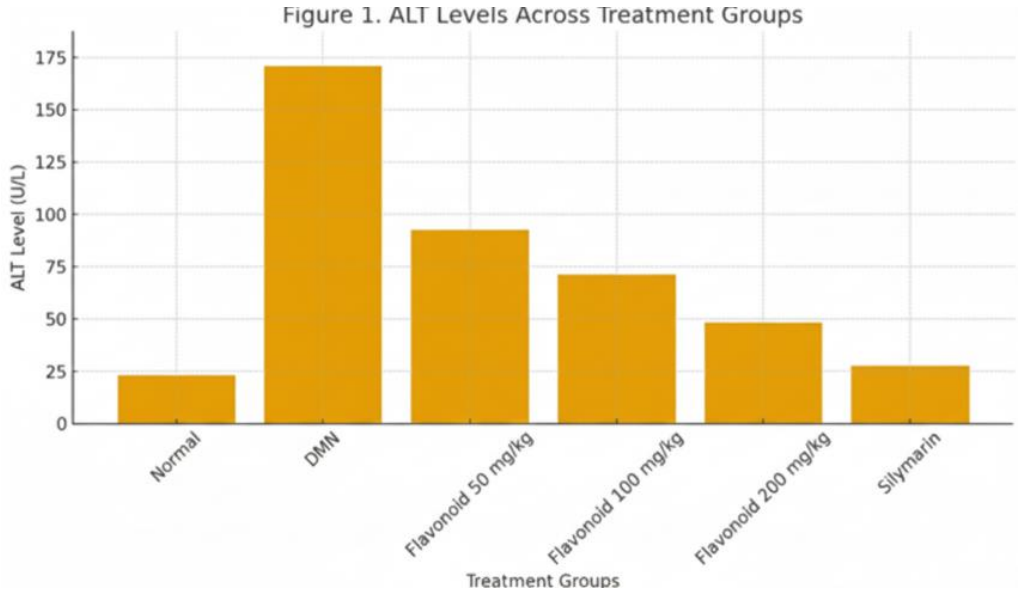

Figure 1. Trend of ALT Reduction Across Treatment Groups

Figure 1. Serum alanine aminotransferase (ALT) levels across experimental groups following dimethylnitrosamine (DMN)-induced hepatic injury and treatment with the flavonoid-containing fraction of *Pometia pinnata*. The DMN control group showed a marked elevation of ALT levels compared with the normal control group, confirming hepatocellular injury. Treatment with the flavonoid-containing fraction significantly reduced ALT levels compared with the DMN control in a dose-dependent manner, with the 200 mg/kg group exhibiting values comparable to the silymarin-treated positive control.

2. Histopathological Findings

Histopathological examination using hematoxylin–eosin (H&E) and Masson's Trichrome staining supported the biochemical findings. Liver sections from the normal control group displayed intact hepatic architecture, characterized by well-organized polygonal hepatocytes with centrally located nuclei, preserved sinusoidal structure, and absence of inflammatory infiltration or collagen deposition. In contrast, the DMN control group exhibited severe hepatic damage, including extensive hepatocyte necrosis, cytoplasmic vacuolation, inflammatory cell infiltration, sinusoidal distortion, and marked collagen accumulation, indicating advanced fibrotic changes.

Treatment with the flavonoid-containing fraction resulted in dose-dependent histological improvement. Rats receiving 50 mg/kg showed partial attenuation of hepatic damage, with reduced necrosis and inflammatory infiltration compared with the DMN control group. The 100 mg/kg dose further improved hepatic morphology, as evidenced by better preservation of lobular architecture and reduced collagen deposition. Notably, the 200 mg/kg dose produced substantial hepatoprotection, with liver tissue morphology approaching normal architecture, minimal inflammatory changes, and markedly reduced fibrosis. The silymarin-treated group exhibited

comparable histological improvement to the high-dose flavonoid group, confirming its established hepatoprotective effect.

Table 2. Representative Photomicrographs of Liver Tissue Following Treatment (H&E Staining, 400× Magnification).

Panel	Group	Description
A	**Normal Control**	Shows normal hepatic lobular architecture with polygonal hepatocytes, centrally placed nuclei, intact cell boundaries, and regular sinusoidal spacing. No signs of inflammation or necrosis are observed.
B	**DMN-Induced Liver Injury**	Demonstrates severe pathological changes including widespread hepatocyte necrosis, inflammatory cell infiltration, cytoplasmic vacuolation, and disrupted lobular organization. Sinusoids appear collapsed or irregular.
C	**Flavonoid 50 mg/kg**	Displays mild histological improvement with reduced necrosis and fewer inflammatory cells compared with the DMN group. Partial restoration of hepatocyte organization is observed.
D	**Flavonoid 100 mg/kg**	Shows moderate regeneration characterized by improved cell morphology, decreased vacuolation, and better-preserved lobular patterns relative to lower-dose treatment.
E	**Flavonoid 200 mg/kg**	Exhibits substantial tissue recovery with near-normal hepatic structure, clear nuclei, minimal inflammation, and well-defined sinusoidal pathways comparable to the normal control.
F	**Silymarin (Standard Drug)**	Shows improved hepatic histological features with reduced pathological alterations compared to the DMN control group, comparable to those observed in the high-dose flavonoid-treated group.

Histopathological evaluation of liver sections, as illustrated in the representative photomicrographs (Figure 2).

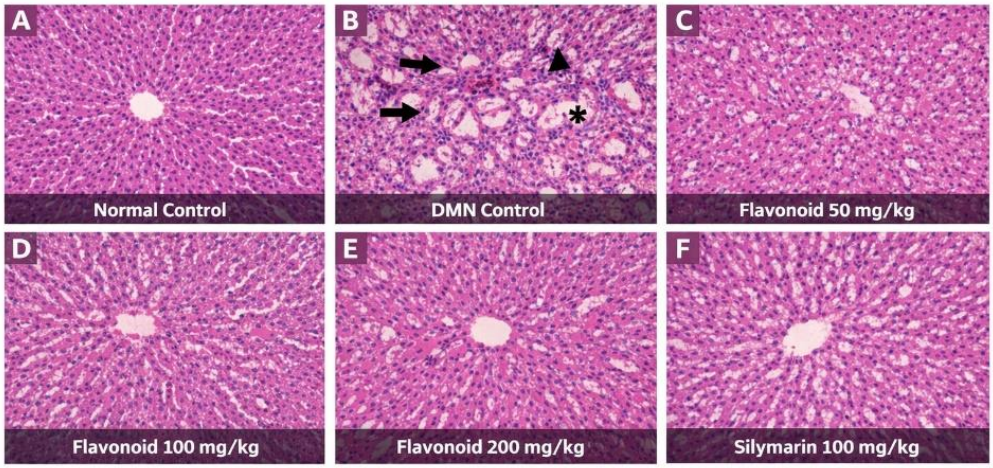

Figure 2. Illustrates Liver Histopathological Changes Across Experimental Groups

The normal control (A) shows intact hepatic architecture with radially arranged hepatocytes and no pathological alterations. In contrast, the DMN control (B) exhibits severe centrilobular hepatocyte necrosis, marked inflammatory cell infiltration, and cytoplasmic vacuolization, confirming DMN-induced hepatotoxicity.

Treatment with flavonoids at 50 mg/kg (C) results in partial improvement, with reduced tissue damage but residual vacuolization and mild inflammation. More pronounced recovery is observed at 100 mg/kg (D), characterized by decreased necrosis and improved hepatic architecture. The 200 mg/kg flavonoid group (E) demonstrates near-normal liver structure with minimal inflammatory changes, indicating a strong dose-dependent hepatoprotective effect.

The silymarin-treated group (F) shows comparable histological improvement to the high-dose flavonoid group, suggesting that flavonoid treatment at 200 mg/kg provides hepatoprotection similar to the standard reference drug.

Revealed distinct morphological differences among experimental groups. The normal control group exhibited preserved hepatic architecture with well-organized polygonal hepatocytes, centrally located nuclei, and regular sinusoidal distribution. In contrast, the DMN control group demonstrated pronounced pathological alterations, including hepatocyte necrosis, inflammatory cell infiltration, cytoplasmic vacuolation, sinusoidal distortion, and disruption of lobular architecture, confirming successful induction of hepatic injury.

Treatment with the flavonoid-containing fraction of *Pometia pinnata* was associated with dose-dependent histological improvement. Rats treated with 50 mg/kg showed partial attenuation of DMN-induced damage, characterized by reduced necrotic areas and mild architectural restoration. The 100 mg/kg dose resulted in further improvement, with decreased cellular degeneration and more organized lobular structure. Notably, the 200 mg/kg group exhibited markedly preserved hepatic morphology with minimal inflammatory infiltration, closely resembling the normal control group.

The silymarin-treated group, which also received DMN induction, demonstrated comparable histological improvement to the high-dose flavonoid group, serving as a positive control for hepatoprotection.

3. Fibrosis Scoring

Masson's Trichrome staining was used to assess collagen deposition and fibrosis severity. Rats treated with the flavonoid fraction exhibited a dose-dependent reduction in collagen accumulation.

Table 3. Liver Fibrosis Scoring Based on Ishak Criteria

Group	Mean Score	Interpretation
Normal Control	0.5 ± 0.3	None
DMN	4.8 ± 0.4	Severe fibrosis
Flavonoid 50 mg/kg	3.2 ± 0.4	Moderate fibrosis

Group	Mean Score	Interpretation
Flavonoid 100 mg/kg	2.1 ± 0.3	Mild fibrosis
Flavonoid 200 mg/kg	1.1 ± 0.2	Minimal fibrosis
Silymarin 100 mg/kg	1.0 ± 0.1	Minimal fibrosis

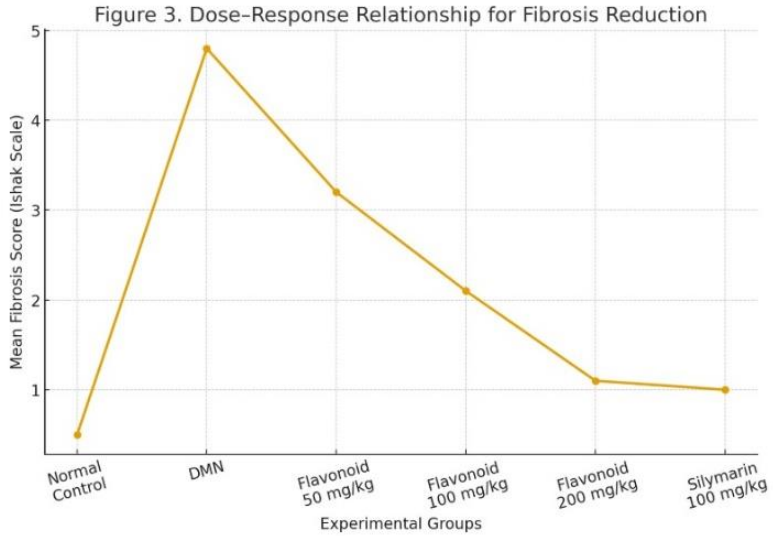

Figure 3. Dose Response Relationship for Fibrosis Reduction

A progressive decline in fibrosis scoring was observed across treatment groups, with the DMN-only group showing the highest fibrosis severity (score: 4.8 ± 0.4). Administration of the flavonoid fraction demonstrated a clear dose-dependent protective effect, with fibrosis scores decreasing from moderate (50 mg/kg: 3.2 ± 0.4) to mild (100 mg/kg: 2.1 ± 0.3) and minimal fibrosis (200 mg/kg: 1.1 ± 0.2). The antifibrotic response observed in the high-dose flavonoid group was comparable to the silymarin reference group (1.0 ± 0.1), indicating strong hepatoprotective potential. Spearman correlation analysis revealed a strong negative association between treatment dose and fibrosis severity ($r = -0.91$, $p < 0.001$), demonstrating a significant dose–response relationship.confirming a significant dose-dependent antifibrotic effect.

DISCUSSION

This study demonstrates that the flavonoid-rich fraction obtained from *Pometia pinnata* fruit peel exerts significant hepatoprotective and antifibrotic effects in a DMN-induced liver fibrosis rat model. The main findings show that treatment with this fraction attenuated liver injury markers, improved histological architecture, and reduced collagen deposition in a clear dose-dependent manner, with the highest dose exhibiting effects comparable to the silymarin reference group.

The DMN model successfully induced hepatic fibrosis, as indicated by marked elevations in serum ALT, AST, ALP, and total bilirubin, accompanied by extensive hepatocellular necrosis, inflammatory infiltration, and collagen accumulation. These findings are consistent with previous reports describing DMN-induced liver damage mediated through oxidative stress, inflammation,

and activation of hepatic stellate cells (Toklu et al., 2007; Wang et al., 2023). The elevated transaminases reflect hepatocyte membrane disruption, while increased ALP and bilirubin indicate cholestatic and fibrotic progression.

Administration of the *Pometia pinnata* fraction significantly improved biochemical parameters in a dose-dependent manner. The 200 mg/kg dose produced enzyme levels approaching those observed in the silymarin-treated group, suggesting strong hepatoprotective activity. This improvement is likely related to the antioxidant and anti-inflammatory properties commonly attributed to polyphenolic compounds, including flavonoids, which are known to reduce oxidative damage and suppress inflammatory signaling pathways (Jiang et al., 2019; Luo et al., 2025).

Histopathological findings further supported the biochemical results. Severe hepatic degeneration observed in the DMN control group was progressively ameliorated following treatment with the flavonoid fraction. The highest dose resulted in marked restoration of hepatic architecture, reduced inflammatory cell infiltration, and minimal necrotic lesions, comparable to the silymarin group. Similar hepatoprotective effects have been reported for flavonoid-rich extracts from other plant sources, reinforcing the role of polyphenols in preserving hepatocyte integrity and limiting fibrosis-related damage (Wei et al., 2020; Abouelezz et al., 2023).

Antifibrotic activity was further evidenced by Masson's Trichrome staining, which showed a substantial reduction in collagen deposition with increasing doses of the fraction. The strong negative correlation between dose and fibrosis score suggests a consistent dose–response relationship. Although molecular pathways were not directly assessed, this antifibrotic effect may involve inhibition of hepatic stellate cell activation and suppression of profibrotic mediators, as reported in other flavonoid-based studies (Pulido-Hornedo et al., 2022; Kumar et al., 2024).

It is important to note that, unlike silymarin which is a standardized extract with well-characterized active components the tested material in this study was a flavonoid-enriched fraction obtained through hexane and ethyl acetate extraction, rather than an isolated pure compound. Therefore, while flavonoids are likely contributors to the observed effects, synergistic interactions with other bioactive constituents cannot be excluded. The comparable efficacy observed relative to silymarin suggests that the fraction possesses strong biological activity, but attributing the effects solely to flavonoids would be premature without further compositional analysis.

Several limitations of this study should be acknowledged. Molecular markers related to oxidative stress and fibrogenesis, such as TGF-β1, α-SMA, MDA, SOD, and GSH, were not evaluated, limiting mechanistic interpretation. In addition, no LC–MS or detailed phytochemical profiling was performed to identify or quantify the active constituents within the fraction. These limitations highlight the need for further studies to clarify the precise mechanisms and bioactive compounds responsible for the observed hepatoprotective effects.

In summary, the flavonoid-rich fraction of *Pometia pinnata* fruit peel demonstrates promising hepatoprotective and antifibrotic activity in a DMN-induced liver fibrosis model. While the results support its potential as a natural therapeutic candidate, further molecular and phytochemical

investigations are required to validate its mechanism of action and identify its principal active constituents.

CONCLUSIONS

This study confirms that the flavonoid-rich fraction of *Pometia pinnata* fruit peel provides significant protection against DMN-induced liver fibrosis in rats, as demonstrated by improved serum liver enzymes, preserved hepatic histoarchitecture, and reduced collagen accumulation in a dose-dependent manner. The 200 mg/kg dose showed efficacy comparable to silymarin, indicating strong hepatoprotective and antifibrotic potential.

These findings support the potential of *Pometia pinnata* fruit peel as a promising source of antifibrotic agents. Future research should focus on detailed phytochemical profiling, molecular mechanism validation, and safety evaluation to support further preclinical development and therapeutic exploration.

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