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Pomelo (Citrus maxima) Peel Ethanolic Extract Mitigates Isoniazid–Rifampicin–Induced Hepatotoxicity in a Male Wistar Rat Model

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Abstract

Background: Tuberculosis continues to pose a substantial public health challenge in Indonesia, which currently ranks second worldwide in disease burden. Although first-line antituberculosis drugs such as isoniazid and rifampicin are highly effective, their prolonged use is frequently associated with hepatotoxicity, which may disrupt treatment continuity and compromise patient safety. Identifying adjunctive strategies capable of reducing liver injury during antituberculosis therapy therefore remains an important research priority.
Objective: This study aimed to examine the concurrent hepatoprotective effect of pomelo (Citrus maxima) peel ethanolic extract in a male Wistar rat model exposed to isoniazid–rifampicin–induced liver injury.
Methods: An experimental posttest-only control group design was applied using thirty-two male Wistar rats distributed into five groups: a normal control, a negative control receiving isoniazid and rifampicin, and three treatment groups administered pomelo peel ethanolic extract at doses of 100, 200, and 400 mg/kg body weight. Hepatotoxicity was induced with isoniazid and rifampicin, followed by extract administration two hours after drug exposure from day 4 to day 10. Liver function was assessed by measuring serum alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT). Phytochemical screening was conducted to identify major secondary metabolites present in the extract.
Results: Phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, triterpenoids, and quinones in pomelo peel extract. Rats receiving isoniazid–rifampicin exhibited marked elevations in SGPT and SGOT levels compared with the normal control group. Concurrent administration of pomelo peel extract significantly reduced these enzyme levels across all treatment doses relative to the negative control. The 400 mg/kg body weight dose produced the greatest attenuation of hepatic enzyme elevation, indicating a dose-related protective response.
Conclusion: Pomelo (Citrus maxima) peel ethanolic extract demonstrates dose-dependent hepatoprotective activity during concurrent exposure to isoniazid and rifampicin in male Wistar rats. These findings suggest that pomelo peel extract may have potential as a supportive agent to mitigate antituberculosis drug–induced liver injury, although further mechanistic, pharmacokinetic, and clinical investigations are required prior to translational application.

Keywords: Tuberculosis, Hepatotoxicity, Grapefruit Peel Extract, SGPT, SGOT

INTRODUCTION

Tuberculosis (TB) remains one of the most persistent global infectious diseases and continues to pose a substantial public health burden in Indonesia. Recent data from the World Health Organization indicate that Indonesia ranks second worldwide in TB incidence, with an estimated 1.06 million new cases reported in 2022, representing approximately 10% of the global caseload (1). This epidemiological context highlights the ongoing need to strengthen TB prevention, treatment, and supportive care strategies. Current TB management in Indonesia primarily relies on standardized short-course chemotherapy administered through the National TB Program, including fixed-dose combination regimens. The four-drug combination of rifampicin, isoniazid, pyrazinamide, and ethambutol remains the cornerstone of first-line therapy; however, it is also frequently associated with adverse drug reactions that may complicate treatment adherence and outcomes (2). In addition to pharmacological therapy, TB control efforts include Bacillus Calmette–Guérin vaccination, directly observed treatment short-course strategies, and the use of second-line agents for multidrug-resistant TB (3).

Despite their clinical effectiveness, first-line antituberculosis drugs are well recognized for their potential to induce liver injury, particularly when used in combination and over extended periods. Drug-induced liver injury (DILI) is the most common serious adverse effect leading to interruption or modification of TB treatment, with reported incidence ranging from 2% to 28% among patients receiving standard regimens. Isoniazid, rifampicin, and pyrazinamide are metabolized in the liver and can generate reactive intermediates or alter hepatic enzyme activity, thereby contributing to hepatocellular damage (4). Isoniazid metabolism, in particular, may result in the formation of hydrazine derivatives that exert toxic effects on hepatocytes through oxidative stress and mitochondrial dysfunction (5). These hepatic complications not only threaten patient safety but may also undermine treatment completion, emphasizing the importance of identifying strategies capable of reducing hepatotoxic risk during TB therapy.

In recent years, increasing attention has been directed toward the hepatoprotective properties of natural products as potential supportive interventions. Numerous plant-derived compounds have demonstrated protective effects against experimental liver injury, largely attributed to their antioxidant and anti-inflammatory activities (6). Citrus species represent a particularly promising group due to their diverse phytochemical profiles. Pomelo (Citrus maxima), a member of the Rutaceae family widely cultivated in Southeast Asia, is commonly consumed for its edible pulp, while its peel is often discarded despite being a rich source of bioactive constituents. Previous phytochemical investigations have identified flavonoids such as naringin and naringenin as major components of Citrus maxima peel, compounds known for their antioxidative and anti-inflammatory potential (Sowmya et al., 2019). Experimental studies have reported hepatoprotective effects of these flavonoids in various models of liver injury, suggesting possible relevance for drug-induced hepatotoxicity (7,8).

Evidence on the hepatoprotective role of pomelo (Citrus maxima) peel extract against antituberculosis drug–induced liver injury is limited. This study evaluated its ethanolic peel extract in male Wistar rats exposed to isoniazid–rifampicin, focusing on phytochemical content, hepatic enzymes, and dose-dependent protective effects.

This study contributes to the field of complementary and integrative health by presenting preclinical evidence on the hepatoprotective effects of pomelo peel ethanolic extract in an experimental model of antituberculosis drug–induced liver injury. The findings add to existing phytotherapy literature by demonstrating a dose-dependent protective response during concurrent exposure to isoniazid and rifampicin. From a nursing and public health perspective, these results highlight the potential role of natural products as supportive strategies to mitigate treatment-related adverse effects; however, they should be interpreted cautiously and not extrapolated directly to clinical practice. The utilization of pomelo peel, an underused agricultural by-product, also underscores opportunities for sustainable resource valorization in health research. Importantly, further studies focusing on phytochemical standardization, safety evaluation, and pharmacokinetic interaction profiling with antituberculosis drugs are required before any clinical application can be considered. The dose-response data generated in this study provide a foundation for future

mechanistic and translational investigations rather than immediate therapeutic recommendation.

METHODS

Study Design

This study employed a pure experimental design using a posttest-only control group design. This design was selected to evaluate the hepatoprotective effects of grapefruit peel ethanolic extract against tuberculosis drug-induced hepatotoxicity by comparing treatment groups receiving different doses of the extract with control groups. The experimental approach allowed for direct manipulation of the independent variable (grapefruit peel extract dosage) while controlling confounding variables through randomization and standardized experimental conditions.

Participants

Male Wistar rats weighing 150–180 g were used as the experimental population. Animals meeting the inclusion criteria were randomly assigned to five experimental groups. Sample size determination was guided by the Federer formula as an initial reference; however, practical and ethical considerations during the experimental period resulted in unequal group sizes. Consequently, a total of 32 rats were allocated, with 6–7 animals per group, to ensure adequate statistical power while minimizing unnecessary animal use.

Intervention Protocol

Preparation of Grapefruit Peel Extract

Pomelo (Citrus maxima) peel samples were obtained from a local market in Ujung Berung, Bandung City, West Java, Indonesia. The peels were thoroughly washed under running water to remove surface contaminants, after which the albedo portion was separated and cut into small fragments. The material was air-dried under sunlight for three days, followed by oven drying at 40–50 °C for 24 hours. The dried peels were then pulverized into a fine powder using a mechanical grinder, as described in previous protocols (Sudto et al., 2009). For extract preparation, 300 g of pomelo peel powder was macerated in 3 L of 96% ethanol at room temperature for three days with occasional stirring. The resulting mixture was filtered, and the solvent was removed under reduced pressure using a rotary evaporator at 50 °C to obtain a concentrated ethanolic extract. This extract was subsequently used to evaluate the concurrent hepatoprotective effect of pomelo peel ethanolic extract during isoniazid–rifampicin exposure in male Wistar rats

Experimental Animals

The acclimatization or adaptation process for mice is carried out for 3 days. Male Wistar mice weighing around 150-180 g are placed in cages maintained under standard laboratory conditions, with a temperature of 22 ± 2 ℃, relative humidity of 60% to 70%, and a light/dark cycle of 12 hours. Food and water are available ad libitum to test animals (Jaydeokar *et al*., 2014). This aims to prevent stress in the male Wistar rats that will be tested. The experiment was conducted under the approval of the National Research and Innovation Agency (NRIA) Animal Care, Jakarta, Indonesia, and according to the code of ethics 151/ KE.0 3/S K/0 7 /2024.

Procedure of Anti-TB drugs Inducing

The types of anti-tuberculosis drugs that will be used in experimental animals are isoniazid and rifampicin, where the doses given refer to previous research by Napitupulu et al. (2023) with a dose of isoniazid of 100 mg/kgBW and rifampin of 100 mg/kgBW for 10 days orally.

Experimental Design

In the control group of 5 animals, male Wistar rats were given standard food and water from day 1 to day 10 and no treatment. In the negative control group consisting of 5 rats, male Wistar rats were given food and water according to standards and acclimatized for 3 days first, then given isoniazid and rifampicin at a dose of 100 mg/kgBW and 100 mg/kgBW on the 4th day to 10th day orally without administering the extract. In the treatment group, male Wistar rats were given food and water according to standards and acclimatized for 3 days first. This group was then given isoniazid and rifampin at doses of 100 mg/kgBW and 100 mg/kgBW orally on days 4 to 10 and grapefruit peel extract at different doses, namely 100 mg/kgBW, 200 mg/kgBW, And 400 mg/kgBW 2 hours after administration of isoniazid and rifampicin.

Preparation of 0.5% Na-CMC Solution

0.5 g of Na-CMC was weighed, and a little hot distilled water was added. The mixture was

stirred until mucilage formed. Then, add distilled water until it reaches a total volume of 100 mL.

Preparation of Doses

After weighing 450 mg, the rifampicin was dissolved in a tiny 0.5% Na-CMC solution until homogeneous. Next, this solution was added with 0.5% Na-CMC to a total volume of 20 mL. 300 mg of isoniazid was weighed and then dissolved in 0.5% Na-CMC until homogeneous. Next, the solution was added to a total volume of 20 mL.

Preparation of Extract Solution

Weigh 4 grams of grapefruit peel extract, then mix it with a small quantity of 0.5% Na-CMC. After the solution becomes homogeneous, add more 0.5% Na-CMC until the total volume reaches 100 mL.

Procedure of SGOT and SGPT Testing

On the 10th day of the experiment, the rats were anesthetized using CO2, and a blood sample of 2 mL was taken from each rat in the five groups' orbital veins. The blood samples were then centrifuged at 4000 rpm for 7 minutes to separate the serum. The serum obtained was stored at -20°C until ready to be used for biochemical analysis of SGPT and SGOT levels. SGPT and SGOT levels were examined using a Microlab 300 photometer. Before measurement, serum samples and working reagents were incubated at 37°C. A total of 50 µL of serum samples was added to a cuvette containing 1 mL of working reagent (mixture of R1 and R2), then the mixture was incubated for 1 minute at 30°C. After incubation, the mixture was measured using a Microlab 300 photometer.

Data Analysis

All data were expressed as mean ± standard deviation (SD) and analyzed using SPSS version 25.0 software. SGPT and SGOT levels across the five experimental groups were compared using one-way analysis of variance (ANOVA) to determine if significant differences existed among groups. When ANOVA revealed significant differences ($p < 0.05$), post-hoc comparisons were conducted using the Least Significant Difference (LSD) test to identify specific group differences and determine which extract doses provided significant hepatoprotective effects compared to the negative control group.

RESULTS

Extraction Results

Grapefruit peel is extracted using the maceration method, which is an extraction method by soaking the sample or simplicia with organic solvents at room temperature (9). The solvent used in this extraction process is 96% ethanol solvent with a simplicia weight of 300 g and obtained a thick extract weighing 60 g. The extract yield was then calculated and obtained a result of 20%. Extract yield is calculated based on the ratio of the final weight (weight of the extract produced) to the initial weight (weight of the simplicia powder used) multiplied by 100% (10). The extract was obtained in the form of thick grapefruit peel extract with organoleptic characteristics of blackish brown color, semi-solid texture, and distinctive citrus smell.

$$\text{Yield (\%)} = (60 \text{ gr}/300 \text{ gr}) \times 100\% = 20\%$$

Figure 1. Extract Yield Results

The greater the yield value indicates the value of the extract produced more and more. The requirement for the yield of thick extracts is that the value is not less than 10% (Indonesian Herbal Pharmacopoeia, 2017). Therefore, it can be concluded that the yield of grapefruit peel extract has met the predetermined requirements. In the extraction process, the maceration method is used due to several reasons that make it has advantages compared to other extraction methods. The procedures and equipment used are simple and thus can maintain natural materials that are not heat resistant (10). Besides, cold extraction also allows many compounds to be extracted, although some compounds have limited solubility in solvents at room temperature (11). 96% ethanol solvent was used in the extraction process because it is universal, polar and easy to obtain. In addition, 96% ethanol was chosen because it is selective, non-toxic, good absorption and high solubilization ability so that it can extract non-polar, semi-polar and polar compounds (12).

Phytochemical Screening Results

Phytochemical screening was conducted to identify secondary metabolite compounds in grapefruit peel extract. Qualitative testing is done using certain reagents that can react with these compounds.

Table 1. Phytochemical Screening Results of Ethanol Extract of Grapefruit Peel

No	Test Parameters	Results	Screening Results	
			Extract	(Suryanita *et al.*, 2019)
1	Alkaloid	Dragendorff = Brick Red Precipitate	+	+
2	Flavonoid	Formed yellow color	+	+
3	Quinones	Formed yellow color change	+	x (not tested)
4	Tannins	FeCl3 tannins = blackish green color formed Gelatin tannins = no white precipitate formed	+	+
5	Triterpenoids	Formed green - blue color	+	+
6	Saponins	Formed unstable foam	-	+

Tabel 2. Rf Value Sample

Mobile Phase	Rf Value	
	A	B
Ethyl acetate : methanol (7:3))	0,12	0,68
	0,56	0,80
	0,76	0,80

Description: A. Grapefruit Peel Extract, B. Quercetin Standard (Natasa, *et al*).

Table 3. Results of Average Levels of SGPT and SGOT

Group	Mean (µ/L) ± SD	
	SGPT	SGOT
Control	43,05 ± 3,09*	89,90 ± 6,98*
Negative Control	89,30 ± 2,83 β	171,18 ± 20,23 β
Extract 100 mg/kgBB	55,85 ± 9,19*β	95,70 ± 19,93*
Extract 200 mg/kgBB	49,23 ± 6,53*	97,70 ± 6,21*
Extract 400 mg/kgBB	43,78 ± 10,15*	70,93 ± 18,09*

Notes:
(*) significantly different from the negative control group (P<0.05)
(β) significantly different from the control group (P<0.05)

Table 4. One Way ANOVA Test Post Hoc LSD on SGPT

Group	Total SGPT (µ/L)		
	Group	*Mean Difference*	*p value*
Control	Negative Control	-46,25000*	0,000
	Extract 100 mg/kgBB	-12,80000*	0,022

	Extract 200 mg/kgBB	-6,17500	0,236
	Extract 400 mg/kgBB	-0,72500	0,887
Negative Control	Control	46,25000*	0,000
	Extract 100 mg/kgBB	33,45000*	0,000
	Extract 200 mg/kgBB	40,07500*	0,000
	Extract 400 mg/kgBB	45,52500*	0,000
Extract 100 mg/kgBB	Control	12,80000*	0,022
	Negative Control	-33,45000*	0,000
	Extract 200 mg/kgBB	6,62500	0,205
	Extract 400 mg/kgBB	12,07500*	0,029
Extract 200 mg/kgBB	Control	6,17500	0,236
	Negative Control	-40.07500*	0,000
	Extract 100 mg/kgBB	-6,62500	0,205
	Extract 400 mg/kgBB	5,45000	0,293
Extract 400 mg/kgBB	Control	0.72500	0,887
	Negative Control	-45,52500*	0,000
	Extract 100 mg/kgBB	-12,07500*	0,029
	Extract 200 mg/kgBB	-5,45000	0,293

Notes:
(*) significantly different from other groups

Table 5, One Way ANOVA Test Post Hoc LSD on SGOT

Group	Total SGOT (µ/L)		
	Group	*Mean Difference*	*p value*
Control	Negative Control	-81,27500*	0,000
	Extract 100 mg/kgBB	-5,80000*	0,607
	Extract 200 mg/kgBB	-7,80000*	0,491
	Extract 400 mg/kgBB	18,97500	0,107
Negative Control	Control	81,27500*	0,000
	Extract 100 mg/kgBB	75,47500*	0,000
	Extract 200 mg/kgBB	73,47500*	0,000
	Extract 400 mg/kgBB	100,25000*	0,000
Extract 100 mg/kgBB	Control	5,80000	0,607
	Negative Control	-75,4750*	0,000
	Extract 200 mg/kgBB	-2,00000	0,859
	Extract 400 mg/kgBB	24,77500*	0,041
Extract 200 mg/kgBB	Control	7,80000	0,491
	Negative Control	-73,47500*	0,000
	Extract 100 mg/kgBB	2,00000	0,859
	Extract 400 mg/kgBB	26,77500*	0,029
Extract 400 mg/kgBB	Control	-18,97500	0,107
	Negative Control	-100,25000*	0,000
	Extract 100 mg/kgBB	-24,77500*	0,041
	Extract 200 mg/kgBB	-26,77500*	0,029

Notes:
(*) significantly different from other groups

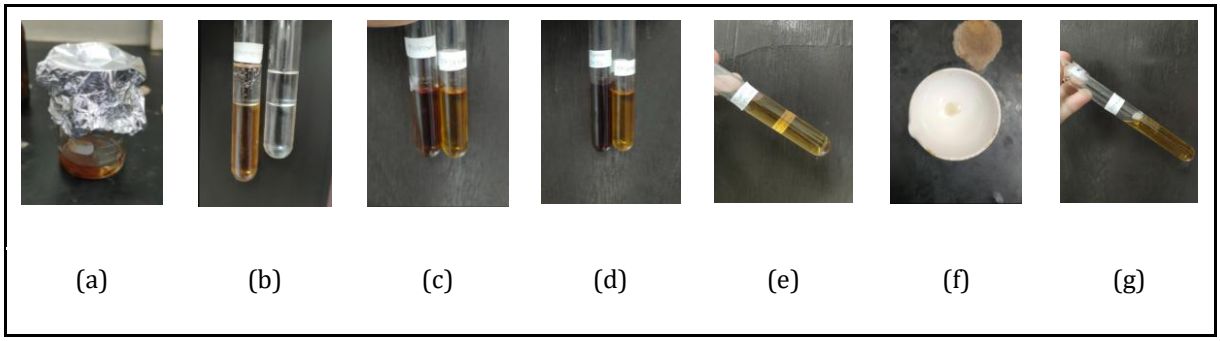

Figure 2. Phytochemical Screening Results (a) Alkaloids (b) Flavonoids (c) Quinones (d) FeCl3 Tannins (e) Gelatin Tannins (f) Triterpenoids (g) Saponin

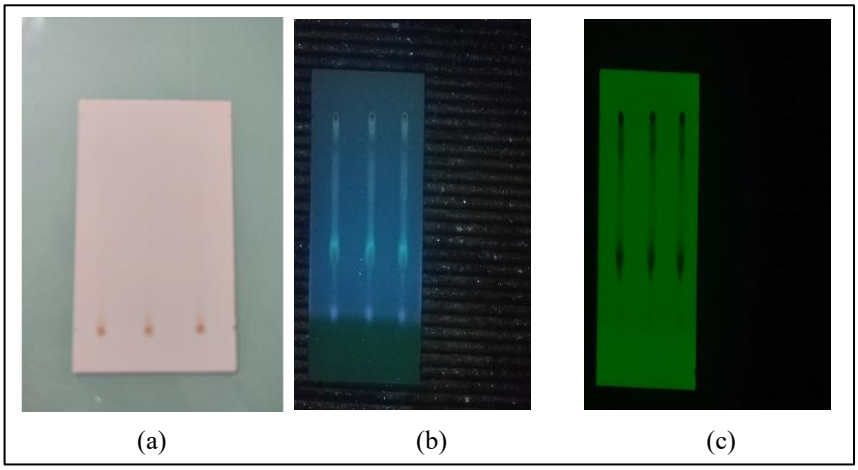

Figure 3. Identification Results of Flavonoid Compounds of Bali Orange Peel Extract Image description: a). Visual b). KLT plate under UV lamp 254 nm c). KLT plate under UV lamp 366 nm.

Data from table 1 and figure 2 show that there are several important secondary metabolite compounds. The formation of orange precipitate was observed after the addition of Dragendorff reagent. Combining magnesium powder and concentrated HCl to identify flavonoids causes the color to become red, yellow, or orange. After the addition of NaOH, the color becomes yellow. Tannin test with 1% FeCl3 produces a blackish green color. Tannin testing was also carried out using gelatin solution, but no white precipitate was formed so that the extract contained only FeCl3 tannins. The formation of green or blue color after the addition of chloroform, acetic anhydride, and concentrated H2SO4 indicates triterpenoids. The saponin test, characterized by unstable foam, showed negative results. These results were generally consistent with the research conducted by Artati (13), except for quinones which were not tested and the absence of saponins which differed from the results of Artati. (13).

Thin Layer Chromatography Analysis Results

Qualitative testing was conducted on grapefruit peel extract to identify flavonoid compounds using thin layer chromatography analysis. Thin layer chromatography has principles including the separation of multicomponent compounds using two phases, namely the stationary phase and the mobile phase (14). The stationary phase used in this analysis is silica gel GF 254 nm and the mobile phase used is ethyl acetate and methanol in a ratio (7: 3). The KLT plate will be observed under UV lamp 254 nm and 366 nm and sprayed by 5% AlCl to identify flavonoid compounds, then the qualitative analysis results are obtained as follows:

Effect of Treatment on SGPT and SGOT Levels

Elevated liver enzymes, especially SGPT and SGOT, indicate liver damage. Higher levels of these two enzymes indicate that liver damage is more severe (5). Normal rat SGPT values range from 10 to 50 µ/L, and SGOT values range from 45 to 100 µ/L, according to the Food and Drug Administration Regulation Number 18 of 2021 concerning Guidelines for Preclinical Pharmacodynamic Tests of Traditional Medicines.

Data from Table 2 above shows that in the negative control group, administration of high doses of rifampicin and isoniazid caused a significant increase in SGPT (89.30 ± 2.83 µ/L) and SGOT (171.18 ± 20.23 µ/L) levels. Significantly compared with the standard control group, which had SGPT levels (43.05 ± 3.09 µ/L) and SGOT (89.90 ± 6.98 µ/L). This finding aligns with Napitupulu (15) finding that mice given isoniazid and rifampin experienced increased levels of SGPT and SGOT. According to Napitupulu (15), this is caused by the formation of toxic metabolites such as monoacetyl hydrazide (MAH) from isoniazid and the pro-inflammatory effect of rifampicin, which stimulates inflammatory mediators and increases cytokine production.

Grapefruit peel ethanol extract showed significant hepatoprotective effects at 100, 200, and 400 mg/kg BW doses, with lower SGPT and SGOT values compared to the negative control group at all doses. At a dose of 100 mg/kg BW, grapefruit peel extract produced lower SGPT (55.85 ± 9.19 µ/L) and SGOT (95.70 ± 19.93 µ/L) values compared to the negative control group, although has not reached normal limits. At a dose of 200 mg/kg BW, the SGPT (49.23 ± 6.53 µ/L) and SGOT (97.70 ± 6.21 µ/L) values were even lower and approached typical values. Therefore, the most effective dose is 400 mg/kgBW, where the SGPT and SGOT values approach the normal range significantly. These results indicate that the higher the extract dose, the more effective it is in preventing an increase in SGPT and SGOT levels, or it can be said that the hepatoprotective effect is dose-dependent.

Further analysis of Tables 3 and 4 strengthens these findings, showing significant differences between the negative control and sample groups with the tested extract dose. The SGPT values (Table 3) at doses of 100, 200, and 400 mg/kgBW were significantly lower compared to the negative control ($p < 0.05$). Similar results were found for SGOT (Table 4), where SGOT values for all extract doses were also significantly lower compared to the negative control; the dose of 400 mg/kg BW showed the most substantial and most significant effect ($p < 0.05$), providing more effective liver protection than other doses.

The active compound content of this extract, including alkaloids, flavonoids, tannins, triterpenoids, and quinones, is responsible for its hepatoprotective properties, which work together to protect the liver. By normalizing liver enzymes such as AST, ALT, and ALP, alkaloids protect liver cells from damage caused by toxins. Flavonoids, as powerful antioxidants, fight free radicals, stop lipid peroxidation, and increase the activity of antioxidant enzymes such as catalase. This protects the liver from oxidative stress. Tannins help reduce oxidative stress and increase glutathione levels, which is important for maintaining healthy liver cells (16). The ability of triterpenoids to inhibit lipid peroxidation and reduce apoptosis and inflammatory responses, thereby reducing the risk of further damage to liver cells (17). Quinones can also function as an inflammatory agent. Additionally, quinones, such as pyrroloquinoline quinone (PQQ), help lower markers of liver damage by activating the Nrf2 signalling pathway, which is critical for antioxidant defence (18). The hepatoprotective effect of grapefruit peel extract is supported by these compounds' antioxidant, anti-inflammatory and cellular protection activities, especially at higher doses.

DISCUSSION

This study provides preclinical evidence that pomelo (Citrus maxima) peel ethanolic extract exerts a hepatoprotective effect in a male Wistar rat model exposed to isoniazid and rifampicin. Concurrent administration of the extract at doses of 100, 200, and 400 mg/kg body weight was associated with a significant attenuation of serum aminotransferase elevations, indicating a reduction in drug-induced liver injury. Among the tested doses, the 400 mg/kg body weight group demonstrated the greatest protective response, with SGPT and SGOT values approaching those of the normal control group. These findings suggest a dose-dependent protective effect under experimental conditions and confirm the suitability of the model for evaluating anti-tuberculosis drug–associated hepatotoxicity (19–21).

The marked increase in SGPT and SGOT levels observed in the negative control group confirms the successful induction of hepatotoxicity following combined isoniazid and rifampicin exposure. This result is consistent with earlier experimental studies reporting liver injury following co-administration of these agents. The hepatotoxicity induced by this regimen is understood to involve multiple overlapping mechanisms. Isoniazid undergoes hepatic metabolism to produce reactive intermediates, including acetyl hydrazine, which may be further bioactivated by cytochrome P450 2E1 to toxic metabolites that damage hepatocytes. Rifampicin can exacerbate this process by inducing hepatic enzyme expression, thereby increasing the formation of reactive species. In addition, oxidative stress, mitochondrial dysfunction, bile acid transport disruption, and inflammatory signaling are recognized contributors to isoniazid–rifampicin–induced liver injury. The elevated aminotransferase levels observed in this study reflect these cumulative pathological processes rather than a single mechanistic pathway.

The reduction of aminotransferase levels in extract-treated groups indicates that pomelo peel extract may mitigate hepatocellular damage during concurrent exposure to antituberculosis drugs. A similar dose–response relationship has been reported in other studies examining citrus peel extracts under different models of liver injury, suggesting that higher doses may be required to achieve sufficient concentrations of bioactive compounds for effective hepatoprotection. The present findings extend previous work by demonstrating that pomelo peel extract confers protective effects in a drug-induced liver injury model relevant to tuberculosis treatment. However, the absence of a positive control, such as silymarin, limits direct comparison of effect magnitude and should be addressed in future studies to strengthen model validation.

Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, triterpenoids, and quinones in the pomelo peel extract. Thin-layer chromatography further supported the presence of flavonoid compounds, consistent with earlier reports on citrus peel phytochemistry. Although these compounds are widely reported to possess antioxidant and anti-inflammatory properties, the present study did not directly evaluate molecular mechanisms. Therefore, any mechanistic interpretation should be considered hypothetical and based on existing literature rather than direct evidence. Previous studies suggest that citrus flavonoids such as naringin and naringenin may reduce oxidative stress, modulate inflammatory signaling, and enhance endogenous antioxidant defenses, potentially through pathways such as nuclear factor erythroid 2–related factor 2 (Nrf2). Similarly, tannins and triterpenoids have been associated with reduced lipid peroxidation and attenuation of inflammatory responses in experimental liver injury models. The collective presence of these phytochemicals may explain the observed hepatoprotective effect; however, confirmation requires targeted biochemical and molecular analyses.

From a translational perspective, these findings should be interpreted with caution. Although pomelo (Citrus maxima) differs botanically from grapefruit (Citrus paradisi), citrus-derived compounds are known to interact with hepatic drug-metabolizing enzymes and transporters. Given the critical role of rifampicin and isoniazid pharmacokinetics in tuberculosis treatment, the potential for herb–drug interactions must be carefully evaluated before considering any clinical application. The present study was not designed to assess pharmacokinetic interactions, bioavailability, or safety profiles beyond short-term biochemical markers. As such, pomelo peel extract should not be recommended as an adjunctive therapy during tuberculosis treatment at this stage.

Several limitations of the study warrant consideration. First, the use of only male rats precludes assessment of sex-related differences in susceptibility to hepatotoxicity and response to intervention. Second, the relatively short duration of drug exposure does not fully replicate the prolonged treatment course used in clinical tuberculosis management. Third, liver histopathology and additional biomarkers of oxidative stress, inflammation, or cholestasis were not assessed, limiting the depth of hepatoprotection characterization. In addition, the extract was not chemically standardized, and quantitative analysis of key flavonoids was not performed, which may affect reproducibility across studies.

Despite these limitations, this study contributes valuable experimental data to the field of complementary and integrative research by demonstrating that pomelo peel, an underutilized agricultural by-product, possesses

hepatoprotective properties in a relevant preclinical model. The dose–response data generated provide a basis for future investigations focusing on extract standardization, mechanistic validation, pharmacokinetic profiling, and interaction studies with antituberculosis drugs. Such research is essential before any consideration of clinical translation.

Translational Considerations and Safety Implications

Although pomelo (Citrus maxima) differs phytochemically from grapefruit (Citrus paradisi), citrus-derived flavonoids and related compounds have been reported to influence hepatic drug-metabolizing enzymes and transporters. Given the clinical importance of rifampicin and isoniazid pharmacokinetics, these findings should be interpreted as preclinical and exploratory. Prior to any clinical application, standardization of extract composition, pharmacokinetic interaction studies, and safety profiling are required to evaluate potential effects on antituberculosis drug metabolism. Therefore, pomelo peel extract should not be recommended for clinical use during tuberculosis treatment without further investigation.

Clinical implications

Although this investigation was conducted in an experimental animal model, the observed dose-related reduction in hepatic aminotransferase levels suggests potential relevance for supportive strategies aimed at minimizing liver injury during antituberculosis treatment. Hepatotoxicity associated with isoniazid and rifampicin remains a major challenge in tuberculosis management, as it may compromise treatment adherence and patient safety. The present findings provide preliminary evidence that bioactive compounds derived from Citrus maxima peel may attenuate biochemical indicators of liver injury, thereby reinforcing the importance of ongoing research into adjunctive approaches that could support treatment continuity.

From a nursing and public health perspective, these results emphasize the critical role of routine liver function monitoring during antituberculosis therapy. Nurses are strategically positioned to identify early signs of hepatotoxicity, educate patients regarding warning symptoms, and facilitate timely clinical evaluation. In addition, the findings underscore the importance of patient counseling related to the use of herbal or natural products during TB treatment. While plant-based substances are often perceived as safe, certain phytochemicals—particularly those derived from citrus species—may influence hepatic drug metabolism. Consequently, this study should not be interpreted as justification for clinical use of pomelo peel extract, but rather as evidence highlighting the need for careful medication history assessment and counseling in clinical practice. Furthermore, the utilization of pomelo peel as a typically discarded agricultural by-product suggests potential value for future translational research focused on sustainable health innovations, provided that safety and interaction profiles are rigorously evaluated.

Study limitations

Several limitations should be acknowledged when interpreting the results of this study. First, the experimental design relied on a rat model, which restricts direct applicability to human populations. Differences in metabolism, drug response, and susceptibility to liver injury between animals and humans necessitate caution in extrapolating these findings to clinical settings. Second, only male rats were included in the experiment, preventing evaluation of potential sex-based differences in drug-induced hepatotoxicity and hepatoprotective responses.

Third, the duration of antituberculosis drug exposure was relatively short compared with the prolonged treatment regimens used in human tuberculosis management. As a result, the study does not fully capture the cumulative or chronic effects of long-term drug administration. Fourth, assessment of liver injury was limited to serum SGPT and SGOT measurements. Although these markers are widely used indicators of hepatocellular damage, the absence of histopathological examination and additional biochemical parameters limits comprehensive evaluation of hepatic protection.

In addition, the extract used in this study was not chemically standardized, and quantitative analysis of specific active constituents was not performed. This may affect reproducibility and comparability across future studies. The lack of a positive control group receiving a recognized hepatoprotective agent also limits direct comparison of efficacy. Moreover, the study did not assess pharmacokinetic interactions between pomelo peel extract and antituberculosis drugs,

an issue that is particularly relevant given the known enzyme-inducing properties of rifampicin. Finally, the relatively small and unequal sample sizes across groups may have influenced statistical robustness. Collectively, these limitations indicate that the present findings should be regarded as preliminary and exploratory, providing a foundation for more comprehensive mechanistic, toxicological, and translational investigations rather than immediate clinical application.

CONCLUSION

Based on the findings of this study, pomelo (Citrus maxima) peel ethanolic extract was shown to contain several secondary metabolites, including flavonoids, alkaloids, tannins, triterpenoids, and quinones, as identified through phytochemical screening. Thin-layer chromatographic analysis further confirmed the presence of flavonoid compounds, indicated by a characteristic fluorescent spot with an Rf value of 0.76. In the experimental rat model, concurrent administration of pomelo peel extract during isoniazid–rifampicin exposure was associated with a reduction in hepatic enzyme elevations. Rats receiving the extract, particularly at a dose of 400 mg/kg body weight, exhibited lower SGPT and SGOT levels compared with the negative control group, approaching values observed in normal animals. These results suggest that pomelo peel ethanolic extract exerts a dose-dependent hepatoprotective effect under experimental conditions, with the highest tested dose demonstrating the greatest protective response. Further studies are required to clarify underlying mechanisms, establish standardization parameters, and evaluate translational safety prior to clinical consideration.

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Author Contributions

Study conception and design: LRA
Data collection : LRA
Data analysis and interpretation: SDA
Drafting of the article: LRA
Critical revision of the article: ATM

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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