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Ethanolic Peel Extract Attenuates Isonazid-induced Hepatic Injury in Rats via Reduced NF-kB, and Caspase 3 Expression

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Isoniazid can induce hepatotoxicity and liver fibrosis via oxidative stress, NF-κB, and caspase-3 pathways. Garcinia mangostana L. (mangosteen) peel extract, rich in xanthones, offers anti-inflammatory and anti-apoptotic properties that may mitigate drug-induced liver injury. To evaluate the effects of mangosteen peel ethanol extract on NF-κB p65, caspase-3 expression, and hepatic fibrosis in isoniazid-treated Wistar rats. In this experimental study, 28 male Wistar rats were divided into four groups: negative control (NC), positive control (PC; isoniazid 50 mg/kg), and two treatment groups receiving isoniazid plus mangosteen extract at 250 mg/kg (I1) and 500 mg/kg (I2) for 30 days. Liver tissues were analyzed using immunohistochemistry (NF-κB, caspase-3) and Masson's trichrome staining (fibrosis). The 500 mg/kg dose significantly reduced NF-κB p65 and caspase-3 expression and mitigated hepatic fibrosis compared to the PC group ($p < 0.05$). The 250 mg/kg dose showed less pronounced effects. Garcinia mangostana L. peel extract, especially at 500 mg/kg, effectively attenuates isoniazid-induced liver injury by downregulating NF-κB and caspase-3 in isoniazid-induced Wistar rats. These findings suggest its potential as an adjunct in preventing isoniazid-induced hepatic injury in a preclinical setting. Additional translational and clinical investigations are needed to determine its efficacy and safety as a potential hepatoprotective agent in humans.

Keywords: Caspase, Garcinia mangostana, Isoniazi, Liver fibrosisd, NF-kB

INTRODUCTION

Hepatic fibrosis is a condition in which the liver produces excessive amounts of extracellular matrix (ECM) in response to chronic liver injury. Hepatocyte death is an initial step in chronic liver disease that triggers inflammation and activation of hepatic stellate cells (HSCs), which then differentiate into myofibroblasts that drive fibrosis (Gao et al., 2023; Wang et al., 2023). Types of hepatocyte death involved in hepatic fibrosis include pyroptosis, apoptosis, necroptosis, ferroptosis, and autophagy-mediated cell death. Apoptosis is a form of programmed cell death that occurs in various types of hepatocytes. In hepatocytes, apoptosis is often triggered by TNF signaling or microRNAs. Conversely, apoptosis of HSCs may prevent fibrosis. If left unchecked, fibrosis progresses to cirrhosis, characterized by deformation of liver structure, portal hypertension, and complications such as ascites and hepatic encephalopathy. In its early stages, fibrosis may regress if the underlying etiology is addressed, through mechanisms such as apoptosis or inactivation of HSCs and ECM degradation by proteolytic enzymes (Berumen et al., 2021; Parola & Pinzani, 2019; Somnay et al., 2024).

The mechanisms underlying isoniazid-induced hepatotoxicity include the generation of reactive oxygen species (ROS), oxidative stress, and mitochondrial dysfunction (Zhuang et al., 2022). These processes lead to hepatocyte apoptosis and the activation of inflammatory pathways, especially the NF-κB signaling cascade (Ahmadi et al., 2021). NF-κB is a central transcription factor in the pathogenesis of liver fibrosis, promoting the expression of pro-inflammatory cytokines such as TNF-α and IL-6, and contributing to hepatic stellate cell (HSC) activation (P. Chen et al., 2023). Caspase-3, an executioner enzyme in the apoptosis pathway, also plays a pivotal role in liver damage by mediating programmed cell death in hepatocytes. Caspase 3 can be used as an indicator for cell apoptosis and mitochondrial damage (Ibrahim et al., 2024; Lu et al., 2023). Given these mechanisms, targeting inflammatory and apoptotic pathways offers a promising

therapeutic strategy for preventing or reducing hepatic fibrosis. Natural antioxidants and anti-inflammatory agents have gained interest in this context. The pericarp of Garcinia mangostana L. (mangosteen), which contains bioactive compounds such as xanthones, has demonstrated potent anti-inflammatory and anti-apoptotic properties in various studies (Afolabi et al., 2022). Xanthones like α-mangostin have been shown to inhibit NF-κB activation, reduce pro-inflammatory cytokines, and suppress the expression of caspase-3 in models of tissue injury (Harlisa et al., 2022; Hassan et al., 2021).

The effect of mangosteen extract from the peel of Garcinia mangostana in preventing liver damage caused by isoniazid administration has not been previously studied. Exploration its effect on preventing isoniazid induced liver injury at the cellular and molecular levels need to be investigated.This study investigates the hepatoprotective effects of ethanol extract of Garcinia mangostana peel in a Wistar rat model of isoniazid-induced liver injury. However, previous studies exploring the hepatoprotective effects of Garcinia mangostana peel extract have largely focused on macro-level parameters, such as serum liver enzymes (ALT and AST) and general histopathological scoring, without deeply elucidating the specific downstream molecular mechanisms involved in drug-induced liver injury (DILI). Furthermore, existing literature often overlooks how these extracts modulate the delicate balance between inflammation and apoptosis when subjected to specific antitubercular toxicity like isoniazid. Evaluating the expressions of NF-κB and caspase-3 in this study contributes crucial new knowledge by uncovering the precise molecular checkpoints through which α-mangostin exerts its therapeutic effects. By quantifying these specific markers, this research provides a mechanistic link showing how the extract directly intercepts the nuclear transcription of inflammatory cytokines and halts the execution phase of hepatocyte apoptosis, filling a critical gap that standard biochemical assays cannot address.

Rats are chosen as experimental models due to their close biological resemblance to humans and the practicality of their handling and maintenance in laboratory conditions. In addition, their physiological similarity and extensive history of use in disease research make them highly valuable for elucidating disease mechanisms and establishing relevant animal models across diverse scientific fields (Smith et al., 2019). Specifically, it evaluates the modulation of NF-κB p65 and caspase-3 expression, as well as the extent of hepatic fibrosis. This research aims to provide scientific evidence supporting the potential use of mangosteen peel extract as an adjunctive therapeutic agent in managing liver fibrosis.

METHODS

Study design

This laboratory-based experimental study employed a post-test-only control group design to evaluate the effects of Garcinia mangostana L. peel ethanol on NF-κB, caspase-3 expression, and liver fibrosis in Wistar rats with isoniazid-induced liver injury. The research was conducted at the Animal Laboratory Unit of the Center for Animal Research, Gadjah Mada University, Yogyakarta, and histopathological and immunohistochemical examinations were performed at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta.

Animal subjects

Twenty-eight healthy male Wistar rats aged 3–4 months, weighing 170–200 grams, were included in the study. Rats that became ill or died during the experiment were excluded. The rats were maintained under controlled environmental conditions (temperature 20–25°C, 12 hour light/dark cycle, 50–60% humidity), and provided with standard feed and water ad libitum.

Sample size and grouping

Sample size was calculated using the Federer formula:

$$(t-1)(n-1)>15(t-1)(n-1)>15 \quad (1)$$

Where t = 4 (number of groups), yielding n ≥ 6. Thus, 7 rats per group were used, totaling 28 rats. Although the calculated minimum sample size was six rats per group (n≥6), a total of seven rats per group (n=7) was utilized to account for a potential 15% attrition or mortality rate during the 30-day intervention period, thereby ensuring that the final statistical analysis maintained adequate statistical power. While this sample size is characteristic of exploratory animal models, it is statistically justified by the Federer formula, which is widely accepted for controlling type I and type II errors in completely randomized designs with multiple treatment groups. Furthermore, to maximize statistical reliability despite the relatively small sample size, high-precision molecular assays (such as IHC or Western blot for NF-κB and caspase-3) and standardized histopathological scoring were employed to minimize within-group variance and enhance the sensitivity of the statistical tests.

The rats were randomly divided into four groups:

a) Group NC (Negative Control): Received intraperitoneal injection of aquadest only.
b) Group PC (Positive Control): Received isoniazid 50 mg/kg BW intraperitoneally for 30 days.
c) Group T1 (Treatment 1): Received isoniazid 50 mg/kg BW and Garcinia mangostana L. Peel Extract 250 mg/kg BW orally for 30 days.
d) Group T2 (Treatment 2): Received isoniazid 50 mg/kg BW and Garcinia mangostana L. Peel Extract 500 mg/kg BW orally for 30 days.

Preparation of *Garcinia mangostana* extract

Mangosteen peel samples were sourced from Girilayu Village, Matesih District, Karanganyar, Central Java. To isolate the active compound, alpha-mangosteen (α-Mangosteen), an ethanolic extract was prepared at the PAU Yogyakarta laboratory. Subsequent quantification of

the extract was performed via HPLC analysis at the MIPA laboratory, Universitas Sebelas Maret, Surakarta. The analysis revealed that a 50 ppm extract contained an α-Mangosteen concentration of 18.98 ± 0.10 ppm, equivalent to 46.23 ± 0.24 µM.

Experimental procedure

All rats, except the negative control group, were induced with hepatotoxicity by intraperitoneal injection of isoniazid (50 mg/kg BW/day) for 30 consecutive days. Treatment groups received the extract doses orally. At the end of the treatment period, rats were sacrificed, and liver tissues were harvested for analysis.

Histological and immunohistochemical analysis

Liver tissues were fixed in 10% buffered formalin, processed for paraffin embedding, and sectioned. Masson's trichrome staining was performed to assess collagen fiber deposition (fibrosis). The immunohistochemical staining was performed using a three-step indirect immunoperoxidase method with the Avidin-Biotin Complex (ABC). Tissue sections were first deparaffinized through a standard series of xylene, alcohol, and water solutions, then washed with PBS (pH 7.4) and treated with 0.125% trypsin at 37 °C for 5–10 minutes to unmask antigens. Endogenous peroxidase activity was blocked by incubation with 0.5% H_2O_2 in methanol for 30 minutes. After additional PBS washes, nonspecific binding was blocked using 3% serum in 1% BSA for 20 minutes. Sections were then incubated with primary murine monoclonal antibodies against NF-κB p65, and caspase-3 (Santa Cruz, USA) diluted 1:300 in Tris-PBS for 30 minutes in a humid chamber. Following PBS washes, sections were incubated with a biotinylated anti-mouse secondary antibody (Dako Kit) for 30 minutes, then with streptavidin-biotin peroxidase for another 30 minutes.

Color development was achieved by applying the chromogenic substrate until brown staining appeared (approximately 15 minutes). Finally, sections were washed, mounted with a cover glass, and examined under a light microscope. Positive expression of target molecules was indicated by brown staining. Positive and negative controls were included in each staining procedure Positive expression was indicated by brown staining in the nucleus or cytoplasm. Evaluation was performed using a semi-quantitative scoring system by two blinded observers, and discrepancies greater than 25% were resolved by consensus reading. Immunohistochemical analysis of liver tissue samples was performed at the Pathological Anatomy Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta. Liver fibrosis was assessed according to the International Association for the Study of the Liver (IASL) grading system and categorized into five stages: grade 0 (no fibrosis), grade 1 (mild fibrosis characterized by fibrous portal expansion), grade 2 (moderate fibrosis with the presence of a few fibrous bridges or septa), grade 3 (severe fibrosis with numerous fibrous bridges or septa), and grade 4 (cirrhosis).

Outcome measures

After 30 days of treatment, the ALT level were also measured. Five milliliters of blood was taken from the ophthalmic plexus vein for the ALT level measurement The ALT level was measured using spectrophotometry. Primary outcomes: Expression of NF-κB (p65) and caspase-3 in hepatocytes (measured by immunohistochemistry), and histological fibrosis scoring (Masson's trichrome).

Statistical analysis

Data were analyzed using SPSS version 22.0. The mean difference of the ALT level between groups is analyzed using One-Way ANOVA. Kruskal-Wallis test was used to compare ordinal data (NF-κB, caspase-3, fibrosis scores). Post-hoc comparisons were performed using the Mann-Whitney U test. A 95% confidence interval was used in this study. Results were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

The lowest ALT level in week 5 was observed in the NC group (which did not receive either isoniazid or mangosteen pericarp ethanol extract), with a value of 18.45 ± 0.52 ng/ml, while the highest ALT level was found in the PC group (which received only isoniazid), at 38.15 ± 0.79 ng/ml. Among the groups treated with Garcinia mangostana L. ethanol extract in week 5, the lowest ALT level was recorded in the I2 group (administered 500 mg/kgBW of the extract), with a mean value of 20.81 ± 0.44 ng/ml, whereas the highest ALT level was observed in the I1 group (administered 250 mg/kgBW of the extract), reaching 28.52 ± 0.72 ng/ml (Table 1).

Table 1.

Alanine aminotransferase levels amongst all groups

Group	ALT levels on 5th week
NC	18.45 ±0.52
PC	38.15 ±0.79
I1	28.52 ±0.72
I2	20.81 ±0.44
p-value	0.001**

Description :
NC: Not Given Isoniazid nor Mangosteen Extract
PC: Only Given Isoniazid
I1: Given 250 mg/kgBW Mangosteen Extract
I2: Given 500 mg/kgBW Mangosteen Extract
ALT: Alanine Aminotransferase

Nuclear factor kappa B expression

The immunohistochemical analysis revealed that rats in the isoniazid-only group (PC) exhibited higher NF-κB (p65) expression, predominantly scoring 3–4,

compared to the negative control (NC). Administration of Garcinia mangostana L. Peel Extract at 500 mg/kg BW (I2 group) significantly reduced NF-κB expression, with most samples scoring 1–2. However, Kruskal-Wallis analysis showed no statistically significant differences between all groups (p = 0.156) (Table 2). Post-hoc Mann-Whitney tests revealed significant differences between NC vs PC (p = 0.032) and PC vs I2 (p = 0.030), indicating a partial effect at higher doses (Table 3).

Table 2.
Nuclear Factor Kappa B Difference Amongst All Group

NF-κB	Group			
	NC	PC	I1	I2
Score 1	1	0	0	1
	12.5%	.0%	.0%	14.3%
Score 2	2	0	4	2
	25.0%	.0%	50.0%	28.6%
Score 3	5	5	2	4
	62.5%	71.4%	25.0%	57.1%
Score 4	0	2	2	0
	.0%	28.6%	25.0%	.0%
p value	**0,156**			

Description :
NC: Not Given Isoniazid nor Mangosteen Extract
PC: Only Given Isoniazid
I1: Given 250 mg/kgBW Mangosteen Extract
I2: Given 500 mg/kgBW Mangosteen Extract

Table 3.
Post Hoc Test on NF-κBp65 Expression

Intervention	p-valueof NF-κBp65 5th Week		
	NC	PC	I1
PC	0.032*		
I1	0.735	0.171	
I2	0.843	0.030*	0.620

Description :
NC = Not Given Isoniazid nor Mangosteen Extract
PC = Only Given Isoniazid
I1 = Given 250 mg/kgBW Mangosteen Extract
I2 = Given 500 mg/kgBW Mangosteen Extract
*Significant at α=0.05

Hepatic fibrosis

Histological evaluation using Masson's trichrome staining revealed marked fibrosis (score 4) in the PC group, while the NC group showed no fibrosis. The I1 group predominantly displayed score 3 fibrosis, whereas the P2 group exhibited milder fibrosis (score 1–2) (Table 4). Kruskal-Wallis test confirmed significant differences across groups (p < 0.001), and post-hoc tests indicated that I2 significantly differed from PC (p = 0.006) and I1 (p = 0.005), but still differed from NC (p < 0.001).

Table 4.
Differences in Liver Fibrosis Based on Treatment Formulations of Mangosteen Peel Ethanol Extract at Week 5

Fibrosis	Group			
	NC	PC	I1	I2
Score 0	8	0	0	0
	100.0%	0.0%	0.0%	0.0%
Score 1	0	0	0	4
	0.0%	0.0%	0.0%	57.1%
Score 2	0	0	0	1
	0.0%	0.0%	0.0%	14.3%
Score 3	0	4	6	2
	0.0%	57.1%	75.0%	28.6%
Score 4	0	3	2	0
	0.0%	42.9%	25.0%	0.0%
P-value	**0.001****			

Description :
NC = Not Given Isoniazid nor Mangosteen Extract
PC = Only Given Isoniazid
I1 = Given 250 mg/kgBW Mangosteen Extract
I2 = Given 500 mg/kgBW Mangosteen Extract
** Significant at α=0.01

Table 5.
Post Hoc Test of Differences in Liver Fibrosis Findings Among Various Treatment Formulations

Treatment	p-value of Fibrosis at week 5		
	NC	PC	I1
PC	0.001**		
I1	0.001**	0.480	
I2	0.001**	0.006**	0.005**

Description:
NC = Not Given Isoniazid nor Mangosteen Extract

PC = Only Given Isoniazid

I1 = Given 250 mg/kgBW Mangosteen Extract

I2 = Given 500 mg/kgBW Mangosteen Extract

** Significant at α=0,01

Liver tissue fibrosis images

The normal control group shows no fibrosis (F0) (A and B); the negative control group shows cirrhosis (F4) (C and D) (Figure 1). Treatment Group 1 shows portal fibrosis with numerous septa (F3) (E and F); and Treatment Group 2 shows portal fibrosis without septa (F1) (G and H).

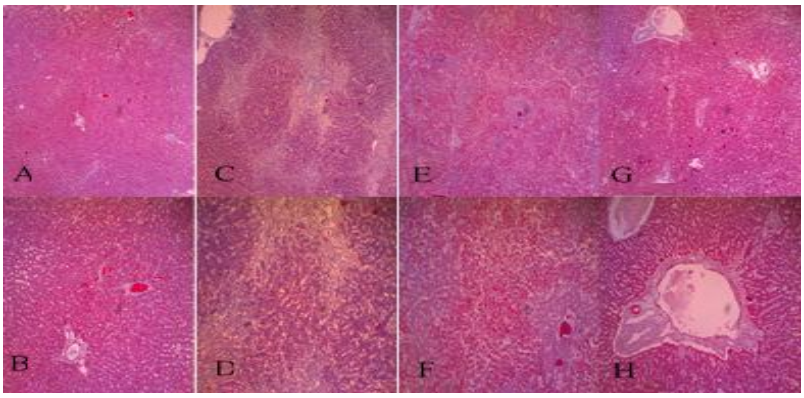

Figure 1. Liver tissue fibrosis histology

Effects of Garcinia mangostana l. peel extract on caspase-3 and hepatic fibrosis

Caspase-3 expression differed significantly among the groups ($p < 0.001$, Kruskal-Wallis). Rats in the PC group exhibited high levels of expression (score 3), while those in the I2 group showed significantly reduced expression (Table 6). Post-hoc analysis confirmed significant differences between NC vs I2 ($p = 0.002$), PC vs I1 ($p = 0.011$), and I1 vs I2 ($p = 0.026$), indicating a dose-dependent reduction in apoptosis marker expression.

Table 6.

Differences in caspase-3 based on treatment formulations of mangosteen peel ethanol extract at week 5

Caspase-3	Group			
	NC	**PC**	**I1**	**I2**
Score 1	7	0	0	1
	100%	0.0%	0.0%	14.3%
Score 2	0	0	3	6
	0.0%	0.0%	37.5%	85.7%
Score 3	0	4	5	0
	0.0%	57.1%	62,5%	22.2%
Score 4	0	3	0	0
	0.0%	42.9%	0.0%	0.0%
P-value	**0,0001**			

Description :
NC = Not Given Isoniazid nor Mangosteen Extract
PC = Only Given Isoniazid
I1 = Given 250 mg/kgBW Mangosteen Extract
I2 = Given 500 mg/kgBW Mangosteen Extract
** Significant at α=0.01

Table 7.

Post Hoc Test on Caspase-3 Expression

Intervention	p-value of Caspase-3 5th Week		
	NC	**PC**	**I1**
PC	0.001		
I1	0.001	0.011	
I2	0.002	0.001	0.026

Description :
NC = Not Given Isoniazid nor Mangosteen Extract
PC = Only Given Isoniazid
I1 = Given 250 mg/kgBW Mangosteen Extract
I2 = Given 500 mg/kgBW Mangosteen Extract
** Significant at α=0.01

Tables 7 demonstrate that the administration of the Ethanol Extract of Garcinia mangostana L. Peel significantly reduces Caspase-3 expression in a rat model of liver injury. The ordinal data from 28 samples, analyzed using the Kruskal-Wallis test, established a highly significant difference in Caspase-3 levels among the treatment groups ($p = 0.0001$). Subsequent post-hoc Mann-Whitney tests further clarified these results by showing significant differences between the negative control group and the high-dose treatment group ($p = 0.002$), and between the lower-dose and high-dose treatment groups ($p = 0.0026$). This robustly indicates that Garcinia mangostana L. Peel Extract possesses a potent anti-apoptotic effect by suppressing Caspase-3 expression.

This analysis reveals that the effectiveness of Garcinia mangostana L. Peel Extract is dose-dependent. Administration of Garcinia mangostana L. Peel Extract at 500 mg/kg BW (group I2) was highly effective in reducing Caspase-3 expression to a level comparable to that of the negative control group. In contrast, the 250 mg/kg BW dose (group I1) demonstrated less effectiveness, with results not significantly different from the positive control group. A similar pattern was observed for NF-κB expression, where the 500 mg/kg BW dose was significantly superior. This finding is consistent with prior research that has confirmed the role of xanthone compounds in mangosteen peel, which possess anti-inflammatory properties by inhibiting NF-κB (Hassan et al., 2021; Setiawan et al., 2023). Mangosteen's ability to inhibit NF-κB is mediated by a reduction in ROS levels and the activation of the Nrf2 antioxidant pathway (Hassan et al., 2021; Morgan & Liu, 2011). Previous studies supporting the connection between α-mangostin, the Nrf2 pathway, and the enzyme SOD include the research by Shehata et al. (2022), which demonstrated that administration of α-mangostin at doses of 25 and 50 mg/kg increased the expression of genes related to the Nrf2 pathway as well as antioxidant enzymes such as NQO1, HO-1, and GCL in a liver injury model induced by Con A. Additionally, Shen et al. (2014) showed that α-mangostin could inhibit Nrf2 expression in 3T3-L1 adipocytes during adipogenesis, helping to reduce inflammation through the regulation of NF-κB. These studies indicate that α-mangostin has the potential to activate the Nrf2 pathway and enhance the activity of antioxidant enzymes like SOD, collectively strengthening the body's defense against oxidative stress (Majdalawieh et al., 2025).

This research reinforces the anti-apoptotic mechanism of Garcinia mangostana L. Peel Extract, which is mainly linked to its bioactive compounds, such as α-mangostin (Harlisa et al., 2022). By suppressing Caspase-3, a key executioner enzyme in the apoptosis pathway, Garcinia mangostana L. Peel Extract directly inhibits programmed cell death (Harlisa et al., 2022; Zonouz et al., 2023). This mechanism is primarily mediated by the extract's strong antioxidant properties, which neutralize ROS and prevent the mitochondrial release of cytochrome c, thereby inhibiting the activation of Caspase-9 and Caspase-3 via the intrinsic apoptosis pathway (El Gaafary et al., 2024; Harlisa et al., 2022; Zonouz et al., 2023). Additionally, mangosteen has anti-apoptotic capabilities by inhibiting the extrinsic pathway, as it can significantly reduce TNF-α levels, which would otherwise lead to Caspase-8 and subsequent Caspase-3 activation. This dual effect of inhibiting both apoptosis and inflammation positions Garcinia mangostana L. Peel Extract as a promising therapeutic agent for protecting the liver from toxicant-induced injury (Harlisa et al., 2022).

This study demonstrates that ethanol extract of Garcinia mangostana L. peel has a hepatoprotective effect in a dose-dependent manner against isoniazid-induced liver injury in Wistar rats. The 500 mg/kg BW dose

significantly suppressed NF-κB and caspase-3 expression and reduced the degree of liver fibrosis, aligning with previous findings on mangosteen's antioxidant and anti-inflammatory effects (Harlisa et al., 2022; Hassan et al., 2021; Zhang et al., 2022).

Regarding its anti-inflammatory action, mangostin downregulates key mediators of inflammation, including NF-κB, TNF-α, IL-1β, and COX-2. By inhibiting NF-κB translocation into the nucleus, it reduces the transcription of pro-inflammatory cytokines and adhesion molecules. Mangosteen peel extract can inhibit NF-κB through the reduction of ROS levels by enhancing the expression of antioxidant enzymes such as superoxide dismutase (SOD).(Cahyani et al., 2025; Hassan et al., 2021) This modulation of inflammatory pathways contributes to its protective effects against tissue injury, including hepatotoxicity.

ROS itself is known to stimulate NF-κB by promoting IκB degradation or alternative phosphorylation.(Morgan & Liu, 2011) This was also explained in a study conducted by Zhang et al. (2022), which found that mangosteen peel extract also downregulated the transcription factor NF-κB in liver tissue by inhibiting the phosphorylation of the IL-1β–induced NF-κB signaling pathway.(Zhang et al., 2022)

NF-κB plays a central role in mediating inflammation by promoting proinflammatory cytokines such as TNF-α and IL-6. Its inhibition observed in the P2 group reflects the extract's potential to modulate key inflammatory pathways. This effect may be attributed to α-mangostin, a major xanthone compound in mangosteen peel, known to block NF-κB signaling and reduce oxidative stress (Setiawan et al., 2023; Tatiya-Aphiradee et al., 2021).

Tumor growth factor (TGF)-β1 is the main profibrotic cytokine that triggers HSC activation in the pathogenesis of liver fibrosis. Increased TGF-β1 expression leads to HSC proliferation (Biswas et al., 2020; Naji et al., 2021; Zhuang et al., 2022). TGF-β1 induces α-SMA expression mediated by C/EBPβ acetylation, which promotes the formation of fibrotic septa in the liver (X. Chen et al., 2021). This activation drives the transformation of HSCs into myofibroblasts that produce ECM, including type I and III collagen (X. Chen et al., 2021; Naji et al., 2021; Zhuang et al., 2022). A reduction in TGF-β1 expression decreases HSC activation, thereby inhibiting ECM production and reducing liver fibrosis. Research by Li et al. (2019) demonstrated that natural compounds derived from certain plants could suppress the TGF-β/Smad pathway to reduce liver fibrosis. They found that these compounds could inhibit TGF-β expression and Smad2/3 activity, leading to decreased collagen production and improvement in fibrosis conditions both in vitro and in vivo models (Liao et al., 2023).

Mangosteen peel extract contains active compounds, particularly xanthones, which have strong antioxidant properties. This antioxidant effect prevents the formation of reactive oxygen species (ROS) induced by stimulating agents. By reducing oxidative stress, mangosteen peel extract inhibits signaling pathways that induce TGF-β1 and α-SMA expression (Yahyazadeh et al., 2024).

Similarly, caspase-3 is a principal executor of apoptosis. The marked decrease in its expression following Garcinia mangostana L. Peel Extract administration, particularly at 500 mg/kg BW, indicates the extract's anti-apoptotic properties. Previous research corroborates that mangosteen extract inhibits caspase activation by enhancing antioxidant defenses and stabilizing mitochondrial membranes (P. Chen et al., 2023; El Gaafary et al., 2024; Zonouz et al., 2023).

The results of this study are consistent with previous research conducted by Harlisa et al. (2022) on an animal model of radiation-induced skin injury, which demonstrated that mangosteen peel extract was able to reduce serum caspase-3 levels (Harlisa et al., 2022). Study by Yi-Jen Liao et al (2023) showed that α-mangostin increased the cleavage of caspase-3, indicating that mangostin can induce apoptosis in hepatic stellate cells (HSCs), which play a critical role in the process of liver fibrogenesis. Activation of caspase-3 helps reduce the number of active and proliferative HSCs, thereby contributing to the alleviation of liver fibrosis (Liao et al., 2023). Previous research supports this effect. For example, a study by Ong et al. (2018) demonstrated that mangostin could stimulate apoptosis in hepatocellular carcinoma cells through the activation of caspase-3, as well as decrease pro-fibrotic expression in fibrosis-induced rat livers. Additionally, Lin et al. (2019) found that mangostin increased the activation of caspase-3 and caspase-9 in hepatocytes under oxidative stress, indicating the role of mangostin in mediating apoptosis through the mitochondrial pathway.This finding is also supported by a study conducted by El Gaafary et al. (2024), which reported that administration of prenylated xanthone compounds such as α-mangostin, γ-mangostin, 9-hydroxycalabaxanthone (9-HCX), and garcinone E, isolated from Garcinia mangostana peel, exhibited similar effects (El Gaafary et al., 2024).

The anti-apoptotic mechanism of mangosteen is mainly associated with its bioactive components, such as α-mangostin, which possess antioxidant and anti-inflammatory properties. These active compounds can suppress caspase-3 activation, a key executioner enzyme in the apoptotic pathway (Harlisa et al., 2022). Oxidative stress is a major trigger of caspase-3 activation, particularly through the intrinsic apoptosis pathway involving mitochondria. Free radicals, such as ROS, cause DNA damage, mitochondrial dysfunction, and the release of cytochrome c into the cytosol (El Gaafary et al., 2024). The bioactive components of mangosteen, such as α-mangostin, act as potent antioxidants capable of scavenging free radicals and inhibiting ROS formation. By reducing oxidative stress, mangosteen prevents cytochrome c release from mitochondria into the cytosol, thereby inhibiting caspase-9 and caspase-3 activation in the intrinsic apoptosis pathway. In addition, mangosteen enhances the activity of antioxidant enzymes such as

superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, which play crucial roles in neutralizing ROS that trigger caspase-3 activation. By suppressing ROS levels, mangosteen reduces the likelihood of intrinsic apoptosis activation. α-Mangostin also contributes to maintaining mitochondrial membrane potential, preventing cytochrome c release, and inhibiting caspase-3 activation via the intrinsic pathway (Harlisa et al., 2022; Zonouz et al., 2023).

Moreover, mangosteen extract exhibits anti-apoptotic effects by inhibiting the TNF-α pathway. TNF-α is a pro-inflammatory cytokine that induces apoptosis through TNF receptor 1 (TNFR1). TNFR1 activation initiates a signaling cascade involving adaptor proteins such as FADD and subsequently activates caspase-8, which in turn activates caspase-3. Studies have shown that mangosteen extract significantly reduces TNF-α levels in experimental animal models. This reduction in TNF-α inhibits the extrinsic apoptosis pathway leading to caspase-3 activation (Harlisa et al., 2022). These findings are also in line with the present study, which demonstrated a decrease in NF-κB expression, a transcription factor involved in TNF-α synthesis.

Fibrosis reduction, as evidenced by lower collagen deposition in liver tissue, also supports Garcinia mangostana L. Peel Extract's antifibrotic activity. This may result from suppressed hepatic stellate cell activation, a process regulated by both NF-κB and TGF-β signaling (Biswas et al., 2020; Braczkowski et al., 2024). The findings emphasize that mangosteen extract not only mitigates inflammation and apoptosis but also interrupts the fibrogenesis cascade, highlighting its multifaceted protective mechanism. While the 250 mg/kg BW dose showed some benefit, it was not statistically different from the isoniazid-only group, indicating insufficient therapeutic efficacy at this dose.

This study presents significant strengths, including its scientific novelty in demonstrating that ethanol extract of mangosteen peel (Garcinia mangostana L) can reduce NF-κB expression and ALT levels, thereby improving liver fibrosis. Clinically, it offers a potential new complementary therapy for isoniazid-induced liver injury, proposes a new research strategy that could form the basis for future clinical trials, and provides a prospective foundation for translating preclinical findings into clinical applications. However, the study has several limitations, such as the use of only one type of extract and limited dependent variables, a narrow dose range that prevents determination of the optimal dose, the absence of toxicity or side effect evaluation, and the use of isoniazid alone without combination with other anti-TB drugs. Additionally, reliance solely on IHC without complementary techniques like Western blot reduces detection sensitivity, only male Wistar rats were used without comparison to females, and the extract was administered orally without exploring active compounds like alpha-mangostin or alternative delivery forms such as infusions or injections

CONCLUSIONS

Administration of the extract at a higher dose of 500 mg/kg BW resulted in significantly lower mean ranks for serum ALT, NF-κB (p65) expression, Caspase-3 expression, compared to the isoniazid-only group, indicating substantial improvements in hepatocellular integrity, inflammatory response, apoptosis inhibition, and fibrotic progression. In contrast, the 250 mg/kg BW dose showed higher mean ranks that were closer to the untreated group and did not differ significantly in several outcomes, highlighting its limited therapeutic effect.

The results support the potential of Garcinia mangostana peel extract as a natural adjunctive therapy for managing drug-induced liver injury. These findings suggest its potential as an adjunct in preventing isoniazid-induced hepatic injury in a preclinical setting. Additional translational and clinical investigations are needed to determine its efficacy and safety as a potential hepatoprotective agent in humans.

SUGGESTION

Future studies should explore a wider dose range of Garcinia mangostana L. peel extract to determine the minimum effective dose and the optimal therapeutic dose for hepatoprotection. The use of complementary molecular techniques such as Western blot and RT-qPCR is recommended to validate and strengthen the immunohistochemical findings of NF-κB and caspase-3 expression. Additionally, investigating the isolated active compound α-mangostin alongside the crude extract would help clarify the specific bioactive component responsible for the observed hepatoprotective effects. Researchers should also consider evaluating the extract's efficacy in combination with standard anti-tuberculosis regimens (e.g., isoniazid combined with rifampicin, pyrazinamide, and ethambutol) to better reflect clinical conditions. Long-term toxicity and safety profiles of the extract should be assessed prior to clinical translation. Finally, including both male and female animal models in future research would improve the generalizability of the findings.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Despite its promising findings, this study has several limitations that should be acknowledged to ensure scientific transparency. First, the Garcinia mangostana L. peel extract used in this experiment was a crude ethanolic extract without comprehensive standardization of its active constituents, and its hepatoprotective effects were not compared directly against the isolated active compound, α-mangostin. Second, while liver structural changes were evaluated using Masson's trichrome staining, a comprehensive, fibrosis-specific biochemical assessment such as the quantification of specific extracellular matrix components like hydroxyproline, α-SMA, or TGF-β1 expression levels via RT-qPCR—was not performed. Third, the sample size (n=7 per group) remains relatively small, representing a typical constraint of exploratory animal models which may limit overall statistical reliability. Fourth, the biomarker panel evaluated was focused primarily on serum ALT, NF-κB, and caspase-3; expanding this panel to include broader oxidative stress markers (e.g., SOD, malondialdehyde) or additional pro-inflammatory cytokines would provide a more complete mechanistic picture. Fifth, the 30-day observation period was relatively short, preventing the assessment of long-term therapeutic efficacy or potential chronicity of liver changes. Furthermore, a dedicated systemic toxicity analysis or side-effect evaluation of the high-dose extract was absent. Finally, this study utilized isoniazid alone to induce hepatotoxicity rather than combining it with other first-line anti-tuberculosis drugs (such as rifampicin or pyrazinamide), and the evaluation was limited exclusively to male Wistar rats, which restricts the immediate generalizability of these findings to broader clinical and translational settings. Future investigations incorporating complementary molecular techniques like Western blot or RT-qPCR, broader dosing regimens, and prolonged treatment durations are highly warranted to validate these results.

Ethics statements

The authors declare that this study received ethical approval from the ethics committee of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, with number 1.724/ VIII/ HREC/ 2025. All procedures were conducted in accordance with the institution's.

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