

The ethanol extract of *Garcinia mangostana L* peel reduces the isoniazid-induced liver damage in rats



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ABSTRACT

Background: The long-term use of Isoniazid (INH) as the Tuberculosis (TB) treatment increases the risk of possible liver damage. The peel from *Garcinia Mangostana L.* has been shown to reduce hepatotoxicity in the rat model. However, the molecular mechanism of this effect is not well-understood

Objective: This study aims to investigate the effect of the ethanol extract of the peel of *Garcinia Mangostana L.* or mangosteen in preventing INH-induced liver damage at the cellular and molecular levels.

Methods: A total of 32 male Wistar rats (*Rattus Norvegicus*) three to four months old with bodyweight between 170 and 200 grams are randomly enrolled into the negative control (NC), Positive control (PC), Treatment 1, and treatment 2 groups. The rats in the positive control (PC) and the treatment groups (T1, T2) were injected with 80 mg INH per kilogram body weight to induce liver fibrosis at the beginning of the study. The rats in the negative control group (NC) are injected with normal saline. The T1 and T2 groups are given 250 mg and 500 mg per kilogram body weight of mangosteen peel ethanol extract, respectively. After 35-days of treatment, blood samples were drawn from the rats, and the SGPT level was measured. Following the sacrifice, the liver tissues were prepared into slides and immunohistochemically stained and the TGF- β 1 expression and fibrosis level were examined.

Results: The expression of TGF- β 1 is significantly higher in the PC and T1 groups than in the NC group (p-value <0.05). Compared with the NC groups, the SGPT level is significantly higher in the PC, T1, and T2 groups with a p-value less than 0.05. The fibrosis scoring in the T2 group is statistically lower than the PC group. (p-value <0.05).

Conclusion: The ethanol extract of *Garcinia mangostana L.* peel at a dose of 500 mg/kg of body weight shows a significant difference in the fibrosis score between the treatment group and the positive control group.

Keywords: mangosteen peel extract, TGF- β 1, SGPT, liver fibrosis.

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INTRODUCTION

The World Health Organization (WHO) in 2014 reported that around 2-3 billion people were infected with Tuberculosis (TB).¹ The report mentioned approximately 9-10 million people suffered from TB complications, which resulted in 2.7 million deaths worldwide.¹ Indonesia has the second-highest TB burden in the world with an increase in TB cases from 360,565 cases in 2016 to 425,089 cases in 2017.^{1,2} Isoniazid (INH) remains to be the first-line drug for TB treatment either as a single or combination drugs, which requires a long period of therapy (6-12 months). The prolonged treatment increases the risk of liver toxicity and potentially fatal liver damage that can range from minimal to severe damage in

liver failure that requires a liver transplant.³⁻⁵ The incidence of Drug-Induced Liver Injury (DILI) related to tuberculosis therapy varies from 2% to 28%.⁵ The liver damage induced by the INH can be prevented with proper management.

The increase in the Reactive Oxygen Species (ROS) productions resulted in liver damage in INH-induced mice.⁶ The oxidative stress caused the failure of oxidative phosphorylation, the decreased levels of Adenosine Triphosphate (ATP), and the increased lipid peroxidation level in the hepatocytes' mitochondrial membrane. The inflammation process in the mitochondria releases the cytochrome-c to induce apoptosis.⁶ The apoptotic signals recruit the inflammatory cells to the damaged liver and the pro-fibrogenic

cytokines such as Transforming Growth Factor- β 1 (TGF- β 1), Tumor Necrosis Factor- α and various interleukins.^{7,8} The TGF- β 1 plays an important role in fibrogenesis activation of myofibroblasts and triggers the secretion of extracellular matrix proteins, especially collagen type 1.⁹ Polymorphonuclear (PMN), a pro-inflammatory cells which contains a higher concentration of lysozyme which could damage the cell walls, resulting in the cell necrosis, including hepatocytes.¹⁰ Thus, it is characterized by the increase in the level of SGPT.¹¹

Several studies reported that the mangosteen peel extract reduces the hepatotoxicity of INH in the rat model. The mangosteen peel is rich in antioxidants, especially anthocyanins, xanthenes, tannins, and phenolic acids.¹² Moreover, mangosteen peel has an antioxidant effect, anti-inflammatory effect and hepatoprotective properties.¹³⁻¹⁸ However, the study focusing on its molecular mechanism was still limited. Therefore, this study is designed to assess mangosteen peel ethanol extract's effect in preventing INH-induced liver damage at cellular and molecular levels as an effort to develop extracts of natural ingredients as DILI therapy caused by INH.

MATERIALS AND METHODS

Our study was experimental research to compare mangosteen peel ethanol extract's effect in the liver cirrhosis induced mice model. The Ethics Committee approved this study for Health Research of Dr. Moewardi General Hospital / School of Medicine, Universitas Sebelas Maret, No. 719/IX/HREC/2018.

The mangosteen peel was obtained from the Girilayu Village, Matesih District, Karanganyar, Central Java, and the ethanol extract was made in the PAU Yogyakarta laboratory to yield alpha mangosteen (α -Mangosteen), the active compound of mangosteen peel. The mangosteen peel extract was analyzed by HPLC in the MIPA laboratory, Universitas Sebelas Maret, Surakarta. The α -Mangosteen concentration was 18.98 ± 0.10 ppm or 46.23 ± 0.24 μ M in 50 ppm extract.

A total of 32 male Wistar rats (*Rattus norvegicus*), three to four months old, with bodyweight between 170 and 200

grams are obtained from *Laboratorium Penelitian dan Pengujian Terpadu* (LPPT), Universitas Gajah Mada, Indonesia. The rats were fed with standard feeding and provided with drinks. They are kept at the temperature of 32°C with 12 hours of dark and light cycle. These rats are randomly allocated into two different treatment groups, and a positive and a negative control group with a total number of eight rats within each group. In our study, two rats were dead, one in the positive control and another in the treatment group 2.

The rats in the positive control (PC) and the treatment groups (T1 and T2) were injected with 80 mg INH per kilogram body weight once a day for 35 days to induce liver fibrosis. The rats in the negative control group (NC) are injected with normal saline once a day for 35 days. The T1 group was given a dose of 250 mg of mangosteen peel ethanol extract per kilogram bodyweight daily, everyday for 35 days, while the T2 group was given 500 mg in the same manner of T1. After 35-days of treatment, the SGPT level, TGF- β 1 expression, and fibrosis level were measured. Five milliliters of blood was taken from the ophthalmic plexus vein for the SGPT level measurement. Next, they were sacrificed and their liver were harvested. The liver tissues were prepared using immunohistochemical staining for assessing the TGF- β 1 expression level and the Massons' Trichrome staining for fibrosis staging.

The immunohistochemical staining of the liver tissue was conducted at The Pathological Anatomy Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta. The liver fibrosis was scored 0, 1, 2, 3, 4, and 5 for no fibrosis, mild fibrosis (fibrous portal expansion), moderate fibrosis (few bridges or septa), severe fibrosis (numerous bridges or septa), cirrhosis, respectively, based on International Association for the Study of the Liver (IASL) grading.¹⁹ While the expression level of TGF- β 1 was scored 0, 1 and 2 for low, moderate, and high level, respectively. The SGPT level was measured using spectrophotometry.

The mean difference of the SGPT level between groups is analyzed using One-Way ANOVA followed by post hoc LSD. The expression of the TGF- β 1 and liver

fibrosis level were analyzed using the Kruskal-Wallis test followed by post hoc Mann Whitney. A p-value of less than 0.05 was considered significant. All analysis was conducted using SPSS version 20 for Windows.

RESULTS

The expression TGF- β 1

There were six rats (75%) and five rats (71.4%) observed in the NC and T2 groups with moderate expression of TGF- β 1. The high expression of TGF- β 1 (score 2) was observed in the PC and T1 groups for 71.4% and 62.5%, respectively. (See Table 1) There was a significant difference in the expression of TGF- β 1 between these groups, with a p-value of 0.008. The expression of TGF- β 1 was significantly higher in the PC and T1 groups than in the NC group (p-value <0.05). The T2 group had a higher expression of TGF- β 1 than the NC group. However, it is not statistically significant (p=0.053).

The SGPT Level

The highest SGPT level (mean \pm standard deviation) was observed in the PC group followed by the T1, T2, and NC groups for 38.11 ± 0.79 , 28.52 ± 0.72 , 20.88 ± 0.44 , and 18.45 ± 0.52 U/L. Compared with the NC groups, the SGPT level was significantly higher in the PC, T1, and T2 groups with a p-value <0.05. The *post hoc* LSD analysis showed the differences in SGPT level in each group compared to the control group (p<0.01).

The Histological Structure of Liver Tissues and The Scoring of Liver Fibrosis

The degree of liver damage between the groups could be seen in Figure 1. The fibrosis scoring in the T1 and T2 groups was 21.63 and 13.43 units, respectively. These were statistically higher than the NC group with a p-value <0.05. The fibrosis scoring in the T2 group was statistically lower than the PC group (p-value <0.05) (Table 2).

DISCUSSION

The result showed that the TGF- β 1 expression in the PC and T1 groups is statistically higher than in the NC group.

Table 1. The Expression of TGF- β 1 between the groups

TGF- β 1 Scoring	Group of Treatment				p-value
	T1	T2	PC	NC	
Score 0	0 (0%)	0 (0%)	0 (0%)	2 (25%)	0.008
Score 1	3 (37.5%)	5 (71.4%)	2 (28.6%)	6 (75%)	
Score 2	5 (62.5%)	2 (28.6%)	5 (71.4%)	0 (0%)	

T1: Treatment group 1, T2: Treatment group 2, PC: Positive control group, NC: Negative control group

Table 2. The Scoring of Liver Fibrosis between Groups

Fibrosis Scoring	Group of Treatment				p-value
	T1	T2	PC	NC	
Score 0	0 (0%)	0(0%)	0 (0%)	8 (100%)	<0.001
Score 1	0 (0%)	4 (57.1%)	0 (0%)	0 (0%)	
Score 2	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)	
Score 3	6 (75%)	2 (28.6%)	4 (57.1%)	0 (0%)	
Score 4	2 (25%)	0 (0%)	3 (42.9%)	0 (0%)	

T1: Treatment group 1, T2: Treatment group 2, PC: Positive control group, NC: Negative control group

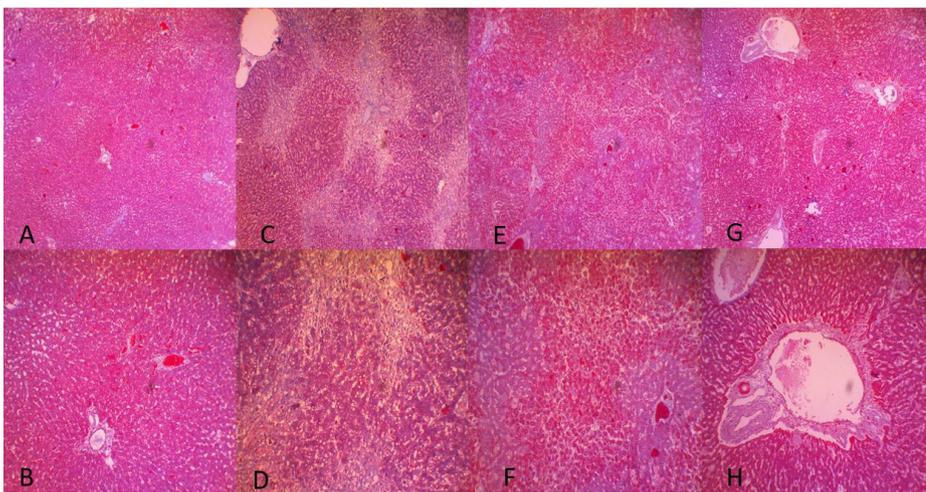


Figure 1. The histological structure from normal to fibrotic liver tissues. The NC group did not show liver fibrosis (F0) (Pic. 3A and 3B). The positive control group histological showed cirrhosis liver structures (F4) (C and D). The T1 group showed portal fibrosis with many septa (F3) (E and F). The T2 group showed a fibrosis portal without any septa (F1) (G and H). A, C, E, G with 40x magnification; B, D, F, H 400x magnification.

This suggests that the induction of INH increases the expression of the TGF- β 1. The increase in the expression of the TGF- β 1 results from the INH metabolites, which can induce the activation of Kupffer cells. The expression of TGF- β 1 is followed by cytokines and chemokine releases which stimulate the stellate cells leading to the fibrous formation.²⁰ Moreover,

the Kupffer cells, antigen-presenting cells (APC), induce the T-cells to release anion superoxide, hydrogen peroxide, nitric oxide, and hydrolytic enzyme, and eicosanoid.²¹ The TGF- β 1 itself plays a role as a key regulator for the development or degradation of extracellular matrix (ECM).²² The mangosteen extract can reduce TGF- β 1 expression in a dose-

dependent manner (Table 1) The α -Mangosteen could be a responsible phytoconstituent related to its suppressing effect on TGF- β 1 expression. The study by Fu et al. 16 shows that α -Mangosteen from *Garcinia Mangostana L* possessed antioxidant and anti-inflammatory effects, improving liver destruction caused by toxic agents.

The AST and ALT are liver enzymes involved in human protein metabolism. Their activation is important indicator of liver injury.²³ The α -Mangosteen, one of the main mangosteen peel extract chemical components, was claimed to have several pharmacological activities, including antibacterial, anti-inflammatory, antioxidant, and anticancer effects.²⁴ A previous study indicated that α -Mangosteen exhibited a significant hepatoprotective effect against APAP-induced acute liver injury in mice, associated with its antioxidant and anti-inflammatory properties.²⁴

The study result shows that the INH caused liver fibrosis in rats. In chronic liver injury, excessive accumulation of extracellular matrix such as collagen tissue is a typical liver fibrosis characteristic.⁷ Oxidative stress and inflammation causes liver injury and activates stellate cells, which increases extracellular matrix (ECM) production and liver fibrosis.^{20,24,25} The mangosteen peel extract at dose 500 mg/kg BW was shown to have lower fibrosis scores than the PC group ($p < 0.05$). The α -Mangosteen is claimed to be able to suppress the fibrogenesis process. It was reported that α -Mangosteen has antiproliferative and antioxidant activity due to its ability to decrease the ratio of pSmad/Smad and pAkt/Akt in TGF- β 1 in vitro induced liver fibrosis.²⁵

The effect of α -Mangosteen was limited to the animal subject test. It is not known if the hepatoprotective effect can be seen in humans. Therefore, there is a need for further research in order to evaluate the hepatoprotective effect of α -Mangosteen.

CONCLUSION

The ethanol extracts of *Garcinia mangostana L* peel at a dose of 500 mg/kg BW reduce the INH-induced liver damage by decreasing TGF- β 1, SGPT level, and liver fibrosis in rats.

ETHICAL CONSIDERATION

Ethics approval has been obtained by the Ethics Committee of Dr. Moewardi General Hospital / School of Medicine Universitas Sebelas Maret before the study.

CONFLICT OF INTEREST

The author declares no conflict of interest related to the material presented in this article.

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AUTHOR CONTRIBUTION

All authors equally contribute to the study from the conceptual framework, data gathering, and data analysis until reporting the study results through publication.

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