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Original Article

Curcuma longa Prevents Hepatotoxicity Induced by Isoniazid and Rifampicin: An Experiment in Wistar Rats

Putri Halleyana Rahman, Resti Gradia Dwiwina, Yuni Susanti Pratiwi, Muhammad Hasan Bashari
Department of Biomedical Sciences, Faculty of Medicine Universitas Padjadjaran, Indonesia

Abstract

Background: Hepatotoxicity induced by the combination of isoniazid (INH) and rifampicin (RIF) in the treatment of tuberculosis (TB) remains a major concern. Oxidative stress has a role in mechanism of hepatotoxicity. *Curcuma longa* has been widely used as a traditional medicine and has shown antioxidant activity. This study aimed to provide evidence of *Curcuma longa* (Turmeric) as protection against oxidative stress induced by isoniazid and rifampicin therapy.

Methods: This was an experimental study on male Wistar rats weighing 150–200 grams, aged 8–10 weeks which were divided into a negative control group (K0), a positive control group with INH+RIF (K1), a treatment group with a dose of 2.2 gr/kg/day *Curcuma longa* powder (K2), and treatment group with INH+RIF and additional 2.2 gr/kg/day *Curcuma longa* powder (K2+). SGOT and SGPT were measured from blood plasma on the 28th day; then hepatic tissue was obtained to measure MDA levels and observed histologically. Statistical analysis was done using ANOVA and continued with Duncan procedure using SPSS ver. 27.

Results: SGOT, SGPT, the highest average MDA level in the liver, and the highest mean necrotic cell count in the positive control group showed a significant difference ($p<0.05$). The treatment group had a smaller average number of necrotic cells than the positive control group with a significant difference ($p<0.05$).

Conclusions: *Curcuma longa* powder has been shown to prevent elevation in SGOT, SGPT, MDA of liver tissue and hepatocyte necrosis, indicating its potential in protecting the liver from oxidative stress.

Keywords: *Curcuma longa*, hepatotoxicity, isoniazid, oxidative stress, rifampicin

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Correspondence:
Putri Halleyana Rahman, dr., M.Kes.
Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Indonesia

E-mail:
putri.halleyana@unpad.ac.id

Introduction

Tuberculosis (TB) is the leading cause of death from a single infectious agent until the COVID-19 pandemic.[1,2] In 2020, approximately 9.9 million people worldwide suffered from TB and 1.3 million of them died due to Mycobacterium tuberculosis infection.[2] High rates of infection and mortality continue to increase despite the availability of efficient treatments, especially in developing countries.[1,3–5] The combination of isoniazid (INH), rifampicin (RIF), and pyrazinamide (PYZ) for two months followed by INH and RIF for the next 4 month is the recommended and most widely used regimen.[1,5,6] Nevertheless, hepatotoxicity induced by the combination of INH and RIF remains a major concern because it can lead to treatment failure and other adversities.[5,7,8]

Hepatotoxicity associated with INH, RIF, and PYZ administration occurs in 3–40% of patients,[5,9] ranging from mild reversible disturbance to fatal liver injury, resulting in elevated serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels.[1,4,5,10] Hepatotoxicity is thought to occur from various mechanisms, but mainly from oxidative stress and lipid peroxidation.[4,5] Metabolites of INH are also reported to have high toxicity and also inhibit the activity of antioxidant response element (ARE).[4,7,8] Increased production of reactive oxygen species (ROS)

and reactive nitrogen species (RNS) induced by INH and RIF places hepatocytes in a state of oxidant-antioxidant imbalance.[4,10,11] This state drives cells into oxidative stress and in turn damages lipid and protein structures, including deoxyribonucleic acid (DNA), and changes signaling pathways.[4,11] Excessive ROS ignites endogenous lipid peroxidation and subsequently damages the integrity of cell membranes.[4,11] This phenomenon can be observed from increased level malondialdehyde (MDA) serum level.[1,4]

Curcuma longa (Turmeric) is a perennial herbaceous plant from the *Zingiberaceae* family, scattered in tropical regions and known as a coloring spice in Asia.[12–14] The main phytochemical compound of *Curcuma longa* is curcumin (diferuloylmethane) which has many derivatives known as curcuminoids.[13,15] Turmeric in the form of curcumin has been widely used as a traditional medicine and has been shown to have antimicrobial, antiviral, antifungal, anti-inflammatory, and antioxidant activities.[13] Both *in vivo* and *in vitro* studies have shown the properties of curcumin to inhibit lipid peroxidation and neutralize ROS and RNS.[12,13] This scavenging activity is thought to contribute to protecting biomembranes from perioxidative damage. In addition, curcumin is considered safe even in higher dose and also has other therapeutic effectiveness.[12–14]

This study aimed to provide evidence of the use of *Curcuma longa L.* by the community with a simple processing method as protection from oxidative stress induced by INH and RIF therapy.

Methods

This study was an experimental animal study using adult male Wistar rats aged 8–10 weeks and weighing 150–200 gram, obtained from the Biofarma Laboratory, Bandung, Indonesia. The rats were optimized and adapted for 7 days, then randomly divided into 4 groups, namely the negative control group (K0); the positive control group (K1) which was given INH+RIF solution 50 mg/kg/day orally through gavage feeding for 21 days followed by 100 mg/kg/day for 7 days to ensure hepatotoxity;[16] the treatment group (K2) was given *Curcuma longa* (Turmeric rhizome) powder at dose of 2.2 gr/kg/day,[17,18] and other treatment group (K2+) was given INH+RIF at the same dose as the positive control and additional *Curcuma longa* (Turmeric rhizome) powder solution 2.2 gr/kg/day. All groups received standard feed and water ad libitum. The intervention was given through gavage feeding in solution form, including the negative control group which was given distilled water. Animal objects were handled in accordance with animal handling standards of the Ethics Committee of Faculty of Medicine Universitas Padjadjaran number 17/UN6.C.10/PN/2018.

Isoniazid from Kimia Farma was pulverized and made into solution by adding HCl until it reached a pH of 3.0, whereas Rifampicin from Indo Farma was pulverized and mixed with distilled water to become a solution.

Curcuma longa (Turmeric rhizome) was obtained from plantations in Tembalang, Semarang, Central Java, Indonesia. The *Curcuma longa* used was 11–12 months old and processed into powder at the Pusat Antar Universitas Institut Teknologi Bandung (PAU-ITB) (KKP Hayati). Curcuma longa L powder was given in a solution of 2 cc of distilled water prepared daily.

Measurements of SGOT and SGPT were conducted on days 0, 7, 14, 21 and 28. Capillary blood was drawn ±1.5 cc from rat's tail and put into Eppendorf tube that had been given ethylenediaminetetraacetic acid (EDTA). The blood was then centrifugated using 3000 rpm for 10 minutes. Each plasma of 100 µl was put into 2 new Eppendorf tubes. A total of 1 ml GOT reaction solution was added and mixed into tube I and GPT reaction solution into tube II. After 1 minute, the increase in absorbance per minute was measured for 3 minutes using a spectrophotometer with a wavelength of 365 nm.

Rats were sacrificed for hepatic tissue histological sampling and MDA measurement. After the animals were sacrificed with ether, an incision was made and the exposed area was perfused with 0.9% NaCl until the center was visible. The hepatic tissue was immediately taken for MDA measurement and the rest was preserved in 10% formaldehyde solution. Tissue MDA levels were measured using the Ohkawa method. The reagents and hepatic tissue homogenate were mixed and heated in a 100°C water bath for 60 minutes, then immediately cooled in ice. The result of hepatic MDA and thiobarbituric acid in the reagent reaction yielded a pink mixture color. Then it was centrifuged at 2,325 rpm for 10 minutes. The resultant supernatant was measured for absorbance at a wavelength of 523 nm.

Hepatic tissue examination was performed using Olympus microphotograph with H and E staining. Observations were done at 40x and 100x magnification. The extent of histological damage to hepatic tissue was characterized

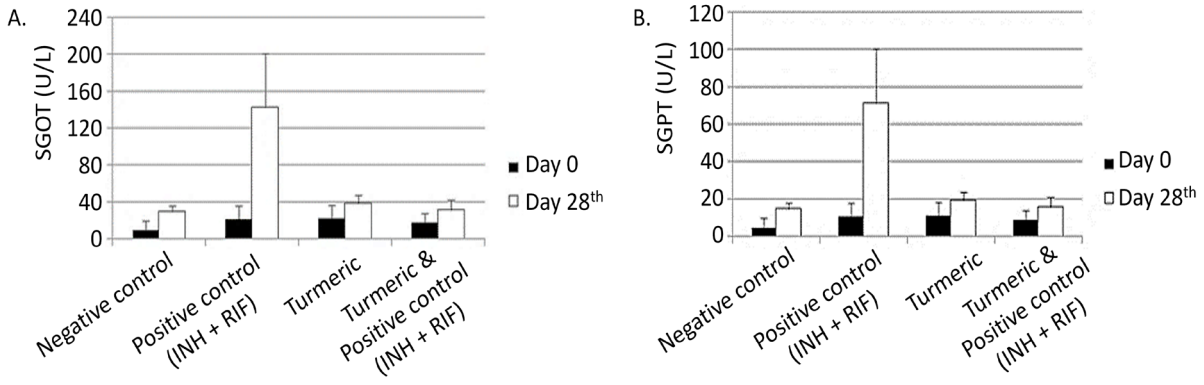

Figure 1 Comparison of SGOT (A) and SGPT (B) Levels between Day 0 and Day 28
Note: * p < 0.05

by necrotic hepatocyte shown as cells with minimal staining, appearing shrunken, having damaged nucleus, or having indefinite cell margin. Necrotic cells were counted in the visual field at 600x magnification. Observations were made around the central vein in 3 different random locations on each preparation.

The distribution of data showed normal result, so it was then analyzed using analysis of variance (ANOVA) and continued with the Duncan procedure using SPSS ver. 27.

Results

Blood levels of SGOT and SGPT on days 0 and 28 increased in the positive control (K1) group and showed a significant difference ($p<0.05$) compared to the negative control group (Figure 1). In the group given Curcuma longa L powder, SGOT and SGPT blood level did not show a significant difference ($p >0.05$) compared to the negative control group.

Level of SGOT in the INH+RIF plus *Curcuma longa* (Turmeric rhizome) group did not show significant difference ($p>0.05$) compared to the negative control group, but showed significant difference ($p<0.05$) compared to the positive control group. SGPT levels in INH+RIF plus *Curcuma longa* (Turmeric rhizome) powder group were lower compared to other groups. The SGPT levels in this group werr significantly lower ($p<0.05$) compared to the positive and negative control groups.

The results of measuring MDA levels in hepatic tissue showed that the highest average levels were found in the positive control group significantly ($p<0.05$) than the negative control

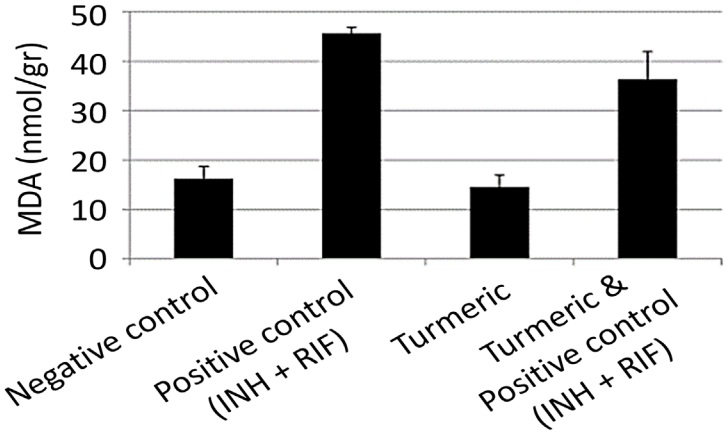

Figure 2 MDA Level in Wistar Rats Hepatic Tissue

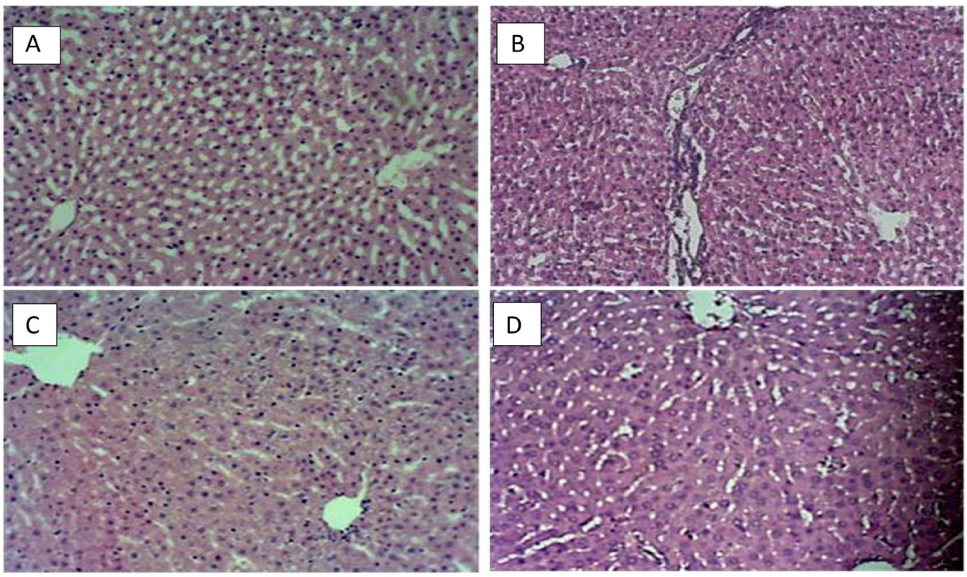

Figure 3 Histological Appearance of Hepatic Lobule Tissue Observed under a Microscope with 100x Magnification.

Note: Hepatocytes can be observed surrounding the central vein (CV), A= (K0) negative control, normal appearance of hepatocytes can be seen with intact nuclear membrane; B=(K1) positive control, dilatation of the hepatic trigone with signs of inflammation was notable, sinusoid appeared irregular; C= (K2) Curcuma Longa L. powder, hepatic lobules were within normal limits and sinusoids appeared regular; D= (K2+) (INH+RIF) hepatic lobules appeared normal with regular sinusoids +

group. This high levels of MDA in the hepatic tissue of this control group were related to INH and RIF treatment which caused oxidative stress from ROS.

The results of measuring MDA levels in hepatic tissue revealed that the group with only turmeric rhizome treatment did not show a significant difference ($p>0.05$) compared to negative control group, suggesting that administration of 2.2 gr/kg/day *Curcuma longa* (Turmeric rhizome) powder in normal conditions did not interfere with the lipid peroxidation process.

Meanwhile, the INH+RIF plus *Curcuma longa* (Turmeric rhizome) powder group showed significantly lower MDA levels compared to the positive control group ($p<0.05$), and also showed a significant difference ($p<0.05$) compared to the negative control group. Histological observations were carried out to study the hepatic portal triad of animal liver at 100x magnification and necrotic cells were counted at 600x magnification.

In the positive control group, inflammation and dilatation of the hepatic trigone were notable. The sinusoid structure appeared irregular, and was notably different from the negative control group. In the *Curcuma longa* (Turmeric rhizome) powder group, the lobules were seen in good condition with a regular sinusoidal structure. In the group with INH+RIF induced plus *Curcuma longa* (Turmeric rhizome) powder, the lobules seemed to be in good condition with regular sinusoid structure.

In terms of the necrotic cell count, the largest average was in the positive control group which was significantly different ($p<0.05$) from the negative control group. The largest average was also significantly different from the treatment group which showed a smaller number of necrotic cells, indicating that *Curcuma longa* (Turmeric rhizome) powder at a dose of 2.2 gr/kg/day might prevent hepatic cell damage induced by INH and RIF.

Discussion

This study analyze the effect of curcuma simplicia instead of curcumin, the active compound that requires a complex mechanism to be isolated and extracted.[15,19] The INH+RIF dose was changed because SGOT SGPT levels on days 7 and 21 showed no difference (data in

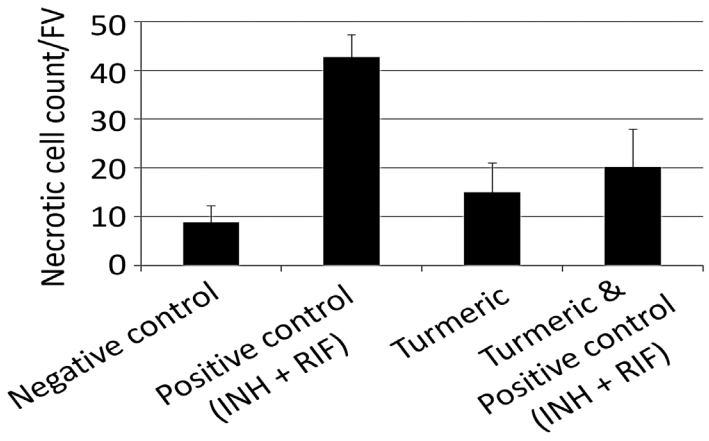

Figure 4 Necrotic Cell Count.
Note: *denotes significant difference between groups

supplementary materials). The study showed that turmeric rhizome powder at a dose of 2.2 gr/kg/day could prevent the increase in SGOT and SGPT serum level induced by INH and RIF administration. Elevated SGOT and SGPT levels are markers of liver injury.[20] Enzymes leak into the circulation when hepatocytes are damage with plasma membrane disruption.[20] In addition, INH and RIF induce oxidative stress and increase ROS production which in turn disrupts hepatic cell membrane integrity.[10] This effect of turmeric rhizome powder is most likely correlated with its active ingredients, curcuminoid. Curcumin in turmeric rhizome powder has strong antioxidant property, thus it helps protect the hepatic cell membrane integrity.[12–15] Other studies have confirmed the protection of curcumin against hepatocyte injury and various oxidative agents such as heavy metal,[18] immobilization-induced oxidative stress,[21] other drugs,[14,22] and even hepatocellular carcinoma.[23]

Lipids are easily oxidized compounds and are the main targets of ROS and RNS through lipid peroxidation.[4,14] This lipid peroxidation yields MDA as one of the end products, and it is a reliable marker of oxidative levels.[4,12] This compound can reach remote tissues due to its more stable property, thus possibly lead to systemic tissue damage.[24] Locally, ROS oxidize polyunsaturated fatty acids in phospholipid bilayer and other lipid membranes.[24,25] This decreases membrane fluidity and integrity, causes lysosomal fragility, and disrupts membrane pumps.[4,21,25] Byproducts of this oxidation such as MDA can also affect signal transduction, gene expression, and cell proliferation even in very small concentrations.[4,9,25] This study has shown that administration of turmeric rhizome powder can prevent the increase in MDA level in hepatic tissue induced by INH+RIF. Curcumin has also been shown to reduce MDA levels in cadmium-induced testicular injury in mice.[26] These results also strengthen other clinical trials with curcumin supplementations that are beneficial against oxidative stress as evidenced by lower tissue MDA levels.[17,21] These benefits are thought to come from curcumin's ability to scavenge free radicals such as superoxide and hydroxyl radicals.[13,14] Curcumin's antioxidant mechanism is correlated with two methoxylated phenol, one enol, and a β-diketone structure.[12,17]

In a study conducted on rats given INH+RIF for 42 days, histopathological changes were more prominent with infiltration of mononuclear inflammatory cells and eosinophils.[11] In comparison, this study, where the intervention time was only 28 days, has shown significant inflammation and dilatation of the hepatic trigone. Positive histological findings in the group with INH+RIF supplemented with turmeric powder further accentuate the benefit of curcumin. Another study has revealed similar reduction in histopathological damage induced by cadmium using curcumin as a supplement in rat seminiferous tubules.[26] The administration of INH and RIF induces oxidative stress in hepatocytes.[4,11] The aftermaths of oxidative stress are decreased amounts of ATP and dinucleotides, DNA damage, protein stability disruption, and membrane damage

through lipid peroxidation and release of proinflammatory cytokines.[4,17,25] Hepatocyte cell membrane damage can lead to cell death which can be prevented by reducing oxidative stress.[4,17,21] Curcumin in the turmeric rhizome powder has strong antioxidant properties.[13–15,23]

The limitation of this study is that the raw material used is not an extract, hence the dose concentration is higher. Therefore, a future study using more refined form is needed.

In conclusion, administration of *Curcuma longa* (Turmeric rhizome) powder can prevent the increased levels of SGOT and SGPT, hepatic tissue MDA, and necrotic hepatocyte numbers in rats induced by oxidative stress through INH+RIF. In addition, INH and RIF at doses of 100 mg/kg/day each can induce hepatotoxicity in Wistar rats. Thus, *Curcuma longa* (Turmeric rhizome) powder has a protective effect towards hepatotoxicity.

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