

The bcl-2 and caspase-3 expression after purple sweet potato treatment on isoniazid and rifampicin-induced liver injury



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ABSTRACT

Backgrounds: Liver injury induced by antituberculosis drugs showed high proportions in many countries and might result in serious effects. Previous studies had proved the increase of reactive oxygen species formation and induction of apoptosis related to antituberculosis treatment. There has been no standard therapy for protecting the liver from damage related to antituberculosis. Purple sweet potato extract is one of natural sources that possess hepatoprotector action regarding its antioxidant activity. This research aimed to prove the action of purple sweet potato ethanol extract on apoptosis related to liver injury caused by antituberculosis.

Method: This research was an experimental study with randomized post-test only control group design. The samples were male rats (*Rattus norvegicus*) strain Wistar, age 8-12 weeks, weight 150-200 grams (divided into two groups: control and experimental group). Purple sweet potato ethanol extract was prepared with maceration technique. The measurement of bcl-2 and caspase-3 expression was conducted with ELISA method. Data analysis was performed using independent t-test.

Results: Our study result revealed that the liver expression of bcl-2 in treatment group was significantly higher than control group (15.83 ± 3.14 vs. 12.11 ± 1.53), whereas the liver expression of caspase-3 in treatment group was significantly lower than control group (1.96 ± 0.38 vs. 2.41 ± 0.30 ; $p < 0.05$).

Conclusion: Purple sweet potato extract potentially protects the liver from apoptosis induced by antituberculosis.

Keywords: antituberculosis, apoptosis, hepatotoxic, purple sweet potato.

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INTRODUCTIONS

Isoniazid and rifampicin (INH-RIF), as main components of antituberculosis regimen used to date, had been assumed to contribute a significant role in antituberculosis toxicity in the liver. Previous study had proved that the mechanism of liver damage induced by isoniazid and rifampicin was strongly related to stress oxidative. Isoniazid produces many toxic substances in its metabolism, namely hydrazine and acetylhydrazine. INH and RIF might induce the action of CYP2E1 enzyme, thus would result in the increased production of reactive oxygen species (ROS) by CYP2E1. This subsequently enhances the formation of isoniazid toxic metabolites, as well as ROS, and aggravate the liver damage.¹⁻³

The excessive free radical production

and lack of antioxidant defense mechanism would lead to oxidative stress. This subsequently causes lipid peroxidation of cell membrane, protein modification, enzyme inactivation and DNA damage.⁴

The oxidative stress related to INH administration might promote cell apoptosis. This was shown by a study conducted by Hassan *et al.* (2016). The study revealed that isoniazid exposure enhanced apoptotic index and caspase-3 expression in the rat liver.⁴ Apoptosis is defined as programmed cell death which may occur through intrinsic pathway (involves mitochondria) as well as extrinsic pathway (via death receptor). Caspase-3 (caspase executioner) is a protease enzyme that plays an important role in apoptosis mechanisms. There are several bcl family proteins that play a role

on an intrinsic pathway, one of which is bcl-2 protein which tends to possess anti apoptotic action. The induction of apoptosis commonly occurs through p53 (tumor suppressor gene) activation. The p53 protein might function as a transcription factor for BH3 only, Bax, TRAIL-R, Fas and p21. Apoptosis induction might also occur through inhibition of the PI3k/Akt pathway. The PI3k/Akt (phosphatidylinositol 3-kinase) pathway is one of important signaling pathway which regulates many cellular processes such as proliferation, metabolism, and survival.⁴⁻⁶ To date, there has been no standard treatment for the management of antituberculosis induced hepatic injury. Therefore, administration of hepatoprotective agents from herbal sources such as purple sweet potato

(Ipomoea batatas) will be very important.

Purple sweet potato ethanol extract had been proved to possess hepatoprotector action against hepatic toxicity related to many substances, such as acetaminophen, ethanol, carbontetrachloride (CCl₄), dimethylnitrosamine (DMN), D-galactosamine, as well as antituberculosis (INH-RIF). The hepatoprotective effect of purple sweet potato extract is shown by its capability to reduce the serum aspartate aminotransferase and alanine aminotransferase level, as well as to improve liver histopathological feature. This is correlated with its wellknown secondary metabolite component, namely anthocyanin. Anthocyanin belongs to the flavonoid group of secondary metabolites, which is already known as an antioxidant. Many studies had revealed that purple sweet potato could decrease malondialdehyde (MDA) level, as well as increase superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT), as the general parameters of oxidative stress.⁷⁻¹² To date, there has been no study regarding the effect of purple sweet potato ethanol extract to apoptosis on toxicity related to isoniazid and rifampicin administration in tuberculosis cases.

There is still controversy regarding the anthocyanins action on apoptosis in hepatic injury cases. The activity of anthocyanin on apoptosis in liver toxicity has been studied in several studies. Study conducted by Zhang *et al.* (2010) showed that purple sweet potato extract was able to significantly reduce caspase-3 expression on rats induced by D-galactose.⁵ Contrary result was reported on study performed by Popovic *et al.* (2016). The study reported that bilberry extract (contained anthocyanin as secondary metabolite) promote apoptosis on hepatotoxic rats induced by carbontetrachloride (CCl₄).¹³ This study aimed to prove the action of purple sweet potato ethanol extract on apoptosis related to liver injury caused by antituberculosis.

METHODS

Study Design

This study was an experimental study with a randomized posttest only control group design. The study was conducted

at Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Bali, Indonesia. Our research has been approved by the Ethics Committee of Medical Faculty, Udayana University/Sanglah Hospital (approval number: 2098/UN14.2.2.VII.14/LT/2021).

Our study samples consist of thirty six Wistar rats (*Rattus norvegicus*), male, age 8-12 weeks, and weight 150-200 grams. The sampling method using a random sampling technique. The samples were randomly divided into two groups: control group (received only bidestilated water and induced with combination of antituberculosis INH-RIF), and treatment group (received purple sweet potato ethanol extract and induced with combination of antituberculosis INH-RIF).

Animal Model for Antituberculosis Hepatotoxicity

For inducing liver damage after antituberculosis treatment, we administered the combination of isoniazid and rifampicin. The INH-RIF combination was administered intragastrically in dose 500 mg/kgBW/day for 28 days.

Administration of Purple Sweet Potato Extract

Purple sweet potato ethanol extract was administered intragastrically in dose 1 gram/kgBW/day. It was starting from 7 days before administration of INH-RIF combination, continuously given for 35 days.¹²

Assessment of Biomarker for Apoptosis

Biomarkers for apoptosis assessed in this study were the expression of bcl-2 and caspase-3 in the liver. The expression

of bcl-2 and caspase-3 in the liver were measured with ELISA technique.

Assessment of Liver Injury

Hepatic damage was identified from the concentration of transaminase enzymes (including aspartate aminotransferase and alanine aminotransferase). The transaminase level was measured with spectrophotometry technique.

Data Analysis

Our data were analysed with statistical software. The comparison of apoptotic biomarkers between groups was analyzed using independent t-test. Numeric data presented as mean \pm standard deviation (SD). The p-value of less than 0.05 was considered as statistically significant.

RESULTS

The mean number of liver expression of bcl-2 and caspase-3 on control and intervention groups were represented in **Table 1**. Our study result showed that purple sweet potato extract could significantly decrease the liver expression of caspase-3, as well as increase the liver expression of bcl-2, on hepatotoxic rats related to isoniazid and rifampicin administration.

DISCUSSIONS

Apoptosis resulting from oxidative stress conditions (related to isoniazid and rifampicin toxicity) might be diminished by administration of purple sweet potato extract. It had been proved to associated with its antioxidant activity related to its major secondary metabolite namely anthocyanin.⁷ As we know that antioxidant might attenuate the oxidative stress condition, which could be identified

Table 1. The bcl-2 and caspase-3 expression in the liver

Variable	Control Group	Intervention Group	p-value
Liver expression of bcl-2 (ng/mL), mean \pm SD	12.11 \pm 1.53	15.83 \pm 3.14	0.004*
Liver expression of caspase-3 (ng/mL), mean \pm SD	2.41 \pm 0.30	1.96 \pm 0.38	0.009*

*represent statistically significant result

by several biomarkers of oxidative stress such as MDA, CAT, SOD, GPX or others.

Some previous studies had demonstrated the hepatoprotector activity of purple sweet potato (*Ipomoea batatas* L.) or anthocyanin. This was performed by in vivo study conducted on liver toxicity induced by carbontetrachloride, acetaminophen, alcohol (ethanol), D-galactose or dimethylnitrosamine (DMN).^{7-9,14}

The activity of purple sweet potato on apoptosis had been studied in liver toxicity induced by D-galactose. The study showed decrease expression of caspase-3 after purple sweet potato extract treatment on rats induced by D-galactose.⁵ Our study result was also in line with the result of a study conducted by Zhang *et al.* (2010).⁵ Our research reported the decrease apoptosis (which was marked by decrease expression of liver caspase-3 and increase expression of liver antiapoptotic protein (bcl-2)) on hepatotoxic rats treated with purple sweet potato extract. Other study conducted by Arjinajarn *et al.* (2017) shown similar result.¹⁵ The study revealed that riceberry bran extract (contained anthocyanin) attenuated oxidative stress, inflammation and apoptosis on gentamicin-induced hepatotoxicity. Apoptosis markers evaluated in this study including caspase-3, Bax and Bcl-XL.¹⁵

In other organs, lingonberry extracts and pure anthocyanins also had cardioprotective effects against hydrogen-peroxide-induced apoptosis.¹⁶ This was shown by study from Isaak *et al.* (2017).¹⁶ Similar evidence was shown by study performed by Huang *et al.* (2017). The study revealed that anthocyanins (from black rice) significantly reduced apoptosis and the associated proapoptotic proteins, and subsequently increased the level of survival protein. Anthocyanin administration was found to enhance cardiomyocyte survival and restore cardiac function.¹⁷

A silico study conducted by Sari *et al.* (2020) demonstrated anti apoptosis potency of anthocyanins. Four types of anthocyanins investigated in the study were cyanidin-3-O-gluco-side, delphinidin-3-O-glucoside, pelargonidin-3-O-glucoside, and peonidin-3-O-glucoside. Those anthocyanins have potential activity

as anti-apoptosis through caspase-3 interaction in BIR2 region and allosteric sites.¹⁸ In silico study conducted by Xiao *et al.* (2021) found that cyanidin-3-O-xylosylrutinoside was more likely to bind and well fitted into the binding domain of Bcl-2, Caspase-9, and cytochrome c. The study investigated anthocyanin derived from black raspberry in alcoholic liver disease.^{19,20}

Caspase-3 is a protein contributed to apoptosis execution including intrinsic and extrinsic apoptosis. Caspase-3 is a proteolytic enzyme which is activated by phosphorylation in p38 at Ser150. Caspase-3 participates in the final step of intrinsic and extrinsic apoptosis pathways, unlike the bcl-2 protein which only plays a role in intrinsic apoptosis pathways. Oxidative stress that occurs due to INH can stimulate apoptosis conditions in cells. This was shown in a study conducted by Hassan *et al.* (2016). In this study, an increase in the apoptotic index and hepatic caspase-3 expression was found in rats induced by isoniazid.⁴

Oxidative stress from any sources (chemical exposure, radiation, free radicals, and others) might result in mitochondrial leakage and swelling that lead to release of cytochrome c. Subsequently cytochrome c will bind to Apaf-1. The cytochrome c/Apaf-1 complex will activate caspase-9 (initiator caspase) from procaspase-9. Thus, caspase-9 will activate the executioner caspase (caspase-3,-6,-7). The executioner caspase then cleaves I-CAD from CAD, thus releasing CAD to enter the nucleus. In the nucleus, CAD will cleaves DNA.^{6,18}

Bcl-2 protein is an anti-apoptotic protein which normally binds to mitochondria membrane, preventing mitochondrial leakage and swelling. Thus, it will inhibit the process that leads to cell death. Bcl-2 inhibits the aggregation of BH123 protein on mitochondrial membrane, thus blocking the release of cytochrome c.^{6,18}

Anthocyanin had already proved to inhibit apoptosis induced by oxidative stress through activation of Nrf2/HO-1 (nuclear transcription factor erythroid-2-like factor/heme oxygenase-1) signaling pathway. Previous study indicated that Nrf2 was involved in reactive oxygen

species (ROS) generation via nicotinamide adenine dinucleotide phosphate (NADP) oxidase. Anthocyanin from *Hibiscus syriacus* potentially increased antiapoptotic protein (PARP and bcl-2), as well as decreased pro-apoptotic protein (Bax and caspase-3) in H₂O₂-induced apoptosis. *Hibiscus syriacus*-derived anthocyanin also concomitantly increases expression of Nrf2 and HO-1 in H₂O₂-induced apoptosis. Activation of Nrf2 would subsequently result in its nuclear translocation, trigger the expression of antioxidant response element (ARE), such as HO-1.²⁰⁻²²

Our study only focused on studying the main protein for apoptosis; bcl-2 and caspase-3. Our study did not study other markers related to apoptosis pathways such as p53 and pAkt. Therefore, it was the limitation of this study. Further research should explore many other markers related to apoptosis pathways to fully understand the mechanisms.

CONCLUSION

Administration of purple sweet potato extract potentially decreased apoptosis in liver toxicity due to INH-RIF which was characterized by decreased expression of caspase-3 and increased hepatic bcl-2 protein.

CONFLICT OF INTEREST

No conflict of interest was declared.

ETHICAL CONSIDERATION

Our research has been approved by the Ethics Committee of Medical Faculty, Udayana University Sanglah Hospital with approval number: 2098/UN14.2.2.VII.14/LT/2021.

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AUTHOR CONTRIBUTION

IAA responsible for research ideas, design of the study, and manuscript preparation. AWI is responsible for design of the study

and review of the manuscript. ISC is responsible for analysis of the data. KWA responsible for manuscript preparation.

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