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ORIGINAL ARTICLE

Hepatoprotective effect of Cinnamon (*Cinnamomum burmanii*) extract on Serum Aspartate Transaminase (AST) of male wistar rats induced with isoniazid

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ABSTRACT

Hepatotoxicity is a condition of liver cell damage that is often caused by drugs such as isoniazid. Isoniazid (INH) is an anti-tuberculosis drug that, when used excessively, can cause an increase in Reactive Oxygen Species (ROS) and trigger oxidative stress, thereby inducing cell necrosis, especially in the liver. Cinnamon is a plant that contains flavonoids, tannins, alkaloids, and essential oils, which are useful as antioxidants and have the potential hepatoprotective properties. This study aims to determine the effect of ethanol extract of cinnamon (*Cinnamomum burmanii*) and its effective dose as hepatoprotection on aspartate transaminase (AST) levels in isoniazid-induced male rats. Extract preparation was carried out using the maceration method. This study was a post-test only control group design laboratory experiment with a sample of 25 male Wistar rats consisting of five groups: the normal control group was given aquabidest (K1), the positive control was induced with 200 mg/kg isoniazid (K2), and three treatment groups were induced with 200 mg/kg isoniazid and given cinnamon ethanol extract at a dose of 100 mg/kg BW, 200 mg/kg BW and 400mg/kg BW for 14 days. At the end of the study, the AST levels were measured. Data were analyzed using the One-Way Anova test and the Tukey's Post Hoc Test. The results showed that the ALT levels of the group given cinnamon at doses of 100, 200, and 400 mg/kg decreased significantly ($p<0.05$) by 93,20 ± 8,55, 85,60 ± 8,23, and 71,20 ± 3,56 U/L, respectively. The cinnamon ethanol extract could prevent increases in isoniazid-induced AST in rats, thus having a hepatoprotective activity.

Keyword: Aspartate transaminase, *Cinnamomun burmanii*, hepatoprotection, isoniazid

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INTRODUCTION

Hepatotoxicity is a condition of liver cell damage by exposure to toxic chemicals. Hepatocyte or bile duct injury from a variety of substances can have a hepatocellular, cholestatic, or mixed pattern. Hepatocellular necrosis is the predominant hepatotoxicity manifestation brought on by isoniazid (INH). High dosages of acetaminophen are just one example of a medication that might harm the liver, but other drugs, like INH, can do it less predictably or in an "idiosyncratic" way. Instead of occurring days to weeks after commencement, INH-induced liver damage often manifests between weeks to months.[1] The most common cause of hepatotoxicity is the use of anti-tuberculosis drugs such as isoniazid.[2] Hepatotoxicity is the most severe side effect of antituberculosis therapy and can result in 11% discontinuation of antituberculosis therapy.[3]

The incidence of hepatotoxicity in developed countries is around 3%, while in developing countries it is around 8% to 39%. Hepatotoxicity due to anti-tuberculosis drugs in Indonesia reaches 22.5-43%.[4,5] The risk of hepatotoxicity in the Indonesian population who have the slow acetylator enzyme NAT2 and people with CYP2E1*c1/c2 due to isoniazid induction is three times higher than other populations in various countries. The potential risk is increased in people who have the combination of slow acetylator NAT2 and CYP2E1*c1/c2.[5] An issue with world health is tuberculosis (TB). A combination of isoniazid (INH), rifampicin, pyrazinamide, and ethambutol is one of the conventional treatments for TB. It is also possible to administer INH on its own to prevent TB as well.[6,7] Isoniazid is an anti-tuberculosis drug that has a mechanism of action by inhibiting the formation of mycolic acid, which is an important component of the mycobacterial cell wall.[1]. Despite the positive effects of INH, INH therapies are linked to serious side effects, particularly peripheral neuropathy and hepatotoxicity. In between 10% and 20% of patients taking INH, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood is momentarily elevated. The majority of people can adjust to it, and without stopping treatment, their serum ALT levels return to normal, although a small percentage (less than 1%–3%) do experience serious liver damage and, in some cases, liver failure.[1,6]

Cinnamon (*Cinnamomum burmanii*) contains many biologically active substances like eugenol, trans-cinnamaldehyde, and linalool. Natural antioxidants found in cinnamon spice are important anti-aging agents and have many properties. Antioxidants are used to prevent episodes of oil oxidation, which reduces the creation of harmful oxidative derivatives and free fatty acids.[8] The antioxidant content of cinnamon is expected to reduce the side effects of isoniazid hepatotoxicity.[8] The purpose of this study was to determine the effect of an ethanol extract of cinnamon (*Cinnamomum burmanii*) and its effective dose as hepatoprotection on aspartate transaminase (AST) levels in isoniazid-induced male rats.

METHODS AND SUBJECT

The study was conducted from July 2022 to February 2023. This research was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, and received ethical approval on October 26, 2022, with the letter number 021/UH2.10/EC/2022. The research is a laboratory experimental study using a posttest-only control group design.

Animal experiment

The experimental animals used were male Wistar rats (*Rattus norvegicus*) with the number of rats per group calculated using the Federer formula: $(n-1)(t-1) \geq 15$. The results showed that the minimum number of samples in each group was five

animals. Experimental animals were obtained from PT Biofarma. This study used 25 male Wistar rats (*Rattus norvegicus*) that met the inclusion and exclusion criteria: having a body weight of 200-250 g, aged 2-3 months, being healthy and active, and having a good appetite. Subjects were excluded if they had experienced a weight loss of more than 10% and shown physical abnormalities after acclimatization. Subjects were dropped out if they died during treatment.

Plant material

The study used the bark of cinnamon (*Cinnamomum burmanii*) obtained from the Manoko Experimental Garden in Lembang, Bandung,

Plant extraction

The process of making cinnamon extract began with 2.5 kg of cinnamon bark that had been washed , then sliced thinly and dried in an oven at 60ºC for two days. The dried cinnamon bark was then crushed using a grinding machine to produce 1 kg of cinnamon powder. Following this, 300 g of cinnamon powder was then macerated for three days by placing it in three Erlenmeyer flasks and adding 900 ml of 96% ethanol solvent. Each flask contained100g of cinnamon powder and 300 ml of 96% ethanol (1:3). After that the solution is filtered or separated using filter paper. Finally, the macerate formed is then evaporated through a rotary evaporator at 90ºC to obtain a thick extract.[9]

Experimental animal treatment

After seven-day acclimatization process, the weight of animal experiments was then measured. They were divided into five groups, with five male Wistar rats in each treatment group. A standard feed of 20-25 g/head/day and drinking water ad libitum were given to each group. The following treatment were given within 14 days in each group:

1. Group 1 (normal): given standard feed (20-25 gr/head/day), drinking water ad libitum, and aquabidest
2. Group 2 (positive control): given standard feed (20-25 gr/head/day), drinking water ad libitum, aquabidest, and 200 mg/kg/day INH
3. Group 3 (treatment 1): given standard feed (20-25 gr/head/day), drinking water ad libitum, cinnamon ethanol extract dose of 100 mg/kg/day, and 200 mg/kg/day INH peroral
4. Group 4 (treatment 2): given standard feed (20-25 gr/head/day), drinking water ad libitum, cinnamon ethanol extract dose of 200 mg/kg/day, and 200 mg/kg/day INH peroral
5. Group 5 (treatment 3): given standard feed (20-25 gr/head/day), drinking water ad libitum, cinnamon ethanol extract dose of 400 mg/kg/day, and 200 mg/kg/day INH per oral

The time interval between the administration of cinnamon ethanol extract and the administration of isoniazid in this experiment was one hour, based on the emptying time of the rats' stomachs.[10] After 14 days of treatment, blood samples were taken to measure AST levels.

Measurement of AST Levels

Blood sampling in the study was carried out once at the end of the study. Blood collection began with an anesthetic process using CO2 for less than one minute by inhalation. Furthermore, blood collection was carried out through the eye or retro-orbital using a capillary tube. Blood samples were collected in EDTA tubes and centrifuged at 3000 rpm for 20 minutes. The plasma obtained was put into the Effendorf tube and the AST levels were measured. The method used in this research was the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) method using ASAT reagents. ASAT reagents consisted of reagent 1 (TRIS buffer, L-Aspartate, MDH, and LDH) and reagent 2 (2-Oxoglutarate and NADH) in a ratio of 4:1 to become monoreagents. 1000µL of monoreagent was then mixed with 100µL

of control plasma, homogenized and allowed to stand for 1 minute.[10]

The AST measurement began with the calibration of the Rayto 1904c photometer.The absorbance results were compared with the AST range that had been set; if it was appropriate then the tool could be used to measure the AST. In the AST measurement, 1000µL of monoreagent was mixed with 100µL of rat plasma, then homogenized and allowed to stand for one minute. The solution was measured using a Rayto 1904c photometer, and the absorbance was read every minute (ΔA/minute) for three minutes at a temperature of 37℃ and a wavelength of 340 nm. After that the absorbance difference was calculated every minute (ΔA/minute).[10]

The data obtained in this study were analyzed using the One-Way ANOVA tests and Tukey post-hoc tests.

RESULTS AND DISCUSSION

The results of the AST measurement in the five groups are described in Table 1. The highest average AST level was found in the group that received isoniazid treatment at a dose of 200 mg/kg (113.80 ± 10,73 U/L). The lowest levels of AST were found in the group that received 200 mg/kg of isoniazid and 400 mg/kg of ethanol extract of cinnamon (*Cinnamommum burmanii*), which was 71.20 ± 3,56 U/L. The levels of AST in treatment groups were still within the normal range. There were significant differences between groups ($p<0.05$).

Data Analysis

Table 1. AST level of all groups

Group	AST level Mean ± SD (U/L)	F (*Anova*)	p value
Normal	73,20 ± 12,69		
Positive control	113,80 ± 10,73		
Cinnamon extract 100mg/kg	93,20 ± 8,55	17,408	0,000*
Cinnamon extract 200mg/kg	85,60 ± 8,23		
Cinnamon extract 400mg/kg	71,20 ± 3,56		

*p<0.05

The results of Tukey's post hoc test as illustrated in Table 2 show that there was a significant difference between the positive control group and the treatment group, which means that administration of cinnamon extract can reduce AST level in INH-induced liver damage. Meanwhile, there was no significant difference between the groups treated with the cinnamon extract at doses of 200 and 400 mg/kg, indicating that with increasing doses, the effectiveness was still equal. Compared to the normal group, the groups with the treatment of the cinnamon extract at doses of 200 and 400mg/kg did not differ significantly ($p>0.05$).

Table 2. Comparison of AST levels between groups

Group		Post Hoc Tukey Test p-value
Normal	- Positive control	0,000*
	- Cinnamon extract 100mg/kg	0,021*
	- Cinnamon extract 200mg/kg	0,253
	- Cinnamon extract 400mg/kg	0,997
Positive control	- Cinnamon extract 100mg/kg	0,017*
	- Cinnamon extract 200mg/kg	0,001*
	- Cinnamon extract 400mg/kg	0,000*
Cinnamon extract 100mg/kg	- Cinnamon extract 200mg/kg	0,697
	- Cinnamon extract 400mg/kg	0,010*
Cinnamon extract 200mg/kg	- Cinnamon extract 400mg/kg	0,142

*p<0.05

Discussion

The increase in AST levels in the INH-induced positive control group indicated that INH was hepatotoxic. Hepatotoxicity due to INH is caused by free radical reactions from the metabolism of isoniazid in the liver in the form of hydrazine and acetyl hydrazine. Free radicals are extremely reactive due to the unpaired electron. Free radicals are all byproducts of normal cellular metabolism and contain a subclass of reactive nitrogen species (RNS), which were formerly assumed to be oxygen-centered radicals called reactive oxygen species (ROS).[11] Reactive metabolites (acetyl hydrazine and hydrazine) will trigger macromolecular acetylation leading to protein binding in the liver and decreases glutathione activity, which is a detoxifier of reactive oxygen species (ROS).[1,12,6, 13]

AST (Aspartate aminotransferase) is an enzyme that can predict cell damage, especially in the liver. AST is an enzyme that can be found in the liver, heart, skeletal muscle, kidneys, and pancreas. Therefore, ALT is one of indicators to identify liver damage. The normal AST level in humans is 12 – 38 U/L. To differentiate whether an increase in AST originates from damage to the liver or heart, additional tests such as ALT are needed. Isoniazid does not cause damage to the heart, so the increase in AST level during isoniazid induction is caused by liver damage.[14]

The results of this study support previous studies that showed a hepatotoxic effect from INH, which was characterized by increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.[10,15]

In this study the treatment groups experienced a significant decrease of AST levels as a result of the antioxidant properties of cinnamon ethanol extract , which can counteract the effects of free radicals induced by isoniazid. There are three main ways that antioxidants protect cells: binding to transition metals to prevent them from reacting with free radicals, which thereby inhibiting their harmful effects; making small molecules that have the ability to scavenge free radicals available; and using specific mechanisms to repair DNA damage caused by ROS.[11]

Previous studies have shown that cinnamon has very strong antioxidant activity *in vitro* and contains high amounts of total phenolics and total flavonoids, making it potential as a food additive (antioxidant) in the food and pharmaceutical industries.[16] The antioxidant activity of flavonoids originates from their ability to donate hydrogen atoms or through their ability to chelate metals.[17] Cinnamon is also a plant that has the greatest hepatoprotection activity.[18]

The protective effect of cinnamon ethanol extract against liver cell damage by isoniazid is thought to be due to the presence of active compounds contained in cinnamon, including flavonoids, tannins, essential oils, and alkaloids which can provide a hepatoprotective effect. Flavonoids are abundant in plant tissues and can act as antioxidants.[8,19] The hepatoprotection effect works by delaying, slowing down, and preventing the process of fat oxidation caused by free radicals thereby preventing damage to liver cells (hepatocytes).[19]

The effective dose is the smallest dose of cinnamon extract that can cause an effect that is significantly close to the normal levels of AST. Thus, it was observed that the cinnamon extract at a dose of 200 mg/kg was an effective dose for the treated group, because the average AST level was not significantly different from normal group, and had a significant difference from positive control group. There was no significant difference between the average levels of AST in group that was given cinnamon ethanol extract with doses of 200 and 400mg/kg. This proves that larger doses do not always have a better effect. There is a phenomenon that increasing the dose is not linear to enhance the therapeutic effect. Therapeutic effects that are not in line with the increase in the dose of the drug given are known as non-monotonic dose-response relationships (NMDR).[20]

Natural remedies have shown tremendous promise in reducing the toxicity of a number of medications. It is time to promote natural remedies as a supplement to medications that also damage cells due to their accessibility and nutritional nature. Even though the vast majority of natural goods that have been researched so far are safe, certain studies have found that some natural compounds can be hazardous to the liver. As a result, choosing natural products wisely is also essential. Natural products are expected to both reduce the likelihood of drug-induced liver damage and offer an alternate method of treating drug-induced hepatotoxicity.[12]

CONCLUSION

A Cinnamon ethanol extract has hepatoprotection activity with an effective dose of 200 mg/kg.

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DECLARATION OF INTERESTS

We have no conflict of interest

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