Research Article

The Effect of *Cinnamomum Burmanii* Ethanol Extract on Isoniazid-induced Serum Levels of Serum Glutamate Piruvate Transaminase (SGPT) Wistar Strain Male Rats

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Published: 4 October 2024

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 4th ICONISS Conference Committee.

Abstract.
Hepatotoxicity can be caused by excessive drug use, triggered by increased oxidative stress which is considered as the initial mechanism of Drug Induced Liver Injury (DILI). One of the causes of DILI is Isoniazid. One of the plants acting as a hepatoprotector in protecting liver cells is cinnamon (*Cinamommum burmanii*). This study aims to determine the effect of 96% ethanolic cinnamon extract as a hepatoprotector against an increase in Alanine Transaminase (ALT) levels induced by isoniazid. The parameters used to assess liver damage were rat plasma ALT. The sample used in this study was rat plasma, which was taken through the retro orbital sinus. This research is an experimental study with a sample of 25 experimental animals divided into five groups, namely negative control (K1), positive control I (K2), and experimental group with cinnamon ethanolic extract at a dose of 100 mg/kgbw (K3), 200 mg/kgbw(K4), and 400 mg/kgbw(K5). The research used a post-test-only control group design. The results were analyzed using the One-way ANOVA test followed by the post hoc Tukey test. The results proved that cinnamon ethanolic extract at doses of 100 mg/kgbw (P = 0.029), 200 mg/kgbw (P = 0.001), and 400 mg/kgbw (P = 0.000) was effective in reducing plasma SGPT levels in isoniazid-induced rats when compared with the positive control group (K2). The most effective dose was at 200 mg/kgbw (K4). Thus, this proves that all of the doses in experimental groups have a hepatoprotective effect against isoniazid-induced liver damage.

Keywords: Alanine Transaminase (ALT), cinnamon ethanol extract, Drug Induced Liver Injury (DILI), isoniazid

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1. Introduction

Hepatotoxicity can be caused by excessive drug use and drug abuse [1]. When exposed to hepatotoxic substances, the liver tends to be susceptible to damage to necrosis due to oxidative stress [2]. Oxidative stress in hepatocytes is the initial mechanism for drug induced liver injury (DILI) [3]. DILI is a condition of liver injury due to the use of drugs, herbs or other toxic substancessuch as in tuberculosis therapy namely rifampicin and isoniazid [4]. Various kinds of TB drugs that must be consumed by patients include a fixed dose anti-tuberculosis drug package (OAT-KDT) which contains isoniazid, rifampicin, pyrazinamide, and ethambutol [5].

Tuberculosis (TB) is a chronic disease that has become a global problem [6]. Circulating global issues have been supported by the fact that in 2019, Indonesia is ranked third with the highest number of TB sufferers in the world [7]. One of the first-line drugs for TB therapy is isoniazid [8,9]. The incidence of liver damage induced by the TB drug isoniazid (INH) is reported to be the highest and is ranked second in the United States [10]. The result of isoniazid metabolism which produces free radicals has an effectvarious natural products such as cinnamon [11]. Cinnamon has secondary metabolites such as flavonoids, alkaloids, tannins, and essential oils which can act asantioxidants [11,12]. The success of the mechanism of action of cinnamon as a hepatoprotector is illustrated by a decrease in serum glutamic pyruvate transaminase (SGPT) levels which has increased due to DILI by isoniazid [13]. Selection of SGPT is an indicator that can increase faster than other enzymes and is the most common enzyme found in the liver so that it is considered the best indicator to assess liver damage. The novelty of this research can be assessed from the use of cinnamon as an ingredient that is often used as a daily things. However, no one has discussed the hepatoprotective effect on DILI cases [14].

2. Materials and Methods

Research begins in July 2022 and ends in January 2023. Cinnamon ethanol extract was made at the FMIPA Laboratory and the Biochemistry Laboratory, Faculty of Medicine, Jenderal Achmad Yani University. The preparation and treatment of experimental animals was carried out at the Animal Laboratory of the Faculty of Medicine, Jenderal Achmad Yani University. After that, SGPT measurements were carried out by the Pharmacology Laboratory of Padjadjaran University. This research was approved by the

Ethics Commission for Health Research, Faculty of Medicine, Jenderal Achmad Yani University and received ethical approval with letter number 021/UH2.10/2022.

2.1. Research design

The type of research used is experimental in nature with the post test only control group design method using a completely randomized design technique (CRD).

2.2. Research subject

This study used 25 male Wistar rats (Rattus norvegicus) obtained commercially from Biofarma which were divided into 5 groups, namely the negative control group (K1), the positive control group (K2), cinnamon ethanol extract at a dose of 100 mg. /kgBB, 200 mg/kgBB, and 400 mg/kgBB.

2.3. Object of research

The object of this study used cinnamon (Cinnamomum burmanii) obtained from the Manoko Experimental Garden in Lembang, West Bandung.

2.4. Research preparation

This study was initiated by preparing 25 male Wistar rats for 5 experimental groups. All animals with homogeneity tried to be acclimatized. for 7 days in the biopharma laboratory. By also preparing cinnamon ethanol extract in three doses consisting of 100 mg/kgbb, 200 mg/kgbb, and 400 mg/kgbb and isoniazid 200 mg/kgbb which has been dissolved to be induced through male rats of the Wistar strain.The procedure for acclimatizing experimental animals at the Animal Laboratory of FK Unjani is according to operational standards. Wistar rats in this study were required to go through an acclimatization period of 7 days at room temperature 26 – 28°C, the cage consisted of 5 rats which were given standard feed of 20-25 g/head/day and drank from a bottle ad libitum. The cage used is 60 x 40 cm in size with a height of 60 cm. High wood shavings±3 cm is needed for the bottom of the cage which will be changed 3 times a day [15].

2.5. The making of cinnamon ethanol extract

Making the extraction in this study using the maceration method. Cinnamon bark as much as 2.5 Kg which is washed thoroughly with water, then cut into small pieces so that it can be dried in an oven at 60°C for 3 days at the Biochemistry Laboratory, FK Unjani. Then it was weighed again to determine the weight by continuing the process of chopping and refining the wood using a milling machine at the Chemistry Laboratory, Faculty of Science and Informatics, Jenderal Achmad Yani University. Furthermore, cinnamon bark powder as much as 1 Kg. In this study, 300 grams will be put into three Erlenmeyer with 100 grams each added with 900 ml of 96% ethanol solvent, so a comparison between cinnamon bark powder and 96% wood ethanol extract will be obtained, namely 1: 3. The maceration process is carried out for 3 – 4 days with stirring, then followed by filtration or separating the solution using filter paper. The maserate that has been formed is then evaporated through a rotary evaporator at 90 °C, so that a thick extract is obtained [16].

2.6. Experimental animal treatment

The number of groups in this study were 5 groups, each group containing 5 rats so that the number of animals in this study used 25 male Wistar rats. Each group was adapted for 7 days and given standard feed. Furthermore, body weight was measured to ensure that the rats met the inclusion criteria. After weighing, cinnamon ethanol extract was given with three different dose variants after 1 hour the rats experienced physiological gastric emptying, then induction of isoniazid with a toxic dose of 200 mg/kgbb [13]. Treatment for the next 14 days in each group including group 1 as a negative control group which was only given aquabidest (K1), positive control group which was only given a toxic dose of isoniazid (K2), treatment group 1 by giving a dose of 100 mg cinnamon ethanol extract /kgBB (K3), treatment group 2 with a dose of 200 mg/kgBB (K4), and treatment 3 with a dose of 400 mg/kgBB (K5). Taking blood samples for measuring SGPT levels after treatment. Then, the rats were destroyed through inhalation technique with carbon dioxide gas (CO2) and determined using an incinerator based on the AVMA Guidelines for the Euthanasia of Animals [17].

2.7. Measurement of SGPT levels

Measurements using the IFCC method using the ASAT reagent. The method of measurement is that 1 ml of blood is taken from the retro-orbital sinus and put in an Eppendorf tube. It takes ASAT reagent with a ratio of 1: 4 to be a monoreagent. Monoreagent taken 1000μL is then mixed with control serum or Trulab-N as much as 100μL, then homogenized and allowed to stand for 1 minute. Followed by measuring the solution with a Rayto 1904c photometer at a wavelength of 340 nm and a temperature of 37°C, after that calculate the difference in absorption per minute (ΔA/min). Then enter into the SGPT range that has been set. In this study, SGPT measurements will be carried out at the Pharmacology Laboratory of Padjadjaran University (UNPAD) [13].

2.8. Data analysis

Data analysis using IBM SPSS application. The analysis begins with a normality test using the Shapiro-Wilk test. This test is used to determine the normality of data if sig > 0.05. Then, Levene's test is used to determine the homogeneity of the variance of the data obtained if sig > 0.05. Next, the One-Way Anova Test will be used to prove that there is a difference in the control group with the treatment. If the One-Way Annova or Kruskal-Wallis tests show a p value <0.05 which indicates a significant difference, then it is continued with Tukey's Post Hoc Test analysis to determine the significance of each group treatment [18].

3. Results and Discussion

3.1. Results of rat plasma SGPT measurements

In assessing the effect of administration of cinnamon ethanol extract from three treatments on increasing plasma SGPT levels caused by isoniazid induction. This can be explained in Table 1.

Table 1 shows an overview of rat plasma SGPT levels from each experimental group. The highest SGPT level was shown by the positive control group (K2) which was induced by isoniazid, which means that this group had liver damage. The lowest SGPT level was shown by treatment group 3 (K5) which was given cinnamon ethanol extract at a dose of 400 mg/kg BW. The results of the descriptive calculations meant that the greater the

Table 1: Test results of rat plasma SGPT average levels.

Group	N	Average (U/L) ± sd
K1	5	63.00 ± 5.15
K2	5	85.60 ± 3.98
K3	5	75.40 ± 5.41
K4	5	71.20 ± 4.60
K5	5	61.20 ± 5.45

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH.

dose of cinnamon ethanol extract, the greater the decrease in plasma SGPT levels in rats induced by isoniazid.

This correlates with the theory that the antioxidant content of cinnamon can inhibit the increase in free radicals or prevent liver damage or necrosis [19,20].

3.2. Effectiveness of cinnamon ethanol extract on isoniazid-induced increases in SGPT levels in male wistar rats

Prior to statistical analysis, for the type of numerical data obtained from the research, a normality test was carried out using the Shapiro Wilk Test to see the distribution of the data. The results of the normality test can be explained in Table 2.

The results of the data normality test using the Shapiro Wilks Test in Table 2 show that rat plasma SGPT levels are normally distributed in all treatment groups (p>0.05). However, to perform data analysis using One Way ANOVA, the data must first be tested for homogeneity of variance. data using levene. After testing the homogeneity of the variance, it turned out that the results obtained were p> 0.05, which means that the data can be stated as homogeneous. Followed by the One Way ANOVA test described in Table 3.

Table 3 shows the results of a comparison of SGPT levels in male rats of the Wistar strain obtained from rat plasma, then analyzed using One Way Anova showing that

Table 2: The normality test results for rat plasma SGPT levels.

Group	Normality Test p*) Value	Homogeneity Test
K1	0.497	0.949
K2	0.911	
K3	0.966	
K4	0.992	
K5	0.671	

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

Table 3: The results of the One Way ANOVA test in rat plasma SGPT levels.

Group	Average (U/L) ± SD	P-value*)
K1	63.00 ± 5.15	0.000*
K2	85.60 ± 3.98	
K3	75.40 ± 5.41	
K4	71.20 ± 4.60	
K5	61.20 ± 5.45	

Description : Shapiro Wilk Test ; p>0.05 (normally distributed)
Levene Statistics ;p>0.05 (homogeneous data)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

there was a significant difference in SGPT plasma rat levels in at least two experimental groups (p=0.000).

3.3. Effective dose of ethanol extract of cinnamon (Cinnamommum burmanii) against isoniazid induced SGPT levels of male wistar rats

To find out which treatment group is better so as to produce an effective dose in reducing SGPT levels in rats experiencing hepatotoxicity, further tests will be carried out using the Post Hoc Tukey test. Post Hoc Tukey test results can be seen in Table 4.

TABLE 4: Tukey Post Hoc test results rat plasma SGPT levels.

Group		Post Hoc Tukey Test	
		P-values	Conclusion
K1	K2	0.000	Different meaning
	K3	0.006	Different meaning
	K4	0.104	Meaningless
	K5	0.977	Meaningless
K2	K3	0.029	Different meaning
	K4	0.001	Different meaning
	K5	0.000	Different meaning
K3	K4	0.039	Different meaning
	K5	0.093	Meaningless
K4	K5	0.699	Meaningless

Description: Post Hoc Tukey ;*) $p<0.05$ (there is a significant difference)
K1 = Negative Control
K2 = Positive Control
K3 = Administration of cinnamon ethanol extract 100 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K4 = Administration of cinnamon ethanol extract 200 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH
K5 = Administration of cinnamon ethanol extract 400 mg/kg on the 8th day for 14 days and after 1 hour continued administration of a toxic dose of INH

Based on the results of the Post Hoc Tukey Test described in Table 4, it can be seen that the ethanol extract of cinnamon at doses of 100 mg/kgBW (p=0.029), 200 mg/kgBW (p=0.001), and 400 mg/kgBW (p=0.000) proved effective in reducing plasma SGPT levels in isoniazid-induced rats when compared to the positive control (K2) group. The most effective dose was assessed in treatment group 2 (K4), which was 200 mg/kgBW.

4. Conclusion

Based on the results of this study, it can be concluded that administration of cinnamon ethanol extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg has a hepatoprotective effect on decreasing SGPT levels in isoniazid-induced male wistar rats. The effective dose of cinnamon ethanol extract as a hepatoprotector against isoniazid-induced Wistar strain rats was 100 mg/kg, 200 mg/kg, and 400 mg/kg. However, the most effective dose was in treatment group 2 (K4), which was 200 mg/kgbb.

Conflict of Interest

There is no conflict of interest in writing this research.

Closing

The author would like to thank the parties who have helped carry out the research and the preparation of this paper, namelyDr. Evi Sovia, dr., M.Si, Gusti Ayu Sinta, dr., M. Biomed., AIFO-K, staff of the Unjani Medical Faculty Biochemistry Laboratory, Unjani Medical Faculty Animal Laboratory, UNPAD Pharmacology Laboratory.

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