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The Hepatoprotective Effect of Cassia Cinnamon (*Cinnamomum cassia*) Ethanolic Extract Against Paracetamol Toxicity in Rats

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ABSTRACT

This study aimed to determine the hepatoprotective effect of Cassia cinnamon ethanolic extract on rats induced with paracetamol toxic dose. This study used 20 male albino rats divided into 4 groups, control group was given a placebo, the paracetamol group was given paracetamol dose at 2000 mg/kg with no extract treatment, the Cassia cinnamon ethanolic extract groups were give extract at the dose of 160 mg/kg and 320 mg/kg prior to and after being induced with paracetamol 2000 mg/kg. The extract treatment was given for 7 days before paracetamol single administration and continued until 10 days. The rat blood samples were taken through the lateral vein and then the levels of SGOT and SGPT were measured before treatment (day 0), post paracetamol administration (day 7), and after the last day of treatment (day 10). Then, a histopathological analysis of the liver was performed. The results showed Cassia cinnamon ethanolic extract could not prevent a significant increase in SGOT and SGPT ($p>0.05$) in rats after paracetamol administration at a toxic dose. However, based of histopathological analysis, the administration of Cassia cinnamon ethanolic extract at a dose of 320 mg/kg can prevent the presence of multifocal necrosis and liver histological changes compared to those treated with paracetamol only. It is concluded that Cassia cinnamon ethanolic extract at 320 mg/kg dose provides protection against liver structural damage due to toxic doses of paracetamol.

1. INTRODUCTION

Cinnamon is a plant that has been used as spices in food and drinks from ancient time. There are 4 types of Cinnamon plants spread all over the world [1], but in general it is divided into two types: the Cassia cinnamon (*Cinnamomum cassia*) and the Ceylon cinnamon (*Cinnamomum Ceylon*). The Cassia cinnamon comes from south China and known as Chinese cinnamon (Saigon) which is planted from the tropical evergreen tree. This type of cinnamon has been planted and cultivated in many areas in China, Vietnam and Indonesia. Cassia

cinnamon originated from Indonesia is the most popular due to its sweeter and softer taste [2].

Cinnamon bark is known to have biological activity to overcome colds, diarrhea, and diseases related to the digestive tract. It also has activity as antioxidant [3]. The cinnamon bark extract with the content of trans-cinnamaldehyde level becomes the source of antioxidant compound with the ability to scavenge free radicals [4]. Reported that the largest chemical components of cinnamon are cinnamic alcohol, coumarin, cinnamic acid, cinnamaldehyde and volatile oil containing sugar, protein, simple fat, and pectin [5]. According to the research by the antioxidant activity of cinnamon oil is indicated by the presence of major compounds, namely cinnamaldehyde (75.32%) and eugenol compounds (8.53%). These major compounds are demonstrated to have the ability to scavenge free radicals [6]. The antioxidant activity was contributed by providing an electron to an oxidant that has free electrons making them less reactive [7].

Paracetamol is an analgesic and antipyretic that is most frequently used by the society since it is considered safe and easily accessed over-the-counter [8]. However, paracetamol can cause serious side effects, especially on the liver if used beyond the recommended dose [9]. One of the most serious adverse effects of paracetamol include hepatotoxicity [10]. Paracetamol is known to produce a toxic metabolite through its metabolism in hepatocytes. Paracetamol metabolism occurs through 2 major pathways, namely 180 glucuronidation and sulfation, and 1 minor pathway with the help of cytochrome P450 enzymes. The latest pathway results in a toxic oxidant metabolite, called N-acetyl-p-benzoquinone imine (NAPQI) [11]. When used in a high dose, the two major pathways will experience saturation, resulting in an increase in paracetamol metabolism in the minor pathway, leading to the accumulation of NAPQI in hepatocytes. This consequently makes the liver more susceptible to oxidative stress leading to cellular damage and death [12- 14]. Since Cassia cinnamon has a putative antioxidant effect, this study aimed to determine the hepatoprotective effect of Cassia cinnamon ethanol extract in rats treated with a toxic dose of paracetamol by measuring the levels of SGOT and SGPT as well as histopathological analysis.

2. Methods

2.1 Extract Preparation

Cassia cinnamon barks were cleaned from adhering dirt using running water then the samples were cut into small pieces, then dried to contain water content below 10%. The sample was sieved with a mesh size of 40 to obtain fine powder and then extracted by maceration method. The dried cinnamon was weighed as much as 500 grams, put into a vessel, then extracted by maceration method using 70% ethanol solvent [15]. In the maceration process, the sample was moistened with 70% ethanol until completely submerged for 15 minutes, after that it was added to 2 liters with 70% ethanol at room temperature for 3 x 24 hours while stirring occasionally. Then separate the macerate by filtration and then re-macerate with the same type and amount of solvent until the final and clearer macerate was obtained. The liquid extract obtained was collected and evaporated to obtain a thick ethanol extract.

2.2 Animal Preparation

The experimental animals used were male Wistar rats (*Rattus norvegicus*) with a body weight of 150-200 grams. The animals were randomly divided into 4 groups, each consisting of 5 rats. Prior to experiment initiation, rats were adapted to the experimental environment for 14 days to avoid stress that could affect the body's metabolism.

2.3 Experimental procedures

Twenty (20) male Wistar rats were divided into four groups. Group 1 served as healthy control; group 2 was

as a negative control group, which received a single administration of paracetamol 2000 mg/kg without any pre-treatment; group 3 and 4 were treatment groups, which received Cassia cinnamon extract treatment, at 160 mg/kg and 320 mg/kg doses, respectively for 7 days, followed by paracetamol administration at 2000 mg/kg dose at day 7, and continued to receive extract treatment for another 3 days (until day 10). The measurements of liver enzyme levels were carried out at 3 stages, i.e. before treatment (day 0), post paracetamol induction (day 7), and after treatment (day 10). After the last day of experiment, the liver was harvested to carry out histopathological analysis.

2.4 Statistical analysis

The biomarker data were analyzed with a comparative analysis using the ANOVA statistical test followed by the Tukey HSD post-hoc test. The liver damage was categorized based on the study conducted by minimal for no or minimum abnormality; mild for the presence of focal degeneration/necrosis; moderate for multifocal degeneration/necrosis; and marked for diffuse degeneration/necrosis [16].

3. Results

In this research, paracetamol was made as an inducer of liver damage to observe the effect of hepatoprotective ethanol extract of cassia cinnamon to the rat by using the parameters of SGOT and SGPT levels and histopathological observation

3.1 Liver Biomarkers

The control group showed normal SGPT (<60 U/L) and SGOT levels (<60 U/L) since the beginning of the experiment until the last day of the study (Table 1 and 2). Meanwhile the paracetamol group had SGPT levels of 90.24 U/L at day 7, which increased twice as much as the pre-treatment level. In the 160 mg/kg extract group, the level of SGPT also increased to 83.63 U/L after the induction of paracetamol, while with extract dose of 320 mg/kg, the SGPT level was also increased to 89.43 U/L. However, following 10 days of treatment, the level of SGPT reduced significantly, but this reduction was also occurred in the paracetamol group that did not receive any treatment from day 8 to 10 (Table 1). The SGOT levels of both extract treatment groups were also elevated at day 7 post paracetamol administration and remained elevated following additional treatment up to day 10 (Table 2). Again, similar pattern was also observed in paracetamol only group, indicating that cinnamon extract did not affect any of these liver enzyme levels.

Table 1. The SPGT levels in each treatment group

Treatment Groups	SGPT levels (U/L)		
	Day 0	Day 7	Day 10
Control	53.01±4.47	39.57±2.89	43.14±7.44
Paracetamol only	44.99±3.21	90.24±39.78*	48.71±6.36
Extract 160 mg/kgBW	46.59±4.70	83.63±18.46*	51.14±13.42
Extract 320 mg/kgBW	46.44±4.12	89.43±26.71*	45.60±6.58

Table 2. The SGOT levels in each treatment group

Treatment Groups	SGOT levels (U/L)		
	Day 0	Day 7	Day 10
Control	57.17±7.98	44,996±4.44	47.64±9.19
Paracetamol only	46.20±7.95	160.18±77.46*	102.15±8.11*
Extract 160 mg/kgBW	56.70±5.97	152.14±37.96*	111.65±15.07*

Extract 320 mg/kgBW	56.35±12.97	159.86±73.45*	103.74±20.61*

3.2 Histopathological changes

The histopathological feature of the liver the control group showed no damage to the liver cells (score 0: normal). Conversely, the negative control group with the adminitration of paracetamol at a dose of 2000 mg/kg experienced a multifocal damage to liver tissue, which was indicated by necrosis (black arrow), sinusoidal dilatation (green arrow), congestion (red arrow), hemorrhage (yellow arrow), and hydropic degeneration (orange arrow). This indicates that a toxic dose of paracetamol (2000 mg/kg) can induce a liver injury in rats.

In the group treated with the Cassia cinnamon ethanolic extract at a dose of 160 mg/kg, the liver cells were also showed a significant damage, which was almost similar to that seen in the paracetamol group. The histopathological changes found in this group including necrosis (black arrow), sinusoidal dilatation (green arrow), congestion (black arrowhead). red), hemorrhage (yellow arrow), and hydropic degeneration (orange arrow). In contrast, the group receiving Cassia cinnamon ethanolic extract at higher dose (320 mg/kg) did not experience a significant damage in the liver tissue, where only showed a sign of pyknosis (blue arrow) but the remaining structure showed normal hepatocytes.

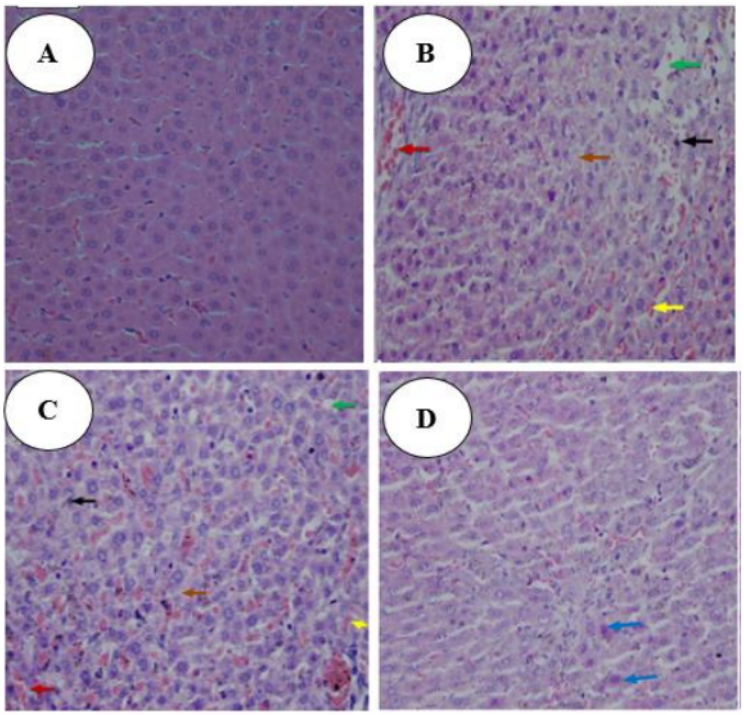

Figure 1. Comparison of the histopathological examination of rat liver with Cassia cinnamon extract with healthy control and paracetamol groups (Hematoxylin-Eosin staining, 400x magnification). A: normal control; B: negative control shows the presence of necrosis (black arrow), sinusoidal dilatation (green arrow), congestion (red arrow), hemorrhage (yellow arrow), and hydropic degeneration (orange arrow); C: extract 160 mg/kg group shows the presence of necrosis (black arrow), sinusoidal dilatation (green arrow), congestion (red arrow), hemorrhage (yellow arrow), and hydropic degeneration (orange arrow); D: the extract 320 mg/kg group shows the presence of pyknosis (blue arrow) only with normal liver structures.

4. Discussion

As the main organ that function as metabolism site, liver is prone to toxic material that is ingested by our body (17). The indicators that are most frequently used to measure the impairment of liver function are the activity of transaminase enzymes, the Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT). Transaminase enzymes are normally found intracellularly, therefore, a damage to the cell causes impaired liver cellular wall, leading to increased permeability. This in turn will result in an increase of SGOT and SGPT levels in the serum [18].

Increased levels of SGOT and SGPT can be caused by the use of excessive doses of paracetamol. Free radicals can be produced by paracetamol in the form of a reactive metabolite acetyl p- benzquinamide (NAPQ1) which will oxidize the macromolecules such as fats and thiol groups on proteins and disrupt calcium homeostasis [19]. The use of hepatoprotective agents may detoxify the toxic compound in the metabolic process, improving the regeneration of damaged liver cells [20].

Cinnamon contains glutathione-S-transferase, an enzyme that could increase serum and liver glutathione [21]. Glutathione has the ability to donate electrons and can function as reducing agents to chelate metal ions and reduce potential radicals in the body [22]. Through the mechanism of antioxidant and the increasing of flotation, the cinnamon can prevent the histological damage in the liver.

5. Conclusions

According to this research result, it could be concluded that although Cassia cinnamon ethanolic extract could not prevent a significant increase in SGOT and SGPT levels but at a dose of 320 mg/kg, the extract could provide significant protection against liver structural damage due to toxic doses of paracetamol.

Conflict of Interest

There is no conflict of interest.

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Author Contribution

All authors are contributed to conceived and design the analysis, collected the data; contributed data and analysis tools; performed analysis and wrote the paper.

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