HEPATOPROTECTIVE EFFECT OF GOD'S CROWN FRUIT (PHALERIA MACROCARPA) 70% ETHANOL EXTRACT AGAINST ACETAMINOPHEN-INDUCED LIVER INJURY IN SWISS WEBSTER MICE

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ABSTRACT

Background: The God's crown fruit has known for its antioxidant and anti-inflammatory properties. These properties might be beneficial in acetaminophen-induced liver injury which is caused by the formation of free radicals through the covalent binding of its metabolites to cytoplasmic proteins. The objective of this study is to determine the effect of the God's crown extracts on hepatotoxicity of acetaminophen.

Method: This study is an animal experiment with positive and negative control and three intervention groups (God's crown 70% ethanol extract with three different doses, 60 mg, 120 mg, and 240 mg per kg weight of each mice respectively). There are five treatment groups, each consisting 6 male Swiss Webster mice in similar initial condition. All groups were given treatment for 14 days, and continued by toxic dose of acetaminophen (300 mg per kg weight) excluding the negative control group on the 15th day. On the 16th day, they were examined for their level of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) through orbital sinus specimen, and histological examination of the liver tissues (after treatment with 10% buffer formaldehyde).

Results and Discussion: The treatment groups have shown lower serum levels of AST and ALT compared to the positive control after exposure of toxic acetaminophen dose, with a dose-response relationship ($p < 0.05$). There is also better histopathological profile of treatment groups compared with the positive control group.

Conclusion: A 70% ethanol extract of God's crown fruit (*Phaleria macrocarpa*) have shown a hepatoprotective property that effectively prevent acetaminophen-induced hepatic injury on mice.

Keywords: *Phaleria macrocarpa*, Protective Agents, Chemical and Drug Induced Liver Injury, Acetaminophen

EFEK HEPATOPROTEKTOR EKSTRAK ETANOL 70% DARI BUAH MAHKOTA DEWA (*PHALERIA MACROCARPA*) TERHADAP HEPATOTOKSISITAS PARASETAMOL PADA MENCIT GALUR SWISS WEBSTER

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ABSTRAK

Latar Belakang: Buah mahkota dewa memiliki khasiat sebagai antioksidan dan anti inflamasi. Parasetamol dosis toksik dapat mencederai hepar melalui ikatan metabolit toksiknya dengan protein-protein sitoplasma yang menyebabkan terbentuk radikal bebas. Penelitian ini ditujukan untuk mengetahui efek proteksi ekstrak buah mahkota dewa terhadap hepatotoksitas parasetamol.

Metode: Penelitian ini adalah eksperimen pada mencit dengan kontrol positif dan negatif, serta tiga kelompok intervensi (ekstrak etanol 70% dari buah mahkota dewa dengan tiga dosis, yaitu 60 mg/kgBB, 120 mg/kgBB, dan 240 mg/kgBB). Setiap kelompok terdiri dari 6 mencit galur Swiss Webster dengan karakteristik serupa. Intervensi diberikan selama 14 hari, yang dilanjutkan dengan pemberian satu dosis toksik parasetamol (300 mg/kgBB) pada hari ke-15. Pada hari ke-16, seluruh mencit diperiksa untuk kadar AST dan ALT serum dari sinus orbita, dan pemeriksaan histopatologi dari jaringan hepar (setelah perlakuan dengan larutan *buffer* formaldehida 10%).

Hasil dan Pembahasan: Kelompok perlakuan dengan buah mahkota dewa menunjukkan kadar AST dan ALT serum yang lebih rendah dibandingkan kontrol positif setelah paparan dosis toksik dari parasetamol, dimana peningkatan kadar mahkota dewa berhubungan dengan penurunan kadar AST dan ALT ($p < 0,05$). Selain itu, pemeriksaan histopatologi juga menunjukkan gambaran yang lebih baik dibandingkan dengan kontrol positif.

Kesimpulan: Ekstrak etanol 70% buah mahkota dewa (*Phaleria macrocarpa*) menunjukkan efek hepatoprotektor yang mencegah cedera hepar karena parasetamol dosis toksik pada mencit.

Kata Kunci: *Phaleria macrocarpa*, agen hepatoprotektif, cedera liver diinduksi obat, Parasetamol

INTRODUCTION

God's crown (*Phaleria macrocarpa*) is one of popular medicinal plants in Indonesia and has been widely used as a traditional medicine. On the contrary to the fruit flesh, there are highly toxic contents within its seed. *Phaleria macrocarpa* has antioxidant and anti-inflammatory property, related to its secondary metabolites which consisted of tannins, saponins, polyphenols, flavonoids, terpenes, and alkaloid.^{1,2} Four components of *Phaleria macrocarpa* which usually used in traditional medicine are the branches, the leaves, the skins of the seeds and the fruit.³ The extract of *Phaleria macrocarpa* fruit have higher antioxidant content than berries.²

Drug-induced liver injury (DILI) disease has been a concern in the practice of medicine until now. Based on its pathogenesis, DILI is grouped into intrinsic (dose-dependent) and idiosyncratic (unpredictable). Acetaminophen or paracetamol (*N*-acetyl phenyl-aminophenol (APAP)) is well known for its toxicity when administered in a high dose. Acetaminophen-induced liver injury (AILI) is the most common DILI found.⁴ *N*-acetyl-P-benzoquinone-imine (NAPQI), which is the toxic metabolite of acetaminophen, would be accumulated after administration of a toxic dose of acetaminophen. This metabolite is detoxified by glutathione through the conjugation process in liver. In its toxic level, NAPQI binds to cellular proteins, which further cause oxidative stress, mitochondrial dysfunction, and cellular necrosis.⁵

Alternative to conventional treatments of hepatotoxicity, some medicinal plants might offer solutions for liver protection. There are three proposed protective mechanisms of these plants, which are elimination of intracellular free radicals, blockade of toxin entry into the cells, interaction

with Cytochrome P450 (CYP450) isoforms, and stimulation of the production of glutathione and other endogenous antioxidants thus reducing the free radical production and lipid peroxidation.⁶

Previous preclinical studies have shown the efficacy of *Phaleria macrocarpa* in treating various conditions. However, the evidence on acetaminophen-induced liver injury is very scarce. This study was undertaken to determine the efficacy of *Phaleria macrocarpa* as a hepatoprotective agents for liver injury related to acetaminophen toxicity. *Phaleria macrocarpa* contains many active metabolites with antioxidant and anti-inflammation activity, which is expected to protect the liver against the toxic effects of acetaminophen.

MATERIAL AND METHOD

This study was conducted at the Food Nutrition and Clinical Nutrition Laboratory, Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, between September and November 2017. This Study has been approved by Research ethic Committee of Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta (Number: 487/C.16/FK/2017)

Animals And Preparation Of Acetaminophen

Thirty male Swiss-Webster mice with average age of 3 months, and average weight $\pm$ 20 grams were obtained from the mice breeding centre within the laboratory. Animals are housed in suitable cages, in good light and ventilation room, room temperature according to laboratory management at 27°C – 32°C. Rat were fed standard rodent pellets and aquadest ad libitum. The cages were clean up everyday.

Acetaminophen preparation were ordered at the Food Nutrition and Clinical Nutrition Laboratory, Center

for Food and Nutrition Studies, Universitas Gadjah Mada. The acetaminophen solution was administered orally through nasogastric tube.

Plant Materials And Intervention Stages

We obtained the *Phaleria* fruit from Merapi Farma Herbal Yogyakarta and prepared the 70% ethanol extract at the Food Nutrition and Clinical Nutrition Laboratory, Center for Food and Nutrition Studies, Universitas Gadjah Mada. The thirty mice were randomly allocated to five groups, Group 1 is a positive control, Group 2 is a negative control, and Group 3-5 are treatment groups. For the first 14 days, each group were given standard oral feed, where Group 1 and 2 did not receive any special treatment, and Group 3-5 received *Phaleria macrocarpa* extract (60 mg per kg weight, 120 mg per kg weight, and 240 mg per kg weight, respectively). On the 15th day, Group 1, 3, 4, and 5 received acetaminophen 300 mg per kg weight.

Biomarker Measurement

Blood specimens were drawn from the orbital sinus while the mice

still alive. We measured the serum level of AST and ALT in the Food Nutrition and Clinical Nutrition Laboratory, Center for Food and Nutrition Studies, Universitas Gadjah Mada.

Histopathology Preparation

The mice were sacrificed on the 16th day of treatment, and had their liver removed and treated with 10% formaldehyde solution in a sealed container. The liver specimens were transported, prepared, and examined in Pathology Anatomy Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada. The microscopic examination was done with Olympus CX21 microscope at 100 times magnification, and the images were taken with Optilab camera.

Histopathological assesment of the liver injury were undertaken by two autors dr. Tejo Jayadi, Sp.PA and Patrick Nalla Nunsio, with specific criteria as described by Blazka⁷ (shown in Table 1). The pathology examiners observed the changes in the histological structure of the liver of mice in each field of view.

Table 1. Grading for the severity of hepatic congestion or necrosis on histopathological examination⁷

Description	Grade
Normal histology	0
Characterized by minimal congestion and necrosis of single hepatocytes, limited to the area immediately around the centrilobular vein; many of the lobules not affected	1
Characterized by moderate congestion and hemorrhage of the area around the centrilobular vein and extending into the midzonal cells; most lobules are affected. Areas of confluent necrosis limited to the liver cells surrounding the centrilobular vein.	2
Characterized by widespread areas of congestion and hemorrhage in the centrilobular and midzonal areas of the liver. Confluent coagulative necrosis involving all hepatocytes in the centrilobular zone; bridging of areas of necrosis between centrilobular zones is common.	3

Statistical Analysis

The baseline characteristics of each groups were described and further analysed for the fulfilment of normal distribution. However, the data did not follow the normality assumptions, thus we used Kruskal-Wallis to test the difference of AST level, ALT level and histopathologic grade. To determine the difference between-pairs, we used Mann-Whitney test.

RESULTS

Effect Of *Phaleria Macrocarpa* Extract On Serum AST And ALT Levels After Toxic Acetaminophen Exposure

The description of serum AST and ALT post exposure in each group

are shown in Table 2. The Kruskal-Wallis test for each AST and ALT level resulted in $p < 0.001$. Further between-pairs analysis with Mann-Whitney test resulted in significance difference ($p < 0.05$) of mean serum AST and ALT level of treatment groups (Group 3,4,5) compared to positive control (Group 1). Toxic acetaminophen exposures related to hepatocyte necrosis is indicated by elevated AST and ALT (Group 1 vs Group 2). The pre-treatment with *Phaleria macrocarpa* extract has shown the hepatoprotective effect and the improvement of AST and ALT level by the increased dose of extract, shown in Table 2.

Table 2. The serum level of AST and ALT of each groups (n=30) after toxic acetaminophen exposure (Group 1,3,4,5) except for negative control.

Group	Treatment (aside from standard feed and drink)	N	Mean serum AST level (IU/L)	Mean serum ALT level (IU/L)
1	Acetaminophen, 300 mg per kg weight	6	72.50 ± 1.91	38.03 ± 0.39
2	No treatment	6	38.11 ± 0.67	19.42 ± 0.43
3	God's crown extract, 60 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	62.31 ± 1.00	31.23 ± 1.64
4	God's crown extract, 120 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	47.34 ± 0.67	27.19 ± 0.61
5	God's crown extract, 240 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	42.64 ± 1.55	23.14 ± 0.85

Effect Of *Phaleria Macrocarpa* Extract On Liver Histopathology After Toxic Acetaminophen Exposure

The grading of histopathological preparations is shown in Table 3, where Figure 1 (A-F) shows the histopathological findings. *Phaleria macrocarpa* showed a

protective effect on liver function and hepatocyte necrosis, including a dose-response relationship. The Kruskal-Wallis test result is statistically significant ($p < 0.001$), and between-pairs test with Mann Whitney showed significant differences in the histopathologic score of the positive control group with treatment 2 and 3 ($p < 0.05$).

Table 3. The grading of pathological changes in each group (n=30) after toxic acetaminophen exposure (Group 1,3,4,5) except for negative control.

Group	Treatment (aside from standard feed and drink)	n	Mean Histopathological grading
1	Acetaminophen, 300 mg per kg weight	6	2,967
2	No treatment	6	0
3	God's crown extract, 60 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	2,867
4	God's crown extract, 120 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	2,367
5	God's crown extract, 240 mg per kg weight and Acetaminophen, 300 mg per kg weight	6	2,067

Figure 1. A: normal liver histology (Grade 0). B: Coagulative necrosis (Grade 3).

C: Bridging necrosis and severe congestion (Grade 3). D: bridging necrosis and inflammation of the porta region (Grade 3). E: focal necrosis to zone 2 and moderate congestion (Grade 2). F: mild congestion mild focal hepatocyte centrilobular necrosis (Grade 1)

DISCUSSION

This study showed that mice pre-treated with God's crown extract show less liver damage than positive control. The higher dose of God's crown extract also showed more protection, suggested a dose-response relationship where higher dose of God's crown extract resulted in lesser

liver damage. There are no previous studies of God's crown effects on acetaminophen induced hepatotoxicity. Two studies found a hepatoprotective effect of proliverenol extracted from *Phaleria macrocarpa* against ethanol-induced hepatotoxicity and carbon-tetrachloride-induced hepatotoxicity.^{8,9} Further analyses discovered the down-

regulation of NF- κ B-TNF α -caspase-8, TGF- β , endothelin, and platelet-derived growth factor production, as possible mechanisms which explain the hepatoprotective effect of proliverenol.^{8,9} Unfortunately, this study did not measure the mediating molecular activity of God's crown hepatoprotective activities on acetaminophen-induced hepatotoxicity, although similar mechanisms to other drug-induced liver injury might occur in this setting. This protective activity is suggested to counter the production of free radicals, protein adduct, and mitochondrial damage which leads to hepatocyte necrosis in acetaminophen-induced hepatotoxicity.¹⁰

The protective effect of *Phaleria macrocarpa* fruit extracts might come from its high content of flavonoids (kaempferol, myricetin, quercetin, naringin, and rutin), phenol (magniferin, gallic acid, and 6-dihydroxy-4-methoxybenzophenone-2-O- β -D-glucoside), tannin, saponin, and terpenes.¹¹ The flavonoids in *Phaleria* possess antioxidant activity which slows or inhibits excessive oxidation and binds to oxidant agents. It also has anti-inflammatory activity which inhibits nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression.¹² Other antioxidant properties of *Phaleria* extract is inducing the production of superoxide dismutase (SOD) which catalyse the decomposition of superoxide into peroxides.¹³ Specifically for gallic acid, an animal study showed that it protected the liver from N-Nitroso dimethylamine-induced inflammation, probably by inducing Nrf2-mediated antioxidant enzymes and attenuating the inflammatory mediator COX-2 through NF- κ B inhibition pathway.¹⁴

CONCLUSION

In this study the 70% ethanol extract of *Phaleria macrocarpa* fruit

showed a protective activity against the hepatotoxic effects of high acetaminophen dose. In addition, we found a dose-response relationship regarding this effect. Further studies are needed to explore the molecular mechanism of hepatoprotection property of *Phaleria* extracts.

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