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The Ethanolic Extracts Therapy of Ceremai Leaves (*Phyllanthus acidus (L.)*Skeels) on Malondialdehyde (MDA) Levels and Histopathology of Hepar of Hypercholesterolemic Rats

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Abstract: Hypercholesterolemia is high condition when the concentration of cholesterol in blood was increased. Ceremai (*Phyllanthus acidus* (L.)Skeels) is a plant which used by Indonesian people as aherbal to treat hypercholesterolemia. This study aimed to determine effects of ethanolic extracts therapy of ceremai leaves (*Phyllanthus acidus* (L.)Skeels) on malondialdehyde (MDA) levels and heparhistopathology of hypercholesterolemic rats (*Rattus norvegicus*). MDA levels were determined through TBA test (thiobarbituric acid) while hepar histopathology was determined with Hematoxylen-Eosin (HE) staining. The ethanoic extracts therapy of ceremai leaves (*Phyllanthus acidus* (L.)Skeels) showed could reduce MDA levels of hepar by 37.17% and also 59.92% with doses of 150 mg/kgBW and 300 mg/kgBW, respectivelly. The histopathology of hepar of showed improvement after the therapy. It was concluded, that ceremai leaves could be used to hypercholesterolemia therapy.
Keywords: *Phyllanthus acidus* (L.) Skeels, Hypercholesterolemia, Antioxidants, MDA, Hepar Histopathology.

Introduction

Hypercholesterolemia is a condition where the concentration of cholesterol in blood is increased exceed the normal value. Blood cholesterol concentrations in humans is considered normal if the total cholesterol <200 mg/dL [1] .

Free radicals will be accumulated in the body of hypercholesterolemic patients. Malondialdehyde (MDA) is one of the most frequently signs of oxidative damage by free radicals on cell membranes[2].Ceremai (*Phyllanthus acidus* (L.)Skeels) is a plant which is used by the majority of Indonesian people as a traditional medicine to treat the hypercholesterolemia and hypertension [3]. Ceremai (*Phyllanthus acidus* (L.)Skeels) has antioxidants activity to inhibit the occurrence of lipid peroxidation [4].

This study aimed to determine the effect of ethanolic extracts therapy of ceremai leaves (*Phyllanthus acidus* (L.)Skeels) on the levels of malondialdehyde (MDA) and histopathology of heparhypercholesterolemic rats (*Rattus norvegicus*).

Experimental

Instruments and Materials

The instruments used in this study were a set of tools cups, spatula, auto-pipette, stirrer, mortar, vortex, oven, water bath, centrifuge, digital scales, UV-Vis spectrophotometer, rotary evaporator, light microscope, rats scales, rats cages, syringes, Easy Touch GCU, -20^{o}C freezer, 4^{o}C refrigerator.

Materials used are dried powder of ceremai leaves (*Phyllanthus acidus* (L.)Skeels), cholic acid, lard, eggs quail, rat's standard feed, distilled water, HCL, Ethanol, Physiological NaCl 0.9%, MDA standard kit, PBS-azide, *Tri Cloro Acetic Acid*(TCA), *Paraformaldehyde*(PFA), Alcohol 70%, 80%, 90% and 95%.

Experimental animals were used male rats (*Rattus norvegicus*) Wistar strain with 150-200 g of body weight which obtained from Animal Model Unit Development (UPHP) UGM Yogyakarta. All conditions of experiment and handling of the animals were conducted following the protocols approved by Ethical Clearences Committe of Brawijaya University (238-KEP- UB). The rats were divided into five groups: control group (A), hypercholesterolemic group (B), and (C), (D) were hypercholesterolemic group with ethanolic extract of ceremai leaves dose of 150 mg/kgBW300 mg/kgBW, respectively.

Induction of Hypercholesterolemia

Rats in group B, C, and D were treated with hypercholesterolemic diet by force feeding. Hypercholesterolemic diet for each rat was prepare from 10% lard (2g), 0.02g of cholic acid and 1g of cooked egg yolk, then mixed with destilated water to 2mL.

Preparationof Ethanolic Extract of Ceremai Leaves (*Phyllantus acidus* (L.)Skeels)

25g of driedceremai leaves (*Phyllanthus acidus* (L.)Skeels) were mixed with 100mL of ethanol in an orbital shaker for 48 h. The filtrate obtained from the extraction was filtered then extraction product was evaporated to separate the solvent from the extract using a rotary vacuum evaporator at 40°C [5].

Measurement of Malondialdehyde (MDA) Levels

Hepar (1g) was grilled by a cold mortar, 500µL of NaCl 0.9% then added and homogenized. Homogenates was centrifugated at 8000 rpm for 20 minute and the supernatant was collected for MDA measurement. 100µL of Supernatant was added with 550µL of distilled water, 100µL TCA, 250µL of 1N HCl, 100µL of 1% Na-Thiothen homogenized with vortex mixer, then centrifuged at 500 rpm for 10 min. The supernatant was incubated in water bath at 100°C for 30 min, and stored to room temperature and measured using UV-Vis at λ max of 532 nm.

HeparHistopahology analysis

Hepar was isolated and washed with 0.9% NaCl-phys. Preparation of hepar tissue section and staining was conducted based on previous methods with slight modification [6]

Result and Discussion

The result ethanolic extracts of ceremai leaves (*Phyllanthus acidus* (L.)Skeels) therapy onhepar of hypercholesterolemic rats (*Rattus norvegicus*) malondialdehyde (MDA) showed decreasing of MDA levels (Table 1).

Table 1.MDA Level of Hypercholesterolemic Rats

Treatment group	MDA (µg / mL)		
	Value	Increased (%)	Decreased (%)
Control (A)	1.429 ± 0.115^{a}	-	-
Hypercholesterolemia (B)	5.203 ± 0.208^{d}	264.10	-
Treatment of 150 mg/kgBW (C)	3.269 ± 0.314^{c}	-	37.17
Treatment of 300 mg/kgBW (D)	2.085 ± 0.408^{b}	-	59.92

The average of MDA levels of control group was applied ol to determine the increasing or decreasing in the MDA levels on other groups. The MDA level of this group was the lowest (1.429 ± 0.115 µg/mL).MDA levels on group with hypercholesterolemic diet showed increasing of MDA levels to be 5.203 ± 0.208µg/mL. It was 264.10% increased from normal. The ethanolic extract of ceremai leaves extract showed decreasing of MDA levels to be 37.17% and 59.92% with therapy dose of 150 mg/kgBW and 300 mg/kgBW, respectively. Statistical analysis also showed a significantly difference between groups ($p<0.05$).

Malondialdehyde(MDA) levels reflected free radicals. Excessive amounts of free radicals has potencyl to damage cellmembran as the result of lipid peroxidation [7]. Theceremai (*Phyllanthus acidus* (L.)Skeels) leavesextract contains flavonoid which play a role as antioxidantsto prevent free radicals [8]. Antioxidants functions are to bind the free radicals and inhibit lipid peroxidation process, so it can reduce the free radicals as the result of hypercholesterolemic diet. Flavonoids are polyphenols whit role as an antioxidant [9]. Flavonoid antioxidant activity has a major function as scavenger of free radical to reduce free radicals levels [10].

The ethanolic extract of ceremai (*Phyllanthus acidus* (L.)Skeels) leaves on heparrats showed in Figure 1.In normal condition, showed normal central venous, sinusoid and hepatocyte. In group with hypercholesterolemia condition, showed an alteration as hepatocyte with fat degeneration, particulary the central vein. In groups with ethanolic extract of ceremai leaves therapy showed an improvement of hepar histopathology showed by decreasing of hepatocyte fat degeneration. In therapy group with dose of 300 mg/kgBW showed a repairing of hepatocyte to normal condition.

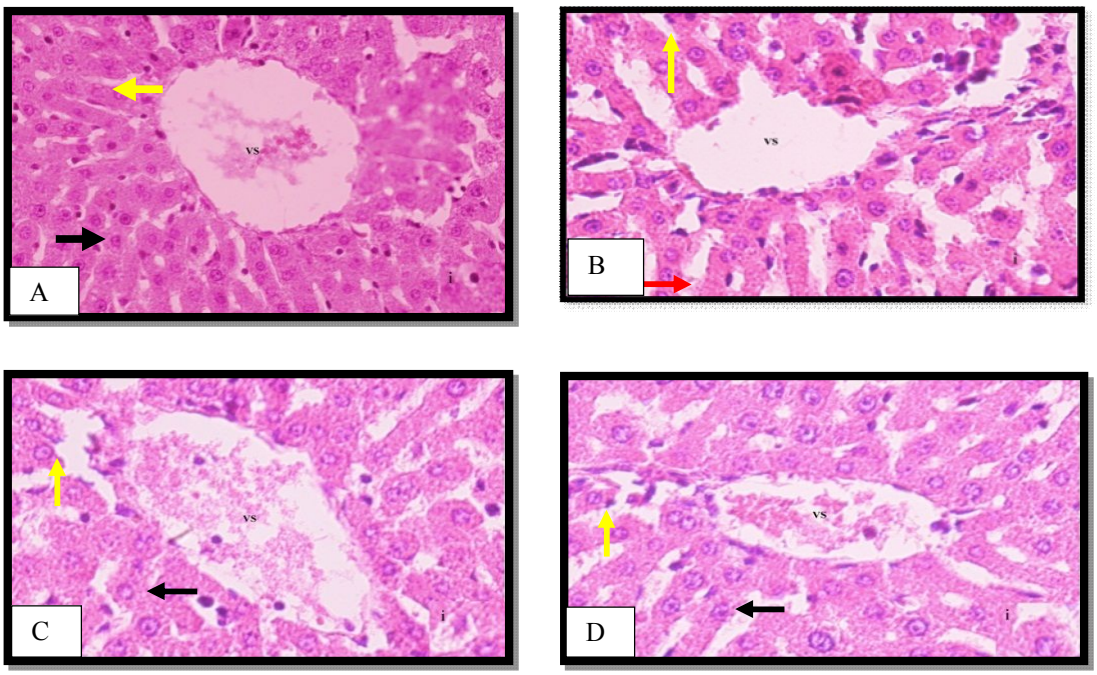

Figure 1. Histopathology of HeparHypercholesterolemia Rats (*Rattus* norvegycus) with Hematoxilen-Eosine Staining (400x).

(A) control, (B) hypercholesterolemia, (C) therapy dose of 150 mg/kgBW and (D) therapy dose of 300 mg/kgBW. Black arrow pointing to normal hepar cell, red arrow pointing to fatty hepatocyte, yellow arrow pointing to sinusoid.

Fat degeneration occurs because of LPL activity enzyme decreases VLDL hydrolysis that caused accumulation of trigliserides granules in hepatocytes. Fat accumulation commonly begins from the portal area that extend toward the central vein. This condition occurred of the blood supply from the intestine to the hepar through the central vein [11]. Heparrepairing showed by improvement of tissue caused of the antioxidant from the ethanolic extract of ceremai (*Phyllanthus acidus* (L.) Skeels) leaves that causing the free radicals decrease by lipid peroxidation inhibition and the reduction of blood cholesterol levels.

Conclusion

The ethanolic extract of ceremai (*Phyllanthus acidus* (L.)Skeels) leaves for hypercholesterolemicrats therapy could decrease MDA levels as well as improvement of hepar histopathology. Dose of 300 mg/kgBW was the best dose for hypercholesterolemia therapy.

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