

Cardioprotective Effect of Ethylacetate Extract of *Zanthoxylum acanthopodium* Dc. against Doxorubicin-induced Cardiotoxicity in Rats

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Abstract: This study was carried out to investigate cardioprotective effect of ethylacetate extract of *Zanthoxylum acanthopodium* DC Lour. fruit (EEZ) against doxorubicin-induced cardiomyopathy in rats. EEZ was prepared by maceration and 300 mg/kg bw as dosage of extract. DOX was administered to rats at dose of 20 mg/bw through intraperitoneal route for two days. Cardioprotective effect was evaluated by measuring biomarkers troponin T (cTnT), CK-MB levels and histopathology of rat's heart tissue was examined. Result of phytochemical screening of extract was found to contain alkaloids, flavonoids, tannin, glycosides, and saponin. DOX raised cTnT and CK-MB levels and were counteracted by administration of vitamin E, rutin, and EEZ. Histopathological analysis of rat's heart tissue resulted in myocytolysis with congestion of blood vessels, pyknosis, cytoplasmic vacuolization and fragmentation. Concomitant treatment with vitamin E, rutin, and EEZ revealed normal muscle fiber. This results suggest that EEZ has cardioprotective effect.

Keywords: *Zanthoxylum acanthopodium* DC, vitamin E, rutin, cardioprotective effect.

1. Introduction

Zanthoxylum acanthopodium DC is an ethnic plant include *Zanthoxylum* genus, Rutaceae family¹. Andaliman fruit contains many compounds, those are antioxidants². The flavonoid compounds are active as a protector for myocardium cells by inhibiting the action of DOX as iron chelation, antioxidant activity, and inhibit carbonyl reductase³. Alkaloids has potential as antioksidant activity⁴

Doxorubicin (DOX) is an anthracycline class of the most effective and broad-spectrum antineoplastic widely used as anticancer on various types of cancer including breast cancer^{5,6} but the use of DOX is clinically irreversible cardiotoxic side effects and cause of death in cancer patients^{7,8,9,10}. Therefore, the use of DOX has been restricted for the purpose of minimizing the incidence of cardiotoxic, however, efficacy as an antitumor decrease^{6,11}. The mechanism of DOX as the cause of cardiotoxicity that is through the formation of free radicals associated with iron and metabolites doxorubicinol^{12,13}.

Myocardium is an organ that is more sensitive to free radicals produced by DOX as a source of endogenous enzymatic antioxidant in the heart, such as *superoxide dismutase (SOD)*, *glutathione peroksidase*, *catalase*, dan *glutathion reductase (GSH)* resulting in irreversible damage to the myocardium cells^{14,15,16}.

Cardioprotective effects of any compound are indicated by measuring levels of biomarkers such as cTnT and CK-MB.

The purpose of this study was to investigate the cardioprotective effect of ethylacetate extract of *Zanthoxylum acanthopodium* DC Lour. fruit (EEZ) in female rats induced doxorubicin.

2. Experimental

This study was carried out experimentally. The animals were divided into six groups; each group consisting of eight rats: Group 1: Rats were injected with CMC Na (negative control). Group 2: Rats received EEZ (300 mg/kg) orally for nine consecutive days. Group 3: Rats in this group was treated intraperitoneally with a single dose (20 mg/kg) of DOX. Group 4: Rats received EEZ (300 mg/kg) orally started 7 days before DOX (20 mg/kg) administration and continued for the next two consecutive days. Group 5: Rats received rutin 50 mg/kg BB and DOX (20 mg/kg). Group 6: Rats received vitamin E 100 mg/kg BB and DOX (20 mg/kg). The administration of each treatment and DOX based on group 4. At the end of the experiment, the rats were killed by decapitation; blood samples were collected into tubes and measured cTnT and CK-MB levels. The hearts were removed, cleaned and washed in ice-cold physiological saline and then fixed in 10% buffered formalin solution at room temperature for histopathological evaluation^{17,18,19,20,21,22}.

2.1 Materials

Doxorubisin (DOX), Ketamine, *Zanthoxylum acanthopodium* DC Lour fruit was obtained from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. *Zanthoxylum acanthopodium* DC Lour. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium. CMC Na, Female Rats (*Rattus norvegicus*) 200-250 body weight.

2.2 Preparation of EEZ

The air-dried and powdered fruit of *Zanthoxylum acanthopodium* DC Lour. (1 kg) were repeatedly extracted by cold maceration with n-hexane (3x3 d, 7.5 L). The powder was dried in the air and extracted with ethyl acetate (3x3 d, 7.5 L) at room temperature on a shake. The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to give a dried extract²³.

2.3 Determining Class of Chemical Compound

Determining the class of chemical compounds carried out on simplex and EEZ^{24,25}.

2.4 Statistical analysis

All data were analyzed using regression using SPSS 19. Test were used for statistical analyses with p values 0.05 were considered significant.

3. Results and Discussion

3.1 Phytochemical screening result

The results of phytochemical screening of *Zanthoxylum acanthopodium* DC Lour is presented in Table1.

Table 1: The result of phytochemical screening

No	Screening	Simplex	EEZ
1	Alkaloids	+	+
2	Flavonoid	+	+
3	Glycosides	+	+
4	Saponins	+	+
5	Antrakuinon glycoside	-	-
6	Tannins	+	+
7	Triterpenoid/ steroid	+	-

3.2 Cardioprotective effect

The results of treatments on cTnT and CK-MB level was presented in Table 2,

Table 2: The result of treatments on cTnT and CK-MB level

Treatments	cTnT ($\mu\text{g/L}$)	CK-MB (U/L)
Control (CMC Na)	0.31	126
DOX	1.89	321
EEZ	0.15	132
Vitamin E + DOX	0.23	135
Rutin + DOX	0.10	122
EEZ + DOX	1.45	230

EEZ did not affect the levels of cTnT. EEZ, vitamin E and rutin can reduce levels of cTnT and CK-MB in DOX-induced. Rutin is a flavonoid compound that acts also as a cardioprotective by means of iron chelate complex formation, antioxidant activity and inhibition of enzymes such as *nitric oxide synthases*, *NAD(P)H oxidase* and *carbonyl reductases*, therefore, it can protect the heart from the effects of DOX-induced cardiomyopathy²⁶. Vitamin E is a neutralizing antioxidants play a role in inhibiting lipid peroxide (RO_2^\bullet)²⁷. By this action, vitamin E possibly has a cardioprotective effect as well.

3.3 Result of heart histopathology

The results of histopathological examination of heart by HE (*haematoxyllin-eosin*) staining is presented in Figure 1.

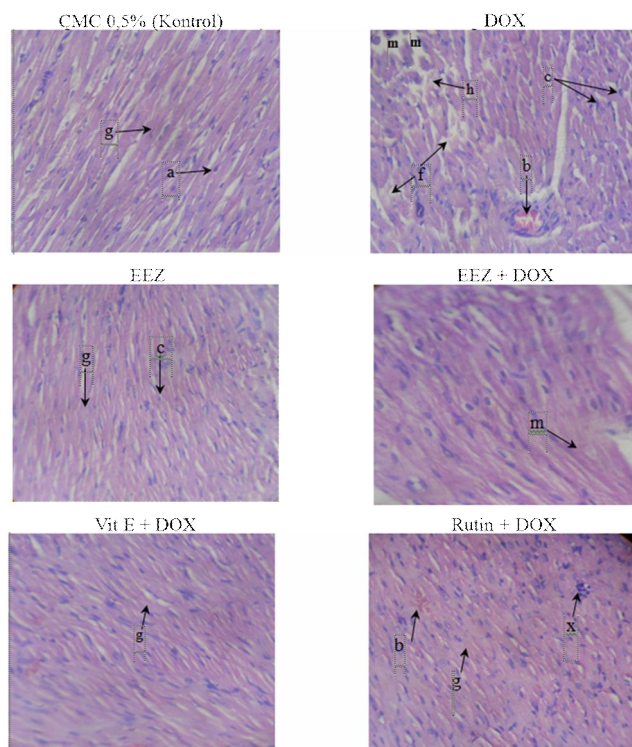


Figure 1: Result of heart histopathology. (a) Normal myosit; (b) congestion of blood vessels (c) picnosis; (m) miositilosis; (f) fragmentation; (g) miofibril normal; (h) Karyolysis; (x) tissue regeneration

Based on the results of histopathological examination of heart by HE staining, a control group treated with CMC Na 0,5% did not seemed causing damage heart muscle cells (normal forms) and the boundary between the cells of the heart muscle fibers clear and regular. In the group of DOX seemed to be bleeding, irregular heart muscle fibers, muscle fiber fragmentation, and pyknosis. Tissue heart muscle cells are

particularly vulnerable to free radicals. Free radicals produced from DOX reacts with unsaturated fatty acids to form lipid peroxides. As a result, changing the structure of lipid bilayer membranes causing cell damage accompanied by cell death. According to the result obtained, EEZ is potential as cardioprotective by decreasing of cTnT and CK-MB levels and protecting cardiomyocyte.

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