

## Effects of Ethanolic Extract of *Curcuma Longa* on Cardiac Biomarkers of Doxorubicin-Induced Rats

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### Abstract

Cardiotoxicity is a condition where damages caused by toxic chemical exposure are observed in heart and blood vessels. Doxorubicin is the most common chemotherapy agents for various types of cancer therapy. However, doxorubicin is converted into doxorubicinol known to trigger cardiac disfunctions and release of several cardiac biomarkers, such as CK-MB and LDH. Turmeric is known to be an alternative medical treatment that has the effect of neutralizing oxidative stress. This study aimed to evaluate CK-MB and LDH levels in doxorubicin-induced rats (20 mg/kgBW) that received turmeric ethanolic extract from curcuma. This was an experimental study conducted in February 2020 in the Faculty of Pharmacy Universitas Sumatera Utara. The rats were divided into six group and each group consisted of wistar albino male rats. The groups were normal (CMC-Na), positive control (Vitamin E 1%+Dox 20 mg/kgBW), negative control (Dox 20 mg/kgBW), treatment I (EEC 100 mg/kgBW+Dox 20 mg/kgBW), treatment II (EEC 300 mg/kgBW+Dox 20 mg/kgBW), and treatment III (EEC 500 mg/kgBW+Dox 15 mg/kgBW). Doxorubicin was given 5 mg/kgBW once a week for four weeks. Results showed that the groups that received 100 mg/kg BW, 300 mg/kgBW, and 500 mg/kgBW of turmeric ethanol extract demonstrated a reducing effect on the biomarkers of cardiac damage, i.e. CK-MB and LDH. Statistically, serum CK-MB and LDH levels at dose 500 mg/kgBW showed no significant differences ( $p>0.05$ ) with the normal and positive treatment group. In conclusion, turmeric has a cardioprotective effect.

**Keywords:** Cardiotoxicity, CK-MB, LDH

## Pengaruh Kardioprotektif Ekstrak Etanol *Curcuma Longa* pada Tikus yang Diinduksi *Doxorubicin*

### Abstrak

Kardiotoksitas adalah kondisi terjadinya jantung dan pembuluh darah akibat paparan kimia. *Doxorubicin* diubah menjadi doxorubicinol yang diketahui menyebabkan gangguan fungsi jantung dan pelepasan biomarker jantung seperti CK-MB dan LDH. Kunyit dikenal sebagai pengobatan alternatif yang memiliki efek menetralkan stres oksidatif. Penelitian ini dilakukan pada Februari 2020 di Fakultas Farmasi Universitas Sumatera Utara. Tujuan penelitian ini adalah mengevaluasi kadar CK-MB dan LDH pada tikus yang diberi ekstrak etanol kunyit yang diinduksi doxorubisin (20 mg/kgbb). Penelitian dibagi menjadi enam kelompok dan tiap-tiap kelompok terdiri atas 4 ekor tikus wistar albino jantan: normal (CMC-Na), kontrol positif (Vitamin E 1%+Dox 20 mg/kgBB), kontrol negatif (Dox 20 mg/kgBB), group I (EEC 100 mg/kgBB+Dox 20 mg/kgBB), group II (EEC 300 mg/kgBB+Dox 20 mg/kgBB), dan group III (EEC 500 mg/kgBB+Dox 15 mg/kgBB). Doxorubicin 5 mg/kgBB diberikan 4 kali. Hasil penelitian menunjukkan bahwa kelompok yang diberi ekstrak etanol kunyit dengan dosis 100 mg/kgBB, 300 mg/kgbb, 500 mg/kgBB memiliki efek penurunan biomarker kerusakan jantung yaitu CK-MB dan LDH. Berdasarkan atas hasil uji statistik kadar CK-MB dan LDH serum pada dosis 500 mg/kgBB tidak terdapat perbedaan yang bermakna ( $p>0,05$ ) dengan kelompok normal dan positif, dapat disimpulkan bahwa kunyit memiliki efek kardioprotektif.

**Kata kunci :** CK-MB, LDH, kardiotoksitas

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## Introduction

Doxorubicin (DOX) is used as one of the most common chemotherapy agents for various types of cancer therapy, which is derived from the class of antibiotics anthracyclines.<sup>1</sup> In clinical use, this class of antibiotics has limitations due to side effects, which can cause cardiotoxicity that is quite severe in cancer patients, both acute and chronic, depending on the dose of administration.<sup>2</sup>

The side effects of doxorubicin are irreversible in chronic use, including the formation of cardiomyopathy, congestive heart failure, nephrotoxic, and hepatotoxic.<sup>3,4</sup> As reported by Wattanapitayakul,<sup>5</sup> the risk of using doxorubicin causes side effects in normal tissue, especially in the heart, and causes the suppression of the immune system. The tissue in the heart organ has an active metabolic. Still, this organ has a low antioxidant ability compared to other organs in the human body. Hence, the heart is very vulnerable to damage caused by free radicals, one of which is doxorubicin.<sup>2</sup> The biological picture is shown in general, and the effect of using doxorubicin is apoptosis or necrosis.<sup>6</sup>

In a study conducted by Clafshenkel et al.,<sup>7</sup> administering doxorubicin 4 mg/kg body weight in animal mice that is carried out once a week for four weeks intraperitoneally (IP) increases cardiac fibrosis when compared to a control group not administered with doxorubicin. Because of doxorubicin's effects, we need an active compound that can protect the heart organ (cardioprotective), which cardioprotective compounds can provide excellent anti-inflammatory and antioxidant effects.

In research conducted *in vitro*, curcumin compounds contained in turmeric are cytotoxic, where the content of curcumin can inhibit the proliferation of cancer cells, and can also reduce pain or wound size from cancer. Therefore, turmeric is very likely to be used as an anti-inflammatory and is very useful for anti-cancer therapy. The potential of turmeric as a medicinal plant can be further investigated so that its utilization as traditional medicine can be further maximized without adverse side effects.<sup>8</sup>

Based on the description above, the long-term cancer therapy with doxorubicin often causes multiorgan damage, one of which can cause cardiotoxicity. Turmeric plants (*Curcuma longa* Linn = Tumeric) with a content that is rich in curcuminoid (curcumin) have properties as a potent free radical reducer, thus encouraging researchers to test the effectiveness of ethanolic

extract of *Curcuma* (EEC) on experimental animals induced by doxorubicin in the heart organ. The parameters measured are CK-MB (Creatine kinase-MB) and LDH (Lactate dehydrogenase) as biomarkers.

## Methods

Microplate Reader, pH meter (OHAUS Starter300 Portable) *Beaker glass* (IWAKI CTE33), *Multiskan Go Reader* (Thermo Fisher Scientific 1510), analytic measure, Eppendorf tube, vial 1 mL, Spatula, Micropipet (1–10 µL, 50–200 µL, 100–1000 µL) (Eppendorf), Thermometer, *automated plate washer*, Tumeric, Vitamin E, Ketamine (Sigma P-4417), Doxorubicin (Merck 109057), and CMC-Na (Sigma P-4417) were used in this study. Animals used in the research were rats (*Rattus norvegicus*), Wistar males of 150–200 g (each group consisted of 4 rats). Before the study commenced, the animal test was adjusted for one week with the room temperature (22–25°C) under the cycle of 12 hours light/dark, with the provision of food and drinking water *ad libitum*. Ethics Commission from health and science commission, University Prima Indonesia approved this study (No. 012/KEPK/UNPRI/II/2020). The present study was conducted in the Faculty of Pharmacy, Universitas Sumatera Utara, and the serum was analyzed at the North Sumatera Regional Health Laboratory.

Air-dried *Curcuma Longa* (800 g) was extracted with 90% ethanol (12 L) three times (2h each) using a soxhlet under reflux. The ethanol extract was concentrated under vacuum to give a crude extract (150 g). Qualitative analytical phytochemical screening of the ethanol *Curcuma* extract method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin, and alkaloids.

*In vivo*, an experiment was conducted by using 24 Wistar rats (*Rattus norvegicus*) male and weight 150–200 g, divided into six groups, and each group consisted of 4 rats. The groups were Normal (Suspension Na-CMC), Negative control (male Wistar rats [*Rattus norvegicus*] induced by doxorubicin 20 mg/kgBW), Positive control (male Wistar rats [*Rattus norvegicus*] induced by doxorubicin 20 mg/kgBW + Vitamin E 1%), Group I (male Wistar rats [*Rattus norvegicus*] induced by doxorubicin 20 mg/kgBW + EEC 100 mg/kgBW), Group II (male Wistar rats [*Rattus norvegicus*] male induced by doxorubicin 20 mg/kgBW + EEC 300 mg/kgBW), and Group III (male Wistar rats [*Rattus norvegicus*] induced by

doxorubicin 20 mg/kgBW + EEC 500 mg/kgBW)

Rats were induced by doxorubicin with an accumulative dose of 20 mg/kgBW for over 21 days, with a 5 mg/kgBW dose once a week. Before treatment, the rats were given the extract with a dose of 100 mg/kgBW, 300 mg/kgBW, and 500 mg/kgBW for 21 days. On the last day of treatment, the rats were not given any food or water for 18 hours before undergoing surgery for the animal test. Rats were administered with ketamine 70 mg/kgBW intraperitoneally, and then underwent surgery. Thoracic dissection was performed and 3 mL of blood was collected and then transferred a microtube to be centrifuged for 10 minutes at 3,000-4,000 rpm of speed until it was separated into serum, and supernatant. Next, 1 mL of serum was collected and put into microtubes to be stored in the refrigerator at a temperature of 4°C for determination of CK-MB and LDH. Measurement of the CK-MB and LDH levels was performed by following the method [9]. As much as 50 µl samples and 500 µl of CK-MB reagent/LDH was mixed in a test tube. The initial absorbance was then read after 1 minute at a wavelength of 340 nm. Next, the absorbance was measured again after 1, 2, and 3 minutes. Test analysis was carried out using one-way analysis of variance (ANOVA), followed by Post Hoc Test using the Tukey HSD test. In this study,  $P < 0.05$  was considered as a statistical significance. Data analysis was performed using the IBM SPSS 20.

## Results

The results of phytochemical screening qualitatively in Curcuma extract were shown in the Table 1. Phytochemical screening of ethanol extract of Curcuma showed the positive result of flavonoids, tannins, saponins, glycosides, alkaloids, and steroids. In this study, an examination of CK-MB from the blood of rats

**Table 1 Results of Phytochemical Screening of Extract Curcuma**

Chemical Component	Result
Tanin	+
Saponin	+
Flavonoid	+
Steroid	+
Glikosida	+
Alkoloid	+

**Table 2 CK-MB Level**

Doses	Mean CK-MB±SD (U/L)
Normal	323.63±5.00 <sup>#</sup>
Negative control	997.53±7.44
Positif control	442.23±3.19 <sup>#</sup>
Group I	751.73±12.67
Group II	427.03±2.46
Group III	379.32±34.57 <sup>#</sup>

Post tukey test  $p < 0.05$  #: has a significant different with negative group;  $p > 0.05$  \*: No significant different with normal

was carried out. The results of serum CK-MB obtained can be seen in Table 2.

Based on Table 2, it is known that the average serum CK-MB value for the normal group was still within the normal range of 323.63±5.00 U/L.

The negative control group (doxorubicin induction) had an average serum CK-MB of 997.53±7.44 U/L. The positive control group had an average serum CK-MB of 442.23±3.19 U/L while the treatment group I had a serum CK-MB value of 751.73±12.67 U/L. The treatment group II had a serum CK-MB value of 427.03±2.46 U/L and the treatment group III had a serum value of 379.32±34.57 U/L.

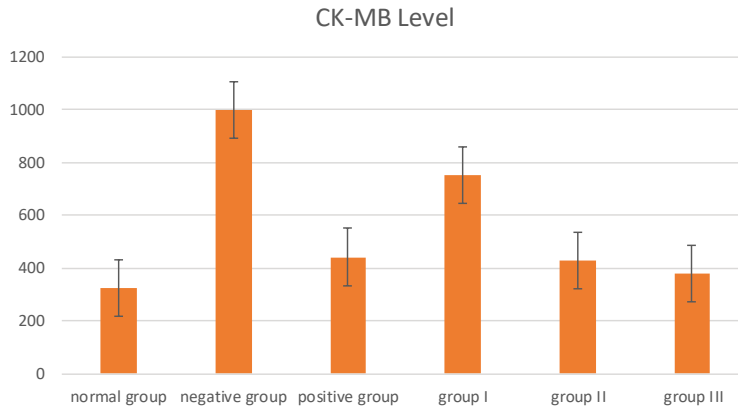
Based on the table, it was revealed that the average serum CK-MB in the most extensive treatment group was 751.73±12.67 U/L in treatment group I. Furthermore, the ordinary serum CK-MB in the smallest treatment group is 379.32±34.57 U/L in treatment group III, as can be seen in Figure 1.

In this study, an examination of LDH in the blood of rats was carried out. The results of serum LDH obtained can be seen in Table 3. Based on the table, it is known that the average serum LDH value for the normal group is still within the normal range of 551.36±5.29 U/L.

**Table 3 LDH Level**

Doses	Mean LDH ± SD (U/L)
Normal	551.36±5.29 <sup>#</sup>
Negative control	2791.36±2.05
Positif control	651.78±2.07 <sup>#</sup>
Group I	1382.01±78.06
Group II	847.46±2.80
Group III	731.21±2.05 <sup>#</sup>

Post tukey test  $p < 0.05$ ; #: has a significant different with negative group;  $p > 0.05$ ; \*: No significant different with normal



**Figure 1 CK-MB Level**

The positive control group (doxorubicin induction) had an average serum LDH of 2153.66 U/L. Treatment group I had a serum LDH value of 1245 U/L. Treatment group II had a serum LDH value of 916.33 U/L. Treatment group III had a serum value of 839.33 U/L, and a positive control group using Vitamin E as a comparison had an average serum LDH of 784 U/L.

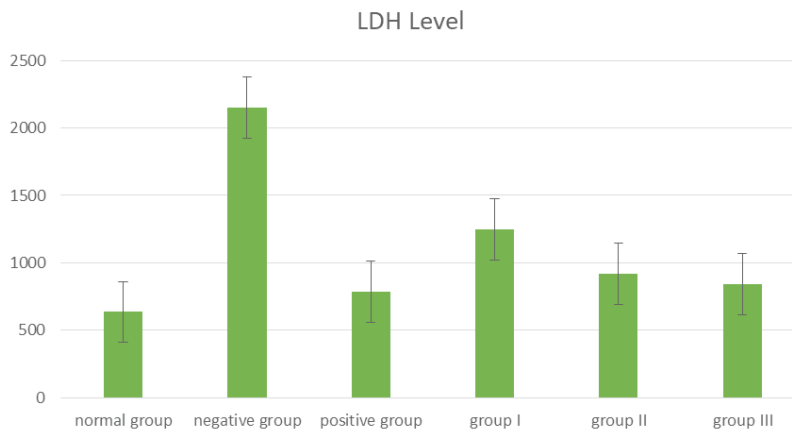
Based on the Table 3, it is known that the average serum LDH in the most extensive treatment group is 1245 U/L in treatment group I. Furthermore, the ordinary serum LDH in the smallest treatment group is 839.33 U/L in treatment group III.

**Discussion**

Based on statistical test results, serum CK-MB levels in the normal group had significant

differences ( $p < 0.05$ ) with the negative control treatment group, treatment group I, treatment group II, and treatment group II and did not have significant differences ( $p > 0.05$ ) with a positive control group with vitamin e supplementation. Serum levels of CK-MB negative control group had significant differences ( $p < 0.05$ ) with the negative control treatment group, treatment group I, treatment group II, and treatment group II, and the positive control group with vitamin e supplementation.

Serum levels of CK-MB positive control group presented significant differences ( $p < 0.05$ ) with the negative control treatment group, the normal group, and the treatment group I and insignificant difference ( $p > 0.05$ ) with the positive control group, treatment group II, and treatment group II. Serum levels of CK-MB in treatment group I had a significant difference ( $p < 0.05$ ) with the negative control group, normal group, positive control



**Figure 2 LDH**

group, treatment group I, and treatment group II. Serum levels of CK-MB treatment group II, with significant differences ( $p < 0.05$ ) with the normal group, negative group, and treatment group I, without significant difference ( $p > 0.05$ ) with a positive control group and treatment group III. Serum levels of CK-MB in treatment group III had a significant difference ( $p < 0.05$ ) with the normal group, negative group, and treatment group I, with no significant differences ( $p > 0.05$ ) with a positive control group and treatment group II.

Based on statistical test results, serum LDH levels in the normal group had a significant difference ( $p < 0.05$ ) with the negative control treatment group, treatment group I, treatment group II, and treatment group II, without significant difference ( $p > 0.05$ ) with the group positive control with vitamin e supplementation. Serum LDH levels in the negative control group had significant differences ( $p < 0.05$ ) with the negative control treatment group, treatment group I, treatment group II, and treatment group II, and the positive control group with vitamin e supplementation.

Serum LDH levels in the positive control group had significant differences ( $p < 0.05$ ) with the negative control treatment group, the normal group, and the treatment group I, without significant difference ( $p > 0.05$ ) with the positive control group, treatment group II, and the treatment group II. Serum LDH levels in the treatment group I had a significant difference ( $p < 0.05$ ) with the negative control group, normal control group, positive control group, treatment group I, and treatment group II. Serum LDH level in treatment group II had a significant difference ( $p < 0.05$ ) with the normal group, the negative group, and the treatment group I, without significant difference ( $p > 0.05$ ) with the positive control group and treatment group III. Serum LDH levels in the treatment group III had a significant difference ( $p < 0.05$ ) with the normal group, the negative group, and the treatment group I, without significant difference ( $p > 0.05$ ) with the positive control group and treatment group II. Turmeric and saffron extract was used to prevent cardiac toxicity caused by doxorubicin. This study identified the cardioprotective effect of turmeric on doxorubicin-induced cardiotoxicity in mice. Curcumin has been used in traditional medicine since it has a cardioprotective, hepatoprotective, and chemopreventive effect on various cancers. Also, it can reduce lipid levels.<sup>10</sup> The mechanism of cardiotoxicity caused by doxorubicin is known from this study. However, a large amount of evidence points towards the formation of

oxygen free radicals, which can damage cells by lipid peroxidation.<sup>11</sup> Damage to heart tissue may be caused by increased oxidative stress and the depletion of similar antioxidants in mice previously reported.<sup>12</sup>

Giving turmeric increases antioxidant levels. Thus, it prevents heart damage, mainly due to the antioxidant action of curcumin. Turmeric ethanol extract prevents doxorubicin-induced histological changes in rat heart tissue by restoring endogenous antioxidant activity or as an antioxidant or both.<sup>13</sup>

In this study, it can be concluded that cardiotoxicity caused by doxorubicin is related to oxidative stress. Anti-proliferation, anti-initiation, and scavenging properties of free radicals from turmeric can increase myocardial integrity and reduce cardiac toxicity.<sup>14</sup>

Turmeric is proven to be cardioprotective, which can be attributed to its strong antioxidant properties. This research shows that turmeric can be considered a potentially useful candidate combined with Dox to limit free radical-mediated organ injury.<sup>15</sup>

In conclusion, the ethanol extract of Curcuma has a cardioprotective effect by reducing the level of heart damage biomarkers, namely LDH and CK-MB. Curcuma was proven to be cardioprotective, which can be potentially developed as herbal medicine as additional therapy while using doxorubicin.

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