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Research Article

The Effect of Oral Andrographolide on Cardiac Biomarkers in Doxorubicin-induced Rats

Efek Andrografolida Oral terhadap Biomarker Jantung pada Tikus yang Diinduksi Doksorubisin

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Abstract

Doxorubicin remains the most prescribed anticancer agent despite its unintended side effects on non-target organs. A limiting-dose strategy is used to lower incidence of cardiotoxicity. Andrographolide has therapeutic effects including antioxidant and anti-inflammatory. This study aimed to assess cardioprotective effects of andrographolide oral on lactate dehydrogenase (LDH), creatine kinase-myocardial band (CK-MB), and relative cardiac weight in doxorubicin-induced rats. Sixteen male rats Sprague Dawley randomized into four groups: receives saline i.p and vehicle orally (Normal), doxorubicin 16 mg/kgBW i.p and vehicle orally (Dox), doxorubicin 16 mg/kgBW i.p+andrographolide 30 mg/kgBW orally (Dox+And30), doxorubicin 16 mg/kgBW i.p+andrographolide 60 mg/kgBW orally (Dox+And60). Blood was collected via cardiac puncture and cardiac organs were weighed after four-weeks administration. Total LDH and CK-MB measured spectrophotometrically. LDH and CK-MB levels significantly elevated, and signs of acute toxicity in Dox group compared with Normal group. Co-treatment with andrographolide at 30 mg/kgBW and 60 mg/kgBW reduced signs of toxicity and significantly attenuated LDH and CK-MB levels compared with Dox group ($P<0.05$ and $P<0.01$). However, body weight and relative cardiac weight were not significantly different in all groups after co-treatment with andrographolide. In conclusion, andrographolide lowered LDH and CK-MB levels, therefore has a protective potency in alleviating toxic effects of doxorubicin.

Keywords*: doxorubicin; andrographolide; LDH; CK-MB*

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Abstrak

Doksorubisin masih menjadi agen antikanker yang sering digunakan meskipun memberikan efek samping terhadap organ nontarget. Strategi pembatasan dosis digunakan untuk menurunkan insidensi kardiotoksisitas. Andrografolida memiliki berbagai efek terapi seperti antioksidan dan anti-inflamasi. Studi ini bertujuan untuk melihat efek kardioprotektif andrografolida secara oral terhadap laktat dehidrogenase (LDH), kreatin kinase-MB (CK-MB), dan berat jantung relatif pada tikus yang diinduksi doksorubisin. Enam belas ekor tikus Sprague Dawley jantan diacak menjadi empat kelompok: yang menerima saline i.p dan pembawa secara oral (Normal), doksorubisin 16 mg/kgBB i.p dan pembawa secara oral (Dox), doksorubisin 16 mg/kgBB i.p+andrografolida 30 mg/kgBB secara oral (Dox+And30), doksorubisin 16 mg/kgBB i.p+andrografolida 60 mg/kgBB secara oral (Dox+And60). Darah diambil melalui tusuk jantung dan organ jantung ditimbang setelah empat minggu pemberian. Total LDH dan CK-MB diukur secara spektrofotometri. Kadar LDH dan CK-MB meningkat secara signifikan dan tanda-tanda toksisitas akut pada kelompok Dox dibandingkan kelompok Normal. Pemberian bersama andrografolida pada 30 mg/kgBB dan 60 mg/kgBB mengurangi tanda-tanda toksisitas dan secara signifikan menurunkan kadar LDH dan CK-MB dibandingkan kelompok Dox ($P<0.05$ dan $P<0.01$). Namun, berat badan dan berat jantung relatif tidak berbeda secara signifikan pada semua kelompok setelah pemberian andrografolida. Simpulan, andrografolida menurunkan kadar LDH dan CK-MB, sehingga memiliki potensi protektif mengurangi efek toksik doksorubisin.

Kata kunci: doksorubisin; andrografolida; LDH; CK-MB

Introduction

Cancer is a leading cause of morbidity and mortality worldwide. It is estimated that the number of cancer cases is set to increase by 69% to 21 million and deaths due to cancer is expected to rise by 72% to 13 million in 2030.[1] Doxorubicin, an anthracycline antibiotic, is a broad-spectrum cytotoxic agent used to treat several cancer including solid tumors, leukemia, and lymphomas.[2] A limiting-dose strategy is enforced in its clinical use because of adverse events and its unintended side effect on non-target organs, particularly cardiotoxicity. The cumulative dose of doxorubicin for adults is 400-700 mg/m^2 and for children is 200 mg/m^2. Anthracycline's dose of up to 450 mg/m^2 could enhance cardiotoxicity incidence.[3] Dexrazoxane is the only FDA-approved cardioprotective agent against DOX-induced cardiotoxicity. It has been used successfully in children with various solid and hematologic cancers to minimize cardiotoxicity. Meanwhile, the quality of available evidence from the most recent clinical trial of dexrazoxane as a cardioprotective agent in adults is low.[4-5] Therefore, there is an urgent need to find a better cardioprotective agent against doxorubicin cardiotoxicity.

Cardiomyocytes are known to be more susceptible to oxidative damage than other cells due to their low antioxidant defense capacity and high density of mitochondria as the main source of reactive oxygen species (ROS) formation. Several studies have shown that oxidative stress, inflammation, and apoptosis are related to doxorubicin-induced cardiotoxicity.[6] There are several proposed molecular mechanisms related to doxorubicin-induced cardiotoxicity including calcium

dysregulation and free radicals causing oxidative stress, mitochondrial dysregulation, autophagy dysregulation, and apoptosis.[7] In addition, nuclear factor kappa B (NF-κB) is one of the main transduction signaling pathways and can either promote or prevent cell death when activated. Doxorubicin activates NF-κB through the formation of intracellular hydrogen peroxide (H_2O_2) and promotes cell death.[8] Thus far, a new strategy is needed to alleviate the toxic effects of doxorubicin to increase its therapeutic effects in chemotherapy. Currently, several studies focus on pharmacology agents such as iron chelator, antioxidant, and anti-inflammatory to ameliorate the side effects of doxorubicin.[9,10]

Andrographolide is one of the major compounds isolated from the *Andrographis paniculate* plant. Extraction of sambiloto leaves using solvent (ethanol:water) is reported to contain 8-10% andrographolide. This labdane diterpenoid is reported to exert various effects including antibacterial, antioxidant, and anti-inflammatory activities.[11-13] In another study, andrographolide administration inhibited the progression of myocarditis through NF-κB and interleukin 6/signal transducer and activator of transcription 3 (IL-6/STAT3) signaling pathway, protecting against oxidative damage through modulating gene transcription and activity of the antioxidant enzyme in various tissues.[14-15] In research using in vivo model, andrographolide ameliorated oxidative stress through upregulated mechanism towards superoxide dismutase (SOD) expression therefore significantly reducing ROS formation.[12] Andrographolide at 25 mg/kg dose is also reported to relieve cardiac dysfunction.[16]

Common parameters used to assess cardiac muscle toxicity are LDH and CK-MB. Lactate dehydrogenase is an enzyme that plays a role in catalyzing the conversion of pyruvate to lactate under anaerobic conditions which is important during periods of high muscle activity. Meanwhile, CK is an enzyme that catalyzes phosphorylation in tissues, especially in skeletal and cardiac muscles.[17] The myocardium has 15% CK-MB isoenzymes, and 85% CK-MM. Creatine Kinase-MB is usually not detected in the blood but when there is damage to the myocardium, the CK-MB value increases. In a study using rats given doxorubicin, there was an increase in LDH and CK-MB which indicated damage to the myocardium.[18] This present study aimed to assess the effects of oral andrographolide administration on the level of cardiac biomarkers, changes in signs of toxicity, and relative cardiac weight to evaluate its potency in alleviating the toxic effects of doxorubicin.

Methods

This present study was conducted under an experimental approach, in vivo, using rats. Male Sprague Dawley, 8 weeks, weighed approximately 100 grams was obtained from Badan

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Pengawasan Obat dan Makanan (BPOM) RI. This study was conducted in *Animal Research Facilities* (ARF) IMERI and Pharmacokinetics Laboratory Pharmacology and Therapeutics Departement, Fakultas Kedokteran Universitas Indonesia (FKUI). This study has obtained approval of ethical review from FKUI's Ethics Committee (KET-1429/UN2.F1/ETIK/PPM.0002/2020).

Sprague Dawley rats were acclimatized for 2 weeks with access to food and water *ad libitum* as well as controlled laboratory condition (temperature, humidity, 12-hour light-dark cycle). Andrographolide active compound with a purity of 90% (Plamed, Xi'an, China) was dissolved with vehicle (carboxymethyl cellulose 1%). Sixteen Sprague Dawley rats were randomized into four group (n=4) which receives saline i.p and vehicle orally (Normal), doxorubicin 16 mg/kgBW i.p and vehicle orally (Dox), doxorubicin 16 mg/kgBW i.p + andrographolide 30 mg/kgBW orally (Dox+And30), doxorubicin 16 mg/kgBW i.p + andrographolide 60 mg/kgBW orally (Dox+And60). Doxorubicin was administered every first day of the week intraperitoneally and andrographolide was administered orally (0,5-1 ml) every day (doxorubicin's day injection excluded). Rat's body weight was recorded daily and characteristics of toxicity such as decreased activity, shortness of breath, nose blood discharge, and ascites were observed. In the fourth week, rats were terminated, the blood sample was collected through the cardiac puncture method for cardiac biomarker parameters, and cardiac organs were weighed.

Total LDH and CK-MB activities were determined spectrophotometrically using commercial kits (DiaSys Diagnostic System GmbH). Total LDH activity (U/L) was dependent on the reaction of lactate with NAD and the NADPH formed was measured spectrophotometrically at 340 nm. The increase in absorbance was measured at 1-minute intervals for 3 minutes. Total CK-MB activity (U/L) was measured in the presence of an antibody against CK-M monomers that inhibited half of the activity of CK-MM and CK-MB without affecting CK-MB. Creatine Kinase-MB activity values were derived based on the absorbance of NADPH at 340 nm. The increase in absorbance was measured at 1-minute intervals for 5 minutes.[18]

Analyzed data were expressed as mean ± standard error of the mean (SEM). Graphic data were analyzed using GraphPad Prism version 9.4.0 software. Statistical analyses were conducted using IBM SPSS Statistics 22 software. The tests consisted of normality, homogeneity, one-way analysis of variance (ANOVA) and, Post-Hoc (LSD or Tukey HSD). Signification differences were proved if the p-value is less than 0.005 and 0.001.

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Results

The body weight, characteristics of toxicity, and mortality were recorded daily throughout the experimental period. No death was recorded in each group. Despite there being no significant difference in body weight among the four groups, a trend of weight loss occurred in the Dox group and Dox+And60. The group which only received doxorubicin treatment showed characteristics of toxicity such as decreased activity, shortness of breath, nose blood discharge, and ascites. Dox group was compared with Dox+And30, it showed that the group which only received doxorubicin was found to develop more ascites as determined and confirmed after necropsy. However, table 1 suggested that there were no significant differences in relative cardiac weight and body weight among the four groups.

Cardiac biomarkers such as LDH and CK-MB were analyzed to assess parameters for cardiac toxicity. As shown in Fig.1 and Fig.2, there were elevations in LDH and CK-MB levels in the Dox group compared to Normal group ($P<0.05$ and $P<0.01$). Fig.1 showed that elevated LDH levels were significantly reduced after andrographolide administration at 30 mg/kgBW ($P<0.05$) and 60 mg/kgBW ($P<0.01$). Meanwhile, CK-MB levels also significantly reduced after the administration of andrographolide at 30 mg/kgBW and 60 mg/kgBW doses ($P<0.01$). The results of this present study suggested that oral andrographolide administration may ameliorate the cardiotoxic effect of doxorubicin in a doxorubicin-induced rat.

Table 1 Body Weight and Relative Cardiac Weight of Rats (Mean ± SEM)

	Groups			
Parameter	Normal	Dox	Dox+And30	Dox+And60
Body weight (g)	324 ± 3,56	302,5 ± 5,38	325 ± 6,61	297 ± 15,97
Cardiac weight (mg)	980 ± 42,23	965 ± 21,02	990 ± 45,28	865 ± 47,35
Cardiac weight:body weight ratio (mg/g)	3,02 ± 0,11	3,19 ± 0,08	3,05 ± 0,14	2,91 ± 0,03

Notes: Normal: control group; Dox: doxorubicin group; Dox+And30: DOX+andrographolide 30 mg/kgBB; Dox+And60: DOX+andrographolide 60 mg/kgBB

$P<0.05$

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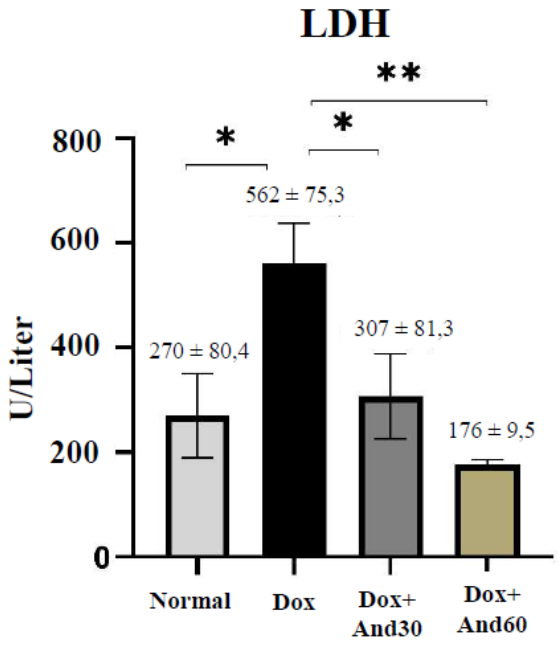

Figure 1 Analysis of Lactate Dehydrogenase Level

Notes: Normal: control group; Dox: doxorubicin group; Dox+And30: DOX+andrographolide 30 mg/kgBB; Dox+And60: DOX+andrographolide 60 mg/kgBB
*: Significally different (P<0.05) compared with doxorubicin group
**: Significally different (P<0.01) compared with doxorubicin group

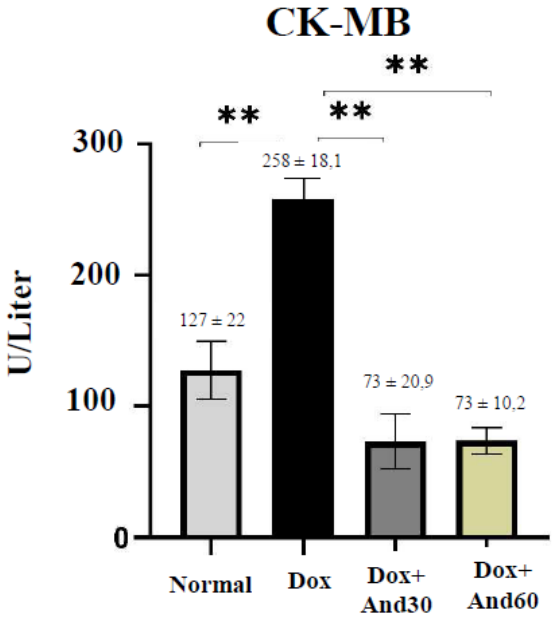

Figure 2 Analysis of Creatine Kinase-MB Level

Notes: Normal: control group; Dox: doxorubicin group; Dox+And30: DOX+andrographolide 30 mg/kgBB; Dox+And60: DOX+andrographolide 60 mg/kgBB
**: Significally different (P<0.01) compared with doxorubicin group

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Discussion

The use of doxorubicin is often accompanied by side effects such as hematopoiesis suppression. Increased ROS production and subsequent decrease in endogenous antioxidants may trigger intrinsic apoptotic pathways in hematopoietic cells and induce platelet cytotoxicity. Furthermore, due to its effects on key factors of lipid and glucose metabolism, doxorubicin may also induce weight loss.[19] The present study showed that a total dose of 16 mg/kgBW of doxorubicin showed a trend of weight loss. Meanwhile, there was no significant difference in the relative cardiac weight in all groups. The characteristics of toxicity such as shortness of breath, nose blood discharge, and ascites were seen in the Dox group. Meanwhile, only nose blood discharge was detected in the Dox+And30 and Dox+And60 groups. Based on an analysis of general signs of toxicity, the administration of andrographolide can reduce symptoms of acute toxicity caused by doxorubicin.

Cardiomyocytes are known to be susceptible to oxidative damage due to their low antioxidant defense capacity. The main structure of doxorubicin is prone to redox cycling and can interact with oxygen to form radical compounds. The main targets of destructive free radicals induced by anthracyclines are cell membranes, which are composed of lipids and susceptible to oxidation.[20,21] Elevation of CK-MB and LDH levels in circulation was one of the characteristics of cardiotoxicity. It indicated the occurrence of damage or injury in cardiac cells due to excessive production of free radicals and lipid peroxidation. Damaged cell membranes caused leakage of cytosolic enzymes.[22-23] CK-MB and LDH enzyme levels were also attenuated in the Dox group which receives a total dose of doxorubicin at 16 mg/kgBW compared with the Normal group. Our results were in accordance with other study using cumulative doses of doxorubicin at 17,5 mg/kg i.p. which significantly increased the level of CK-MB and LDH activities.[24]

Oral administration of andrographolide at 30 mg/kgBW ($P<0.05$) and 60 mg/kgBW ($P<0.01$) significantly reduced LDH level compared with Dox group. The CK-MB level also attenuated after oral administration of andrographolide at 30 mg/kgBW and 60 mg/kgBW ($P<0.01$). Lactate dehydrogenase (LDH) is an enzyme that played an essential role in catalyzing the conversion of pyruvate into lactate under an anaerobic condition which important during a period that requires high muscle activity.[17] In another study using mice showed that administration of andrographolide reduced the level of serum LDH in a liver damage model through reducing hepatic oxidative stress and antioxidative defense mechanism.[25] Creatine kinase (CK), an enzyme that catalyzed the phosphorylation in tissue, is mainly found in skeletal and cardiac muscle. In the myocardium alone, there were 15% of isoenzyme CK-MB.[18] Decreased serum CK levels were

also found in a muscular dystrophy model after andrographolide administration. Andrographolide prevented muscle damage and fibrosis progression.[26]

In a study with an isoproterenol-induced myocardial infarction model, it was shown that andrographolide reduces cardiac dysfunction and oxidative damage through free radical scavenging activity. Andrographolide has free radical scavenging activity through the mechanism of donating allylic hydrogen from an unsaturated lactone ring and followed by displacement of hydrogen from the constituent carbon atoms.[27] In addition, a study conducted that received pre-treatment with andrographolide can increase cellular glutathione (GSH) activity. The glutathione antioxidant system is essential for the defense against ROS in the heart. GSH also plays a role in maintaining thiol, tocopherol, and ascorbic groups in cells.[28] Andrographolide also exerted antioxidant activity through its ability in inhibiting oxidative stress biomarker such as 8-hydroxy-2-deoxyguanosine (8OHDG) and increase expression of the antioxidant enzyme such as catalase, SOD, glutathione peroxidase (GPx), and glutathione reductase (GR).[29] Based on the results of examining cardiotoxicity parameters using cardiac biomarkers, it can be seen that andrographolide has the ability to protect the cardiac organ through antioxidant activity.

The anti-inflammatory activity of andrographolide was demonstrated through its role in NF-κB signaling pathway mechanism. Nuclear factor kappa B (NF-κB) as a transcription factor has a role in various expressions of pro-inflammatory genes.[30] Protein pro-inflammatory formation is prevented by forming a covalent adduct at p50 NF-κB, therefore, lowering transcriptase activity.[31] NF-κB also suppressed ROS accumulation as its protective effect during oxidative stress conditions.[32] Decreased level of cardiac biomarkers LDH and CK-MB in this study is possibly related to the protective effect of andrographolide in the form of anti-inflammatory.

The limitation of this study was the sample size, so that further research were needed to improve the reliability of the data. This study confirmed that oral co-administration of andrographolide with doxorubicin can prevent the elevation of cardiac biomarkers. Further research might focus on the exact mechanism of action in andrographolide as cardioprotective through the NF-κB pathway, anti-inflammatory pathway, and expression of related genes from cardiomyocytes in a rat model of cardiotoxicity.

Conclusion

Oral administration of andrographolide reduced signs of toxicity; however, body weight and relative cardiac weight were not significantly different in all groups. Oral administration of

andrographolide at 30 mg/kgBW and 60 mg/kgBW doses attenuated LDH and CK-MB levels in doxorubicin-induced rats.

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