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INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS AND CARDIOPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF BEETROOT (*Beta vulgaris.* L) ON DOXORUBICIN INDUCED TOXICITY IN RAT

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ABSTRACT
Doxorubicin is used extensively to treat cancer. Oxidative stress condition is closely related to doxorubicin therapy, which is characterized by the formation of lipid peroxidation which has the potential to cause cell damage and induced cardiotoxicity. This study was to determine the cardioprotective effect of beetroot ethanol extract on a rat induced by doxorubicin. As many as 30 Male white rats as weighing 180-200 grams were divided into six treatment groups. Group, I was the normal control group without treatment. Group II was a negative control group that only injected doxorubicin with a cumulative dose of 15 mg/kgBW for 15 days. Group III was the positive control group that treated by vitamin E 100 mg/kgBW, Group IV-VI was the test group that treated by beetroot ethanol extract at doses of 100, 200 and 400 mg/kgBW respectively for 15 days together with doxorubicin induction. The group treated with beetroot extract at a dose of 100 mg/kgBW could improve the condition of rat in all parameters including Creatine kinase-MB (CK-MB) level, Lactate Dehydrogenase (LDH) level and hematology profile and it was significantly different to the negative control ($p < 0.05$). All parameters showed that the beetroot extract had a cardioprotective and it showed a dose-dependent manner. This research proves that the compound contained in beetroot ethanol extract has protective activity in rats that induced by doxorubicin.
Keywords: Doxorubicin, Beetroot, Cardioprotective, CK-MB, LDH, Hematology.

INTRODUCTION

Doxorubicin is anticancer that widely used as chemotherapy in cancer therapy. Doxorubicin is a cytotoxic anthracycline compound that was an isolated compound from Streptomyces peucetius var. Caesius[1]. Conditions of oxidative stress are closely related to doxorubicin therapy, which is characterized by the formation of lipid peroxidation which has the potential to cause cell damage[2]. Doxorubicin toxicity events can cause cell damage to organs such as the heart, kidneys and liver[3].

The chemical structure of doxorubicin has the potential to form free radicals through various mechanisms. Quinone groups in the tetracyclic doxorubicin ring can release several superoxide radicals[4]. Doxorubicin metabolism also causes the formation of reactive aglycone metabolites and alcohol metabolites (doxorubicin-o-rubisinol), which can disturb the balance of Fe intracellular concentrations[5]. The administration of doxorubicin can increase the production of reactive oxygen species (ROS) and disrupt the balance of antioxidant defenses so that it could trigger a cell damage[6]. ROS is normally produced in the body by metabolic processes, to obstruct ROS, the body has a defense system against ROS by producing antioxidant enzymes such as superoxide dismutase, catalase, glutatione and others[7]. Excessive production of free radicals and reduced antioxidant defense systems will cause a cell damage.

There were many studies that prove the toxicity of doxorubicin to organs especially the heart organ[8-11]. It is necessary to have antioxidant supplementation to ward off free radicals from doxorubicin. One of the plants that have high antioxidant is one of beetroot (*Beta vulgaris* L.)[12]. Beetroot contains phytochemical

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compounds such as terpenoids, alkaloids, flavonoids, steroids, tannins and saponins, and also betanin content which have high antioxidant effects [13] . Based on the description mentioned, this study was aimed to finding out the cardioprotective effects of beetroot ethanol extract (Beta vulgaris L.) in doxorubicin-induced toxicity in rat.

EXPERIMENTAL

Materials

Distilled water, beetroot, white male rats, ethanol 96% (Merck), CMC-Na (Bratachem), vitamin e (Genero) and doxorubicin (Kalbe).

Plant Collection and Extraction of Beetroot

The plant samples were collected from a local market at the district of PB Selayang II, North Sumatera, Indonesia. The beetroot samples were authenticated by the Indonesian Institute of Science, Research Center of Biology, Bogor, Indonesia. An amount of 300 g dried beetroot was crushed then macerated in ethanol 96 % for 5 days. Results evaporated with a rotary evaporator at a temperature of ± 50 ℃, then dried on a water bath.[14]

Investigation of Phytochemical Constituents of Ethanol Extract of Beetroot

Phytochemical constituents investigation were carried out on beetroot extract includes examining the chemical secondary metabolites such as saponins, flavonoids, tannins, alkaloids, glycosides, steroids and terpenoids.[15-17]

Animals and Blood Sample

The 30 male Wistar rats weighing 180-220 g were used in this study. The blood samples were collected from sinus retro orbital.

Treatment Regime

Thirty rats were divided into 6 groups and each group consisted of 5 mice, namely:

i. **Normal Group:** Animal groups without treatment
ii. **Negative Control:** Rats were induced by an accumulative dose of doxorubicin 15 mg/kgBW + Na-CMC suspension
iii. **Positive Control:** Rats were induced by accumulative dose of doxorubicin 15 mg/kgBW + suspension of Vitamin E 100 mg / kgBW
iv. **Dose 100:** Rats were induced by accumulative dose of doxorubicin 15 mg/kgBW + extract dose of 100 mg / kgBW.
v. **Dose 200:** Rats were induced by accumulative dose of doxorubicin 15 mg/kgBW + extract dose of 200 mg / kgBW
vi. **Dose 400:** Rats induced by accumulative dose of doxorubicin 15 mg/kgBW + extract dose of 400 mg /kgBW

Rats were induced by doxorubicin with an accumulative dose of 15 mg/kgBW for 15 days by giving doxorubicin 5 times in 1 week with a dose of 1 mg/kgBW intraperitoneally[18] and also simultaneously given beetroot extract for 15 days. On day 16th, the animals were anesthetized and blood samples were collected and determined the (CK-MB) level, Lactate Dehydrogenase (LDH) level, and hematology profile.

Statistical Analysis

Analysis of the study was performed by ANOVA with Tukey's Multiple Comparison Test. The significance *P* values were set at 0.05. The values for all measurements are expressed as the mean ± SD.

RESULTS AND DISCUSSION

Phytochemical Constituent Result of Ethanol Extract Beetroot

Phytochemical screening results showed that ethanol extract beetroot positively contains flavonoids, alkaloids, saponins, tanins, glycosides and steroids/terpenoids (Table-1).

Table-1: Phytochemical Screening Result

No.	Content	Dried Sample	Extract
1.	Flavonoids	+	+
2.	Alkaloids	+	+
3.	Saponins	+	+
4.	Tanins	+	+
5.	Glycosides	+	+
6.	Steroids/Terpenoid	+	+

Effect of Beetroot Ethanol Extract on CK-MB, LDH and Hematology Profile

Beet root showed cardioprotective activity on CK-MB and LDH levels in doxorubicin-induced toxicity. Tables-2 and 3 shows that treatment at a lower dose of 100 mg/kgBW was significantly different from negative control ($p<0.05$). Treatment of beetroot ethanol extract showed improvement in most of the hematological parameters compared to doxorubicin-induced group (Table-4).

Table-2: Effect of Beetroot Treatment on CK-MB Level

Groups	Mean CK-MB Levels (U/L) ± SD
Normal Control	660.75± 40.62*
Negative Control	1190.5± 41.2
Vitamin E Control	608.5± 47.42*
Beetroot extract at Dose of 100 mg/kgBW	1101.75± 45.93*
Beetroot extract at Dose of 200 mg/kgBW	840.5± 41.27*
Beetroot extract at Dose of 400 mg/kgBW	796.5± 52.61*

Data expressed as mean ± SD ($n = 6$)
*Significant Difference Vs. Negative Control Group at $p < 0.01$

Table-3: Effect of Beetroot Treatment on LDH Level

Groups	Mean LDH Levels (U/L) ± SD
Normal Control	1453± 189.27*
Negative Control	4811± 785.38
Vitamin E Control	2502.2± 138.87*
Beetroot extract at Dose of 100 mg/kgBW	3493.6± 169.85*
Beetroot extract at Dose of 200 mg/kgBW	2665.2± 99.03*
Beetroot extract at Dose of 400 mg/kgBW	2451±148.73*

Data expressed as mean ± SD ($n = 6$)
*Significant Difference Vs. Negative Control Group at $p < 0.01$

Beetroot extract (100 mg/kg) co-treated rats showed significant ($p < 0.01$) improvement in most of the hematological parameters compared to doxorubicin-induced group These observations were confirmed by an increased level of WBC, RBCs, HGB, HCT, MCV, MCH, and platelets counts (Table-4).

Table-4: Effect of Beetroot Extract Treatment on Hematology Profile.

Parameters	Groups (Mean Value ± SD)					
	Normal Control	Negative Control	Vitamin E Control	Beetroot extract (100 mg/kgBW)	Beetroot extract (100 mg/kgBW)	Beetroot extract (100 mg/kgBW)
WBC (10^3/uL)	6 ± 2.07*	1.64 ± 0.39	4.98 ± 2.23*	3.54 ± 2.53*	4.12 ± 2.40*	4.24 ± 0.34*
RBC (10^6/uL)	11.032 ± 1.63*	5.1 ± 0.36	8.764 ± 1.54*	8.162 ± 0.81**	8.468 ± 0.57*	8.582 ± 0.58*
HGB (g/dL)	12.36 ± 1.91*	9.14 ± 0.60	13.47 ± 2.23*	11.34 ± 1.27*	13.08 ± 0.46*	14.26 ± 1.31*
HCT (%)	51.6 ± 4.50*	31.14 ± 1.90	49.47 ± 8.79*	42.18 ± 3.13*	45.2 ± 3.68*	47.15 ± 2.18*
MCV (fL)	48.23 ± 1.44*	39.2 ± 3.27	45.65 ± 3.47*	43.16 ± 1.96*	45.16 ± 1.23*	45.95 ± 1.41*

MCH (pg)	13.54 ± 0.69*	10.54 ± 1.29	13.58 ± 0.54*	13.24 ± 0.15*	14.17 ± 0.35*	14.12 ± 0.31*
MCHC (g/dL)	32.23 ± 1.25*	24.93 ± 2.16	26.32 ± 0.81	24.22 ± 1.16	26.18 ± 1.86	26.9 ± 0.99*
PLT (10^3/uL)	1112.2 ± 133.22*	594.4 ± 14.37	1004.6 ± 132.84*	863 ± 103.18*	9033.4 ± 118.18*	9095.6 ± 39.07*
NEU (%)	8.6 ± 4.47*	35.6 ± 4.13	7.4 ± 4.72*	10 ± 11.55*	7.8 ± 2.13*	7.5 ± 1.58*
LYMP (%)	76.3 ± 7.36*	45.4 ± 3.47	72.1 ± 1.77*	55.8 ± 11.12*	63.5 ± 3.19*	71 ± 3.19*
MONO (%)	6.8 ± 4.17	5.2 ± 0.83	7.8 ± 7.13*	6 ± 7.03	6.8 ± 1.48	6 ± 1.13
EOS (%)	0.4 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.2	1.1 ± 0.1
BAS (%)	8.7 ± 3.78	9 ± 2.23	9.3 ± 7.76	14.8 ± 3.34	12 ± 3.46	11.2 ± 1.82

Data expressed as mean ± SD ($n = 6$)
*Significant Difference Vs. Negative Control Group at $p < 0.01$

Creatinine kinase (CK) is an enzyme that is found in heart muscle and skeletal muscle damage. Creatinine kinase is a dimeric molecule consisting of a pair of different monomers namely MM, MB, and BB[19]. CK-MB is a heart muscle that can increase if there is cell damage in heart muscle[20]. Lactate dehydrogenase (LDH) is an intracellular enzyme found in almost all metabolized cells. LDH with the highest concentration is found in the heart, skeletal muscle, liver, kidney, brain, and blood cells. The increase of LDH level in serum suggests an increased oxygen demand[21]. Other conditions that can cause increased LDH serum include hypoxia, tissue injury, and necrosis[22-23]. These experimental results also showed a toxic effect of doxorubicin in hematology parameters.

Many studies have been reported the role of medicinal plants in inhibiting the cardiotoxic effect[24-26]. The antioxidant activity might play a role in the cardioprotective effect by inhibiting ROS. Beetroot contains specific compounds namely betanin which is a red pigment in beetroot, beetroot also contains some phytochemical compound in the form of tannins saponins, alkaloids, flavonoids, terpenoids and steroids. Betanin has the antioxidant activity[27]. Betanin is a heterocyclic compound found in beetroot (Beta vulgaris L.), is showed its red-violet pigment[28,29]. The antioxidant activity of betanin is in the presence of cyclic amine groups and hydroxyl, which are hydrogen and electron donors, with the efficacy to stabilize the reactive species[30]. This shows that betanin is a powerful antioxidant that can prevent ROS (Reactive oxygen species) free radical reactions caused by doxorubicin interactions which can cause lipid profile abnormality. The content of other antioxidants such as flavonoids and tannin which is a group of polyphenol also plays a role in improving the condition of rats which were induced by doxorubicin. Flavonoids can reduce CK-MB and LDH reduce the oxidative stress of macrophages by inhibiting cellularly oxygenated and activating cellular antioxidants[31]. Thus, flavonoids and tannins are natural antioxidants that can protect against Reactive oxygen species.

CONCLUSION

Beetroot ethanol extract has cardioprotective activity in rats that are induced by doxorubicin and it showed dose-dependent.

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