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Amelioration of doxorubicin induced toxicity in rat by *Beta vulgaris* L. extract supplementation: haematology and lipid profile evaluation

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Abstract

Doxorubicin can cause disruption in the lipid profile due to the free radical process. In a free radical reaction, lipid peroxidation is the introduction of a functional group comprising two catenated oxygen atoms into unsaturated fatty acids. The lipid profile is a risk factor for coronary artery disease. Antioxidants play an important role in preventing ROS (Reactive oxygen species) free radical reactions caused by doxorubicin interaction. The purpose of the study was to investigate the possible protective effects of ethanol extract of *Beta vulgaris* L. on doxorubicin-induced toxicity by analysing the haematological and lipid profiles. Extracts were determined for their phytochemical content and antioxidant activity then continue to in vivo study using rats as animal models. Rats were administered doxorubicin at an accumulative dose of 15 mg per kilogram of body weight (kg BW) for 15 days. Doxorubicin was administered 5 times a week with an intraperitoneal dose of 1 mg/kg BW, and *B. vulgaris* L. extract at a dose of 12.5 mg, 25 mg, 50 mg and 100 mg /kg BW, each of which was given for 15 days. On the 16th day, the animals were anaesthetized, and blood samples were collected for the determination of the haematological profile and lipid profile. The result of antioxidant activity showed that the IC_{50} value of *B. vulgaris* L. extract was 37.366 µg/mL. All doses of treatment showed protective activity against doxorubicin-induced toxicity, at a dose of 50 mg/kg BW is the minimum dose that provides significant protection compared to the negative control group ($p<0.05$) in all parameters. It is concluded that *B. vulgaris* L. ethanol extract has protective effects in rats against doxorubicin-induced toxicity.

1. Introduction

Doxorubicin is anthracycline antibiotic group that frequently used in chemotherapy to treat a variety of malignancies, including breast cancer, lymphoma, and leukaemia (Sohail *et al*., 2021). Typical adverse effects of doxorubicin include low blood cell counts, mouth ulcers, hair loss, fatigue, nausea, and vomiting. Cardiotoxicity, which can result in heart failure, arrhythmias, and other cardiac complications, is an additional adverse effect of doxorubicin (Basak *et al.,* 2021). Moreover, doxorubicin can cause disruption in the lipid profile due to the free radical process leading to dyslipidaemia and an increased risk of cardiovascular disease (Subashini *et al*., 2007). Lipids are fats and fatty compounds that are present in the body and include phospholipids, cholesterol, and triglycerides. These lipids are vital parts of cell membranes and are essential for many physiological activities, such as hormone production, energy storage, and cell signalling (Emami *et al.,* 2020).

Changes in the lipid profile has the potential to cause a number of metabolic problems and health issues. Free radicals, which are very reactive and unstable molecules that may disrupt cell membranes and other biomolecules, including lipids, are known to be induced by doxorubicin. Increased cholesterol levels play a role in the production of free radicals accelerated by oxidative stress reactions (Niki, 2008; Nathan and Cunningham-Bussel, 2013). Lipid peroxidation, which occurs when free radicals attack lipids and produce lipid peroxides as a result. Moreover, oxidative stress reactions cause biological macromolecular damage including oxidation of low-density lipoprotein (oxidized-LDL), triglycerides, endothelial dysfunction, and increased inflammatory

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response that begins with the oxidation of unsaturated fatty acids in the lipid layer of the cell membrane (Fereidoon and Ying, 2010). This reaction initiates the occurrence of chain lipid oxidation that causes damage to cell membranes (Logani *et al*., 1983). Antioxidants play an important role in preventing ROS (Reactive oxygen species) free radical reactions caused by doxorubicin interactions.

Antioxidants are compounds that have the ability to inhibit or prevent the process of lipid oxidation (Logani *et al*., 1983). When lipid oxidation occurs, cholesterol easily passes through the artery walls and blocks it. Antioxidants stabilize free radicals by complementing the lack of electrons possessed by free radicals and inhibit the chain reaction of free radical formation. Free radicals are one of the processes that can cause cell damage and death (Nimse *et al*., 2015). Furthermore, the antioxidant defense system of the body, which typically guards the cells from the damaging effects of free radicals, can be disrupted by doxorubicin. Free radicals can be neutralized by antioxidants like glutathione, vitamin E, and various phytochemical constituent that also stop the oxidation of lipids.

Beta vulgaris L. contains phytochemical constituent such as tannin saponins, alkaloids, flavonoids, terpenoids, and steroids as well as betanin which have antioxidant effects (Ninfali and Angelino, 2013). The experimental animal model can be developed in rats by administering high dose Doxorubicin injection. The relatively high frequency of doxorubicin administration can induce significant toxicity and adverse effects in animal models (Osataphan *et al.,* 2020). In animal models, common doxorubicin side effects include injury to the heart, liver, and kidneys, suppression of the immune system, and alterations to the lipid profile. The primary goal of this study was to examine the phytochemical constituent of beetroot extract, antioxidant properties and protective effects of *Beta vulgaris* L. extract on doxorubicin-induced toxicity in rats by examine the hematology and lipid profile.

2. Materials and methods

2.1 Materials

Ethanol 96% (Merck), Methanol (Merck), Doxorubicin (Kalbe), Sodium Carboxymethyl cellulose (Bratachem), Vitamin E (Genero) and distilled water, 1,1 -diphenyl-2-picrylhydrazyl (DPPH) (Sigma), *Beta vulgaris* L. tuber, male Wistar rats

2.2 Plant collection and extraction of Beta vulgaris L. tuber

Beta vulgaris L. tuber was collected from the local market at Padang Bulan, Sumatera Utara, Indonesia on March 2020. Identification was carried out at Herbarium Medanesse, the University of Sumatera Utara with an identification number: 1194/MEDA/2020. The *B. vulgaris* L. (300 g) was dried, crushed and macerated in 5 L ethanol 96% for 5 days. The filtrate was evaporated in a rotary evaporator at 50^{o}C and then dried in a water bath at 50^{o}C (Departemen Kesehatan Republik Indonesia, 1995).

2.3 Phytochemical screening of ethanol extract

The extract was screened for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids using the standard procedure for qualitative determination (Cooper-Driver and Harborne, 1974; Farnsworth, 1966).

2.4 Determination of DPPH radical scavenging activity

An aliquot of 1 mL of each concentration (100, 50, 25, 22.5, and 6.5 g/mL) of the test sample was added to 1 mL of DPPH solution. After that, 3 mL of methanol was added. It was shaken until homogenous, and then incubated for 30 mins in a dark environment. Then the absorbance was measured using a UV-Vis spectrophotometer at 513 nm. The value of antioxidant activity (IC_{50}) is calculated based on the linear regression equation between the percentage of inhibition and sample concentration or fraction, where the x-axis is the concentration while the y-axis is the percentage of inhibition. The regression equation $y = bx + a$ is obtained. Then the y value is replaced with 50. Where IC_{50} is defined as the concentration of the sample needed to inhibit 50% of the DPPH radical (Bobo-García *et al*., 2015).

2.5 Animals and blood sample

Animals used in the study were thirty-five male Wistar rats (10-12 weeks old), with a sample weight of 180-220 g 180-220 g. The rats and pellet diet were obtained from Rat Breeding Centre, Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Rats were housed under a standard room temperature environment with a constant relative humidity under 12-hr light/dark cycles. The animals were fed a standard laboratory of pellet diet with tap water. Acclimatization of tested animals was carried out for 1 week prior to the study. The blood sample was taken from a cardiac puncture. The study has been approved by Animal Research Ethics Committees (AREC) Universitas Sumatera Utara with approval number 0214/KEPH-FMIPA/2020.

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2.6 In vivo study procedure

Rats were used in the treatment of the 35 Rats were divided into 7 groups and each group consisted of 5 Rats. The division of the treatment group is as follows:

I. Normal Group: groups without any treatment.

II. Negative control: Animals administered doxorubicin at a dose of 15 mg/kg BW without extract treatment.

III. Positive control: Animals administered doxorubicin at a dose of 15 mg/kg BW + suspension of Vitamin E (100 mg/kg BW).

IV. Extract: Animals administered doxorubicin at a dose of 15 mg/kg BW + extract (100 mg/kg BW).

V. Extract: Animals induced by doxorubicin at a dose of 15 mg/kg BW + extract (50 mg/kg BW).

VI. Extract: Animals administered doxorubicin at a dose of 15 mg/kg BW + extract (25 mg /kg BW).

VII. Extract: Animals induced by doxorubicin at a dose of 15 mg/kg BW + extract (12.5 mg/kg BW).

Rats were administered doxorubicin at a dose of 15 mg/kg BW accumulatively for 15 days. Doxorubicin was given 5 times a week at a dose of 1 mg/kg BW by intraperitoneal injection (Viswanatha *et al*., 2011; Nugraha *et al*., 2020). Meanwhile, *B. vulgaris* L. extract was given for 15 days. On the 16th day, the animals were anaesthetized for blood sample collection which was then used for the determination of the haematological profile and lipid profile (LDL, HDL, total cholesterol, and Triglycerides).

2.7 Statistical analysis

The statistical analysis of the study was performed by ANOVA with Tukey's Multiple Comparison Test. The significant P-value was set at 0.05. The values for all measurements are expressed as the mean ± standard deviation (SD).

3. Results and discussion

3.1 Phytochemical screening result of ethanol extract

Qualitative phytochemical screening results showed that the *B. vulgaris* L. ethanol extract contained flavonoids, alkaloids, saponins, tannins and steroids/ triterpenoids (Table 1).

3.2 Determination of DPPH radical scavenging activity

Antiradical power of the plant samples was measured in terms of hydrogen donating ability using DPPH, which is a stable, nitrogen-centred free radical and produces deep purple colour in methanol solution. Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character It is important to point out that a low half-maximal inhibitory concentration (IC_{50}) value reflects a high antioxidant activity of the fraction since the concentration necessary to inhibit the radical oxidation in 50% is low. Table 2 shows that the results of IC_{50} investigation in *B. vulgaris* L. extract were 37.66 µg/mL.

3.3 Assessment of haematological profile and lipid profile

Beta vulgaris L. showed protective activity on the lipid profile of rats in doxorubicin-induced toxicity, it is shown in Tables 3 to 6. Our results also reveal significantly lower lipid levels in the test as compared to the negative control group, there were significant differences in the treatment groups' and control group's lipid levels, it is indicating *B. vulgaris* L. tuber lowered the lipid levels in the test group. This agrees with the work of Singh *et al*. (2015) in which beetroot juice supplementation decreased LDL cholesterol levels in physically active individuals. Also, another study reported anti-lipidemic effect of beetroot extract on hypercholesterolemia rats (Naeem Rabeh and Marwa Ibrahim, 2014).

Phytochemical content found in beetroot includes tannins, saponins, alkaloids, flavonoids, terpenoids, and steroids. Antioxidants such as the phenolic group played a function in decreasing cholesterol, LDL, and triglyceride levels in doxorubicin-induced mice. Phenolic groups such as tannins and flavonoids showed an active reduction in the total lipid cholesterol, LDL and triglycerides. Betanin is a heterocyclic compound found in beetroot (Baião *et al*., 2017; da Silva *et al*., 2019). The cyclic amine and hydroxyl groups, which are hydrogen and electron donors, contribute to betanin's antioxidant

Table 1. Phytochemical screening result.

Content	Reagent	Dried sample	Ethanol extract
Flavonoids	HCl (c), Mg powder. Amyl alcohol	+	+
Alkaloids	Mayer	+	+
	Bouchardat	+	+
	Dragendorf	+	+
Saponins	Foam test	+	+
Tannins	$FeCl_3$	+	+
Steroids/terpenoid	Liberman Burchard	+	+

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Table 2. IC_{50} of antioxidant activity of *Beta vulgaris* L.

No	Sample concentration (µg/mL)	Absorbance	% Inhibition	Regression equation	IC_{50} (µg/mL)
1	Blank	0.9923	0	y = 0.8152x + 19.374	37.366
2	6.25	0.8227	17.09161	r^2 = 0.9315	
3	12.5	0.7451	24.91182		
4	25	0.5228	47.31432		
5	50	0.2901	70.76489		
6	100	0.0523	94.72942		

Table 3. Level of total cholesterol.

Groups	Total Cholesterol (mg/dL)					Mean±SD
	1	2	3	4	5	
Normal Groups	81	83	78	76	74	78.4±3.64*
Negative Control	93	97	98	105	110	100.6±6.80
Vitamin E 100 mg/kg BW	65	65	72	75	68	69±4.41*
Extract 100 mg/kg BW	73	75	77	78	68	74.2±3.96*
Extract 50 mg/kg BW	76	77	72	69	82	75.2±4.96*
Extract 25 mg/kg BW	81	82	85	81	92	84.2±4.65*
Extract 12.5 mg/kg BW	92	94	103	98	106	98.6±5.89

Values are presented as mean ± SD (n = 5). *Statistically significant difference compared to negative control group at $p < 0.01$.

Table 4. Level of HDL.

Groups	HDL (mg/dL)					Mean±SD
	1	2	3	4	5	
Normal Groups	38	36	40	43	38	39±2.64
Negative Control	42	41	39	40	42	40.8±1.30
Vitamin E 100 mg/kg BW	51	57	54	62	49	54.6±5.12*
Extract 100 mg/kg BW	52	54	60	62	47	55±6.08*
Extract 50 mg/kg BW	43	45	43	42	46	42±1.64*
Extract 25 mg/kg BW	42	43	41	45	41	42.4±1.67*
Extract 12.5 mg/kg BW	43	41	39	34	34	38.2±4.08

Values are presented as mean ± SD (n = 5). *Statistically significant difference compared to negative control group at $p < 0.01$.

Table 5. Level of LDL.

Groups	LDL (mg/dL)					Mean± SD
	1	2	3	4	5	
Normal Groups	20	22	25	18	20	21±2.64*
Negative Control	29	29	30	39	32	31.8±4.20
Vitamin E 100 mg/kg BW	18	17	16	17	23	18.2±2.77*
Extract 100 mg/kg BW	10	8	9	10	9	9.2±0.83*
Extract 50 mg/kg BW	12	10	12	10	10	10.8±1.09*
Extract 25 mg/kg BW	13	10	11	12	12	11.6±1.14*
Extract 12.5 mg/kg BW	12	10	13	12	14	12.2±1.48*

Values are presented as mean ± SD (n = 5). *Statistically significant difference compared to negative control group at $p < 0.01$.

Table 6. Level of triglycerides.

Groups	Triglycerides (mg/dL)					Mean ±SD
	1	2	3	4	5	
Normal Groups	131	121	110	128	126	123.2±8.22
Negative Control	241	221	236	228	239	233±8.33
Vitamin E 100 mg/kg BW	110	103	107	104	120	108.8±6.83*
Extract 100 mg/kg BW	137	152	142	130	165	145.2±13.66*
Extract 50 mg/kg BW	154	152	143	149	147	149±4.30*
Extract 25 mg/kg BW	210	219	224	197	213	212.6±10.26
Extract 12.5 mg/kg BW	235	231	228	241	212	229.410.87

Values are presented as mean ± SD (n = 5). *Statistically significant difference compared to negative control group at $p < 0.01$.

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activity. This is efficacious to stabilize the reactive species. The antioxidant activity of betanin increases according to the number and position of the hydroxyl groups in the molecule. The position of C-5 hydroxyl group in the aglycone, in this case, is responsible for increasing their antioxidant activity (Cai *et al*., 2003; Madadi *et al*., 2020).

Antioxidants such as flavonoids and tannin play a role in lowering cholesterol, LDL and triglyceride in rats that were induced by doxorubicin. Previous reports have shown that flavonoids can affect the process of LDL cholesterol metabolism by increasing the ability of LDL to bind to its receptors. LDL that is bound to receptors will be metabolized into esters form in the tissues (Ighodaro and Omole, 2012; Cammerer *et al*., 2018). Besides, flavonoids also lower LDL. Flavonoids are also known to reduce LDL lipid peroxidation and reduce the oxidative stress of macrophages by inhibiting cellular oxygenation and activating cellular antioxidants (Fuhrman and Aviram, 2001). Thus, flavonoids are natural antioxidants that have the ability to protect against lipid peroxidation in the arteries. By decreasing LDL, the formation of foam cells will be inhibited, thereby reducing the risk of atherosclerosis.

Several studies have reported that doxorubicin causes toxicity effects on haematological parameters, such as neutropenia and thrombocytopenia anaemia and leukopenia (O'Keefe and Schaeffer, 1992). *Beta vulgaris* L. showed a protective effect on haematological parameters. This is described in Table 7.

Beta vulgaris L. extract showed protective activity due to its haematopoiesis activity. Several studies reported that *B. vulgaris* L. also has an activity to increase the haematopoiesis process, by increasing the production of blood cells which has a correlation in the protection of haematological parameters (Babarykin *et al*., 2019).

4. Conclusion

Beta vulgaris L. ethanol extract showed antioxidant activity and protective activity in parameters of haematology and lipid profile of Rats Induced by doxorubicin. It showed a dose-dependent manner.

Conflict of interest

The authors declare no conflict of interest.

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Table 7. Haematological profile.

Parameters	Groups						
	Normal Group	Negative Group	Vitamin E Group	Extract (100 mg/kg BW)	Extract (50 mg/kg BW)	Extract (25 mg/kg BW)	Extract (12.5 mg/kg BW)
WBC (10^3/µL)	8±4.09*	1.54±0.42	4.59±3.13*	3.38±1.43*	2.88±2.13*	2.1±3.43	1.4±2.59
RBC (10^6/µL)	12.043±2.74*	5.2±0.16	8.439±1.34*	7.49±0.91*	6.182±0.67*	5.137±2.14	5.5±2.86
HGB (g/dL)	13.47±2.81*	8.25±0.50	12.63±1.43*	10.64±1.47*	8.84±2.50*	8.64±2.50	8.54±3.47
HCT (%)	61.6±3.40*	29.14±1.90	43.77±7.89*	40.26±2.23*	35.13±1.83*	32.72±2.92	30.12±3.4
MCV (fL)	47.44±2.42*	37.3±3.42	43.75±4.32*	48.26±2.76*	38.23±1.46*	37.2±2.41	36.4±4.82
MCH (pg)	13.54±1.81*	11.24±1.36	12.68±1.54*	14.34±0.25*	13.32±1.45*	12.28±1.61*	12.90±2.70
MCHC (g/dL)	31.13±1.17*	19.83±1.46	26.14±0.93	24.22±2.26	24.13±3.31	22.43±4.51*	20.75±3.63
PLT (10^3/µL)	1122.5±128.14*	663.8±16.37	1024.8±119.28*	884±111.12*	824±108.4*	724±125.7*	602±241.7
NEU (%)	8.9±3.56*	33.6±2.43	8.4±3.92*	14±3.55*	16±2.65*	18±5.85*	27±4.93*
LYMP (%)	78.5±6.47*	44.4±6.57	73.1±1.47*	53.8±9.12*	49.7±6.42*	45.9±3.86	43.6±2.49*
MONO (%)	6.4±3.67	4.2±0.63	7.9±1.13*	6±2	5.8±0.56	5.3±2.1	4.2±2.2
EOS (%)	0.3±0.1	1.3±0.3	1.1±0.1	1.3±0.2	1.3±2.2	1.4±1.2	1.3±2.6
BAS (%)	7.7±3.58	8±2.23	8.4±7.76	14.6±3.14	13.6±1.29	11.6±1.59	10.3±2.62*

Values are presented as mean ± SD ($n = 5$). *Statistically significant difference compared to negative control group at $p < 0.01$.

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