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Research Article

Effect of andaliman (*Zanthoxylum acanthopodium DC.)* ethanol extract on doxorubicin-induced toxicity on hematology in male rats

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Abstract

Introduction: Doxorubicin is one of the chemotherapy drugs that have harmful effects on blood hematology. Blood hematological diseases are caused by its adverse effects and anticancer properties. The antioxidant properties of several plants have been reported to be closely related to reduced toxicity in blood hematology. Andaliman *(Zanthoxylum acanthopodium)* ethanolic extract (AEE) is thought to reduce the toxicity of doxorubicin due to the antioxidant properties of its secondary metabolite content. This study aimed to determine the effect of AEE on doxorubicin-induced rats and its effect on blood cells. **Materials and Method:** A total of 24 male rats were divided into 6 groups: (1) Normal group; (2) 0.5% Na-CMC group; (3) 50 mg/kg BW quercetin group; (4) AEE 75 mg/kg BW group; (5) AEE 150 mg/kg BW group; and (6) AEE group 300 mg/kg BW administered orally for 9 days. On days 8 and 9, doxorubicin 10 mg/kg BW was administered. Rats were sacrificed for blood collection, and measured at the Integrated Laboratory of the USU Hospital. **Results:** AEE reduces the toxicity of doxorubicin on blood parameters. Furthermore, it affects Hb, white blood cells, platelet cells, monocytes, lymphocytes, neutrophils, and several related protein metabolisms as well as organ damage. Dose 150 or 300 mg/kg AEE reduce all blood toxins to near-normal levels and decrease the lymphocyte and neutrophil suppressive activity of doxorubicin. **Conclusion:** Andaliman ethanol extract can improve Hb count, white blood cells, platelet cells, monocytes, lymphocytes, neutrophils, protein metabolism, and organ damage. Furthermore, AEE can be used in combination with doxorubicin to reduce its hematological toxicity.

Keywords: toxicity, hematology, doxorubicin, andaliman, *Zanthoxylum acanthopodium,* extract

INTRODUCTION

Cancer is characterized by the presence of malignant and uncontrolled abnormal cells/tissues. Furthermore, it is the leading cause of death, responsible for almost 10 million deaths in 2020.[1] The International Agency for Research on Cancer (IARC) estimated that 1 in 5 people in the world will develop cancer during their lifetime.[2]

Doxorubicin is the medication is used to treat cancer. The anticancer properties of doxorubicin are mediated through the generation of free radicals, and the formation of oxidative stress which ultimately causes tumor cell death.[3] Furthermore, it has numerous negative side effects, including hematotoxicity, in addition to being effective as an anticancer. The side effects include anemia, leukopenia, neutropenia, and thrombocytopenia.[4]

The uses of natural compounds as adjuncts in cancer treatment is currently increasing. Similarly, natural compounds reduce the toxicity of chemotherapy regimens administered as part of standard cancer therapy.[5] Plants used as an additional cancer treatment include grapes.[6] Furthermore, many species of *Zanthoxylum have* been reported to have anticancer properties.[7] One of the plant species found in North Sumatra is andaliman *(Zanthoxylum acanthopodium DC.).* The fruit contains flavonoids, terpene alkaloids, benzophenanthridine alkaloids, pyrroloquinoline alkaloids, quaternary isoquinoline alkaloids, aporphine alkaloids, the terpenoid group, namely geranyl acetate, and is dominated by limonene as well as citronellol. Other components are β-myrcene, β-ocimene, linalool, and E-1-decenal.[8] Previous reports showed that andaliman ethyl acetate extract has cardioprotective effects[9] and free radical scavenging activity.[10] It is believed that the ethanol extract of andaliman can counteract the hematotoxic effect of doxorubicin.

MATERIAL AND METHOD

Material

In this study, the materials used was an andaliman fruit from Onan Runggu sub-district, Samosir Regency, North Sumatra Province, Indonesia, and identified in the Medanense Herbarium (MEDA), Faculty of Mathematics and Natural Sciences, University of North Sumatra, 60% ethanol, Doxorubicin (PT. Kalbe), 0.9% NaCl, quercetin (Sigma), and Na CMC.

Extract preparation

Extraction of andaliman fruit was carried out with 60% ethanol. Furthermore, 1,500 grams of simplicia powder was placed in a vessel and poured with 11.25 liters of 60% ethanol. The vessel was then closed and left for 5 days protected from light with occasional stirring. After 5 days, it was filtered and the dregs were squeezed out. The dregs were further washed with 60% ethanol, stirred, and sprinkled to obtain 15 liters. The macerate was collected in a closed vessel, left in a cool place protected from light for 2 days, and then poured. The

concentration of the extract was performed using a rotary evaporator and then the extract was dried with a freeze dryer.

Experimental animals

The experimental animals used were male rats aged 3-4 months with a body weight of 100-200 g. Before the experiment, the rats were acclimated to a standard diet and drinking water for 2 weeks.

Experiment protocol

The male rats were divided into 6 groups, each group consisting of 4 rats. The treatment was carried out as follows: (1) The normal group received no treatment; (2) Group (Na CMC 0.5%); (3) The quercetin group received a dose of 50 mg/kg BW; (4) the AEE group received a dose of 75 mg/kg BW; (5) AEE group received a dose of 150 mg/kg BW; and (6) The AEE group received a dose of 300 mg/kg BW, which was administered orally for 9 days. Days 8 and 9 received doxorubicin at a dose of 10 mg/kg BW according to the test group. On the 10th day or at least after 12 hours of doxorubicin administration, the rats were anesthetized and sacrificed. Blood was collected by cardiac puncture for hematological analysis. Meanwhile, the protocol was approved for study ethics by the Animal Research Ethics Committee (AREC), the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (Number of approval 0564/KEPH-FMIPA/2022).

Hematology Analysis

Hematological analysis was carried out at the Clinical Integrated Laboratory, University of North Sumatra Hospital using the Sysmex 550 in the calculation of the whole blood examined.

Statistical Analysis

One-way ANOVA was used for the analysis of normalized data using SPSS version 22. Data are presented in Mean ± SEM. The value of $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Results

Doxorubicin toxicity has been widely reported, including hematotoxicity, nausea, and vomiting, hair loss, irreversible cardiomyopathy,[11] decreased kidney function with increased creatinine and urea, as well as decreased liver function,[12] Additionally, cancer patients undergoing chemotherapy are more susceptible to infections due to immunological changes exhibited by chemotherapy drugs.[13,14] Changes occur in the lymphatic, neurological, respiratory, kidney, liver, and several other parts of the body.[12,15]

Table 1: Hematologic values from the study on Doxorubicin-induced rats

Hematology	Normal	Na CMC+Dox	Quercetin 50 mg/kg BW+Dox	AEE 75 mg/kg BW+Dox	AEE 150 mg/kg BW+Dox	AEE 300 mg/kg BW+Dox
Hb (g/dl)	12.83±0.05^{bc}	10.08±0.25^{def}	12.8±0.31^{def}	9.58±0.21^{bc}	12.95±0.06^{bc}	12.65±0.1^{bc}
Leukocytes (10^3/µl)	5.15±0.46^{bcef}	1.68±0.23^{acdef}	3.99±0.04^{abf}	4.04±0.01^{abf}	4.65±0.06^{abf}	2.86±0.03^{abcde}
Erythrocyte(10^6/µl)	6.93±0.30	6.89±0.24	6.84±0.19	7.54±0.16	6.49±0.11	6.74±0.22
Segment neutrophils (%)	24.75±2.56^{bdef}	62.90±3.51^{ac}	29.55±0.95^{bdef}	49.18±4.04^{ac}	56.45±1.94^{ac}	58.03±4.40^{ac}
Lymphocytes (%)	72.5±5.16^{bdef}	23.75±2.06^{acef}	52.95±1.68^{ab}	39.9±0.84^{ac}	45.75±8.12^{ab}	49.30±4.37^{ab}
Monocytes (%)	22.98±0.89^{bde}	28.5±0.73^{acef}	21.35±0.57^{bde}	26.85±0.06^{abcf}	25.43±0.21^{abcf}	23.65±0.19^{bd}
Platelets (10^6/µl)	673.75±66.31^{b}	476.25±11.43^{abcef}	641.75±40.34^{bd}	560±9.23^{ce}	476.25±43.55^{bd}	544.5±11.66^{b}

Hb: Hemoglobin; Values represent the mean ± SEM.$p < 0.05$ significantly different compared to a=normal,b=Vehicle+DOX, c= Quercetin 50 mg/kg BW+Dox, d= AEE 75 mg/kg BW+Dox, e= AEE 150 mg/kg BW +Dox, f= AEE 300 mg/kg BW+Dox

Hematological parameters including red blood cells, platelets, white blood cells, lymphocytes, neutrophils, monocytes, and basophils were measured for each treatment group in experimental animals after treatment with doxorubicin. The results showed that the CMC + DOX group experienced a decrease in Hb, leukocyte, and lymphocyte levels while the neutrophils and monocyte levels increased. Erythrocytes were not affected by the administration of CMC+DOX for 2 days. Compared to the normal group, the administration of CMC+DOX significantly suppressed/decreased Hb by 1.17-1.32 times, white blood cells/leukocytes at 1.9-5.1 times, and lymphocytes at 2.0-4.25 times. Meanwhile, the number of neutrophils and monocytes significantly increased in the CMC + DOX group, around 1.1-3.4, and 1.1-1.4 times compared to normal rats. When DOX-treated rats were treated with AEE, Hb, white blood cell/leukocyte, lymphocyte, neutrophil, monocyte, and platelet counts were slightly restored to near-normal levels. Furthermore, AEE 150 mg/kg BW increased the number of white blood cells by 0.75-1.13 times compared to the CMC+DOX group. AEE 150 mg/kg BW increased Hb by 1.18-1.35 times compared to the CMC+DOX group. Moreover, AEE 150 mg/kg BW increased the platelet count to normal (Table 1).

DISCUSSION

AEE can reduce doxorubicin-induced hematotoxicity by affecting the levels of Hb, white blood cells, platelets, monocytes, lymphocytes, and neutrophils, which are associated with protein metabolism and organ damage. Doses of 150 or 300 mg/kg AEE can restore blood parameters to near-normal levels and attenuate the lymphocyte and neutrophil suppressor activity of doxorubicin. The antioxidant properties of limonene, β-osimene, β-myrcene, linalool, citronellal, β-citronellol, neral, geranyl acetate, sesquiterpenes, geraniol, geranial in andaliman affect its hematotoxicity reducing properties [14]. Furthermore, secondary metabolites contained in andaliman are alkaloids, flavonoids, glycosides, saponins, tannins, and steroids. Flavonoids in plants in the

same genus, namely *Zanthoxylum zanthoxyloides*, or eriocitrin, are included in the glycosylated flavanone group.[16] The compounds contained may have influenced the activity of the ethanolic extract of andaliman by reducing doxorubicin-induced hematotoxicity in rats. Andaliman ethanol extract was also reported to have anticancer effects by inhibiting MCF-7 cell proliferation.[17] In addition to its anticancer activity, its hematotoxicity-reducing properties make it a choice as an adjuvant to doxorubicin chemotherapy.

CONCLUSION

Andaliman ethanol extract reduces the hematological toxicity of doxorubicin, especially on hemoglobin, leukocytes, neutrophils, lymphocytes, monocytes, and platelets. This study reveals the potential for using AEE as a companion therapy for doxorubicin treatment.

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Conflict Of Interest: The author declared that there was no conflict of interest during the cause of this study and producing and submitting this manuscript for publication.

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