

Antioxidant and Hepatoprotective Activity of Garlic Chives (*Allium tuberosum*) Ethanolic Extract on Doxorubicin-Induced Liver Injured Rats

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Abstract—The plant phenolic compounds such as flavonoids and isoflavons have an important role in the treatment of many diseases and some of them induce a potent antioxidant and hepatoprotective effect. The objective of this study is to investigate the antioxidant and hepatoprotective activity of flavonoids compound from ethanolic extract of *Allium tuberosum* (EAT) against doxorubicin-induced hepatotoxicity in male Wistar rats. Preliminary phytochemical tests of EAT were done by Thin Layer Chromatography. Antioxidant and hepatoprotective activity of EAT was evaluated by treating groups of rats with 500 & 1000 mg/kg body weight per oral of EAT for 14 days and at the same time (day-1 and day-8) challenging with 4.67 mg/kg body weight of Doxorubicin. On day-15 the hepatoprotective and antioxidant effects of EAT were evaluated by measuring liver function and MDA serum spectrophotometrically using standard procedures. Thin Layer Chromatography indicate the presence of the flavonoids on EAT. Results showed that intraperitoneal injection of Doxorubicin caused a significant ($p<0.001$) elevation in the serum levels of SGOT and SGPT. However, elevations in the measured biochemical parameters were significantly ($p<0.05$ and $p<0.01$) attenuated in rats treated with EAT, in dose related fashion. Oral administration of EAT also decreases levels serum MDA ($p<0.01$). It can be concluded that ethanolic extract of *Allium tuberosum* has a significant hepatoprotective and antioxidant activity. In addition, *Allium tuberosum* may be useful for adjuvant chemotherapy doxorubicin.

Index Terms—*Allium tuberosum*, antioxidant, doxorubicin, SGOT, SGPT

I. INTRODUCTION

Chemotherapy is the primary modality of cancer therapy, and the most frequently used is doxorubicin. This drug is effective in treating various types of cancer such as breast, lung, prostate, cervical, bone, etc., [1] but doxorubicin has many side effects including hepatotoxic, cardiotoxic, nephrotoxic, and immunosuppression. [2], [3]

Doxorubicin affects the hepatobiliary system by inducing an imbalance of oxygen free radicals and antioxidants. Impaired balance of oxidants results in damage of liver tissue. [4] This hepatotoxic activity can be measured using SGOT, SGPT and MDA.

The plant phenolic compounds such as flavonoids and isoflavons have an important role in the treatment of many diseases and some of them induce a potent antioxidant and hepatoprotective effect. [5] One of the potential plants is garlic chives (*Allium tuberosum*). Garlic chives are smallest species of the family Alliaceae, native plants in Europe, Asia and North America. The aroma of garlic chives is more like garlic than chives, so that in English is called garlic chives. This plant is used as an antibacterial, anti-emetics, treatment of urinary incontinence, bladder weakness, etc. [6] Garlic chives can be harvested up to two times more compared to other types of onions. The price is also very affordable.

Garlic chives are known to contain flavonoids, especially allicin that is proven to increase the number of CD4+, but that he also has the effect to increase the pro-inflammatory mediators (IFN- γ , TNF and NO). [7],[8] Allicin also has hepatoprotector effect, but the mechanism is not certain. [9] The aims of this study is to prove the potential of *Allium tuberosum* as a hepatoprotective through an antioxidant mechanism of male Wistar rats induced by doxorubicin.

II. MATERIAL AND METHODS

A. Plants

Garlic chives (*Allium tuberosum*) were collected from P.T GMN Food, Singosari, Malang, Indonesia, in March 2016. The plant was authenticated by a taxonomist at Departement of Botany, Faculty of Mathematics, University of Jember, Indonesia. Garlic chives specimens stored in the herbarium of Faculty of Mathematics, University of Jember.

B. Animals

Wistar rats (weighing 100-150 g) were housed at aconstant temperature ($25\pm2^\circ\text{C}$) with a constant relativehumidity ($60\pm10\%$) on the an automatically

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controlled 12:12 h light-dark cycle (light on at 7:00 a.m.). Rats were fed with standard rat chow and water *ad libitum*. The rats were acclimatized and quarantined for at least one week prior to the experiment.

C. Research Design

Thirty seven male Sprague Dawley rats are divided into five groups consisting of 6-7 rats each as follows: 1) normal controls, rats were given 0.9% NaCl (Widatra, P.T Otsuka, Indonesia) intraperitoneally, 2) negative control, Doxorubicin rats were given 4.67 mg/kgW in 0.5% CMC-Na po, 3) extracts controls, rats were given EAT 1000 mg/kgW in a solvent 0.5% CMC-Na po, 4) first group treatment, Doxorubicin-induced rats 4.67 mg/kgW ip and at the same time got EAT 500 mg/kgW, 5) Second group treatment, Doxorubicin-induced Rat 4.67 mg/kg i.p. and at the same time got EAT 1000 mg/kgW. Induction Doxorubicin (Actavis, obtained from P.T. Actavis Pharmaceutical Bogor, Indonesia) performed on days 1 and 8. The treatment of EAT were administered for 14 days.

D. Extraction Method

Garlic chives which have been dried in the oven smoothed by using a blender. A total of 599.59 grams of chives powder macerated using ethanol 70% with a ratio of 1: 3. The macerate was filtered using filter paper to obtain the filtrate which then evaporated to obtain as much as 175.56 grams of concentrated extract.

E. Measurement of SGOT and SGPT Serum

After 2 weeks, the animals were killed and blood samples were obtained using cardiac puncture method. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) levels were measured using spectrophotometry method (Dialab).

F. Measurement of MDA Serum

100 mL of blood serum and 10 mL of BHT solution (TBARS Assay kit, Bioassay) mixed in a glass tube. Successively added 700 mL of 1% orthophosphoric acid and 200 mL of 2-thiobarbituric acid (TBA) of 0.6%. Tubes were incubated into a tub of hot water of 95 °C for 45 minutes. After incubation, the tube is cooled in cold water. 1 mL of n-butanol was added to the tube, then centrifuged at 2000 rpm for 10 minutes. The top layer is taken and measured by a spectrophotometer λ 535 nm. TBARS (thiobarbituric Acid Reactive Substance) which is condensed bioproduct MDA with TBA calculated in μ M using the extinction coefficient of $1,56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

G. Statistical Analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA). Significance was measured using Fisher's least significant for the exact p values and significant differences are noted in the results. Differences with $p < 0.05$ were considered significant.

III. RESULTS

A. Presence of the Flavonoid on Thin Layer Chromatography

Thin layer chromatography (TLC) indicates the presence of the flavonoid on EAT (Fig. 1). The static phase was using silica gel 60_{F254}, while the motion phase was using butanol, acetic acid, and water with a ratio of 8: 2: 10.

B. EAT Decreases SGOT and SGPT Serum Level

SGOT and SGPT serum was measured as an indicator of cellular injury of liver. Table I shows a significant increase ($p < 0.01$) of SGOT and SGPT enzymes in doxorubicin-induced group compared to control group. Levels both decreased significantly ($p < 0.01$) in the group receiving treatment EAT dose of 1000 mg/kgW and received induction doxorubicin.

C. EAT Decreases MDA Serum Level

The repair mechanisms of liver serum is confirmed by measuring levels of MDA. MDA is used as an indicator of a damage by lipid peroxidation. The increase of MDA indicate oxidation processes or membrane damage caused by free radicals. Fig. 2 shows the doxorubicin significantly increase the MDA serum levels ($p < 0.01$). Meanwhile, EAT administration after Doxorubicin induction can lower MDA serum levels ($p < 0.05$ at a dose of 500 mg/kgW and $p < 0.01$ at a dose of 1000 mg/kgW).

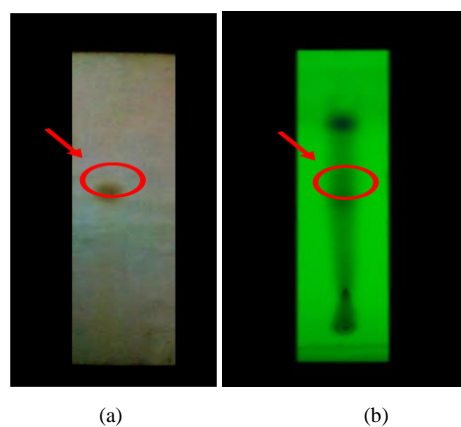


Figure 1. TLC chromatogram in (a) visible light, (b) UV (366 nm) for the samples of EAT, arrows indicate flavanoids component.

TABLE I. EAT EFFECT ON SGOT AND SGPT SERUM LEVEL

Groups	SGOT (U/L)	SGPT (U/L)
Control	40.38 \pm 2.43	20.52 \pm 4.03
DOX	56.26 \pm 4.04 ^a	36.19 \pm 2.60 ^a
EAT 1000	40.16 \pm 4.47	20.74 \pm 3.10
DOX + EAT 500	45.01 \pm 4.38 ^b	28.68 \pm 1.84
DOX + EAT 1000	40.82 \pm 2.63 ^b	25.90 \pm 7.32 ^b

^aSignificant difference ($P < 0.01$) compared to the control group value. ^b

Significant difference ($P < 0.05$) compared to the Dox group value. Data were expressed as Mean \pm SD (n=6-7 rats)

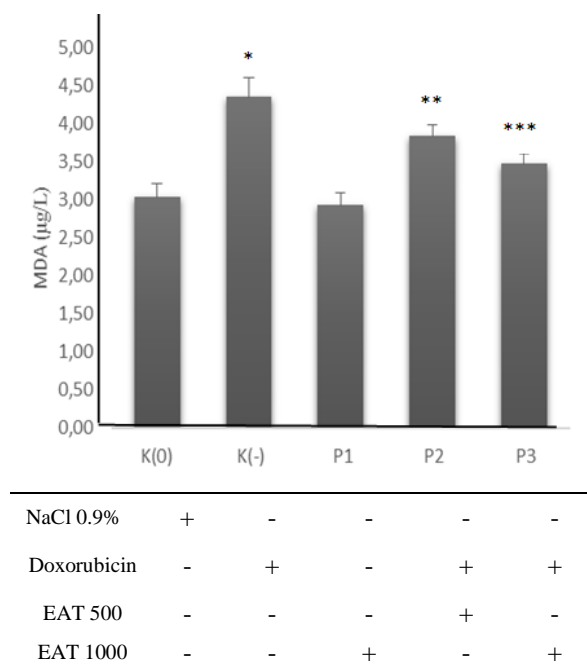


Figure 2. Effect of EAT on MDA serum level. *Significant difference ($P<0.01$) compared to the control group value. **Significant difference ($P<0.05$) compared to the Dox group value. *** Significant difference ($P<0.01$) compared to the Dox group value. Data were expressed as Mean \pm SD (n=6-7 rats)

IV. DISCUSSION

Induction of doxorubicin dose 4.67 mg / kg day 1 and 8 resulted hepatotoxicity and increased oxidant levels. Oxidative stress and reactive oxygen species (ROS) are involved in the pathogenesis of hepatotoxicity induced by doxorubicin. Doxorubicin increases production of free radicals such as superoxide radicals and hydrogen peroxide hydroxyl which has a major role in lipid peroxidation. [10] Previous study showed that doxorubicin lead to liver damage with an increased SGOT, SGPT and γ -GT serum levels. [11], [12]

EAT restore liver function which had suppressed due to the induction of doxorubicin. SGOT and SGPT are specific enzymes as indicators of hepatic injury. In the case of hepatotoxicity due to infection or other inflammatory reactions, SGPT levels higher or the same height as SGOT. [13] This study showed the opposite, SGOT serum levels increase higher than SGPT. It because of cardiotoxicity effect of doxorubicin prior to the hepatotoxicity effect. Increased significant levels of SGOT without increased of SGPT levels are common in cardiac injury. [14]

Malondialdehyde (MDA) is a metabolite of lipid peroxidation by free radicals. Malondialdehyde (MDA) formed when the free radical hydroxyl such as ROS reacts with fatty acid component of cell membrane. [15] The measurement of MDA serum levels can be used as an indicator of the reaction of lipids peroxidation in liver. It also confirm the path-repair mechanisms in cells of the liver hepatocytes. This study shows that the induction of doxorubicin alone significantly increases serum levels of MDA. MDA serum levels decrease after treatment of

EAT, indicating that lipid peroxidase pathway plays a role in the formation of liver injury.

Phytochemical component contained in garlic chives are alkaloids, phenolic content, glikosid, protein, saponins, flavonoids, etc. Flavonoids component especially allicin associated with the activity of antioxidants or thiol disulfide exchange. Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging ROS; and (3) upregulation or protection of antioxidant defenses. [16]

Thin layer chromatography indicates the presence of the flavonoid on EAT. Flavonoid action involves most of the mechanisms mentioned above. Some of the effects mediated by them may be the combined result of radical scavenging activity and the interaction with enzyme functions. Flavonoids inhibit the enzymes involved in ROS generation, that is, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase, and so forth. [17] Previous studies shown that allicin reacts with free thiol groups and entered the cell membrane. [18] One of the non-protein thiol groups in *Allium tuberosum* is glutathione (GSH). Allicin reduce free radicals to reduce lipid peroxidation. Allicin reverse hepatocytotoxicity by increasing hepatic glutathione (GSH) and GSH-related enzyme. A study explains that Allicin selectively express genes glutathione S-transferase (GST) in the liver. [19]

V. CONCLUSION

It can be concluded that doxorubicin causes hepatotoxicity and treatment of EAT is able to restore hepatic function through antioxidant pathways.

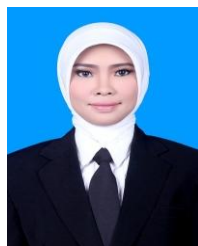
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