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DEWANDARU (Eugenia uniflora L) FRUIT EXTRACT'S HEPATOPROTECTIVE EFFECT TO THE $CC1_4$ INDUCED MICE

Pharmacy

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ABSTRACT

Oxidative stress can be occurred by chemical compound such as CCl_4, the increasing of SGOT, as the factor of liver malfunction and increase the malondialdehyde levels as the oxidative stress' symptom. Antioxidants play a great role for the health of human's body because it can inhibit and neutralize oxidation reaction which involved free radicals. Dewandaru fruit (*Eugina uniflora L.*) has a bioactive compound that is flavonoid sequiterpen antioxidants, vitamin C and anthocyanin. This research aims to test the activity of hepatoprotective effect from Dewandaru fruit extract and the decreasing of oxidative stress through the malondialdehyde levels. Research method post test randomized post test only control group design. The research result is giving ethanol extract 50 mg/kg dosage, 100 mg/kg dosage, 200 mg/kg dosage had decreased SGOT, SGPT, and MDA significantly ($p < 0,05$) to the CCl_4 injected mouse. The highest level of reduction occurred in 200 mg/kg b.w dosage.

KEYWORDS

SGOT, SGPT, MDA, Dewandaru (*Eugenia uniflora L*)

Introduction

Free radicals have an important role in damaging cells and tissues in the body. Free radical occur due to the oxidations reaction of stable compound become unstable and also reactive compound. Oxidative stress is a condition where there is no balance between free radical production or reactive oxygen species (ROS) and antioxidants, where the free radical level is higher than antioxidant (Kurkgu et al., 2010). This condition eventually will impact extensively in the human's body such as cancer and other chronic diseases (Waris and Ahsan, 2006)

Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. (Li, et al 2014).

The source of radical chemical compound that is oxidant and possible to damage the liver (hepatotoxic) is CCl4. In this research, CCl4 used as liver damage inductor (Zuraida, et al, 2015). The giving of CCl4 can increase the urinary creatinine levels protein and urobilinogen and histopathologically can lead to steatosis, centrilobular necrosis, and cirrhosis in the liver, as well as acute tubular necrosis in the kidneys. The lipid accumulation in the liver is followed by the biochemical changing in the blood, which can be seen from alanin aminotransferase (ALT) changing activity and aspartat amino-transferase (AST) in the serum (Khalid, et al, 2017). The examination used to recognize the increasing of transaminase enzyme by checking glutamate piruvat transaminase (SGPT) serum or glutamate oksaloasetat transaminase (SGOT) serum, however the glutamate piruvat transaminase (SGPT) serum checking is more specific since it produced more in the liver than in the enzyme glutamate oksaloasetat transaminase (SGOT) serum The level of cells/tissues oxidative damage in the body because of the free radical is also can be determined by measuring the Malondialdehyde (MDA) in the blood and pentane the respiratory which is the indicator from lipid peroxidation (Sha, et al 2015).

Among the plants that is being used and researched is dewandaru (Eugenia uniflora L.). Dewandaru leafs and fruits have proven empirically or scientifically as the medication to heal fever and stomachache. Dewandaru contained saponin, tannin, vitamin C, atsiri compound such as sineol, sitronela, sesquiterpen, flavonoid, and antosianin (Einbond, et al, 2004). Many researches had been done and showed that ethanol extract from Dewandaru fruit has anti bacteria activity, activity examination of antioxidant from dewandaru fruit extract showed a very strong antioxidant Phytochemical results of dewandaru fruit showed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, and terpenoids and had LD50 toxicity 2408.3 mg / Kg (Onwudiwe, et al., 2013).

The aim of this research is to test the activity of dewandaru fruit extract as hepatoprotector and determine the oxidative stress level (MDA decreasing) from an experimental animal after induced by carbon tetrachloride.

Materials and Method

Dewandaru fruit (Eugenia uniflora L) which obtained from Kawi mountain area, Kepanjen, Malang, East Java. The ethanol solvent, CCl4, experimental animal is mouse, standard feed. The research design used in this research is pure experiment randomized post test only control group design

The research Procedure is as follows:

The mouse is being adapted with the research environment for 14 days. 24 mice are taken and categorized into 4 groups, each group consist of 6 mice. The group consists of control group and treatment group which given ethanol extract of dewandaru fruit with the dosage of 50 mg/kg, 100 mg/kg , 200 mg/kg and CCl4 5% .

Each groups are given treatment as follows: Group P1 as negative control given CMC-Na + CCl_4. Group P2 is given ethanol extract of dewandaru fruit with the dosage of 50 mg/kg + CCl_4. Group P3 is given ethanol extract of dewandaru fruit with the dosage of 100 mg/kg + CCl4. Group P4 is given ethanol extract of dewandaru fruit with the dosage of 200 mg/kg + CCl4. On the 8th day (24 hours of treatment) the mice is being anesthetized (ketamine + xylazin, 0,1 ml, i.m) and taken the blood sample through orbital sinus as much as ± 1 cc. The measurement of SGPT, SGOT levels, used the method based from IFCC (International Federation of Clinical Chemistry). The enzyme activity read on 370C and stated in U/L. The MDA measurement is by Elisa method.

Result and Discussion

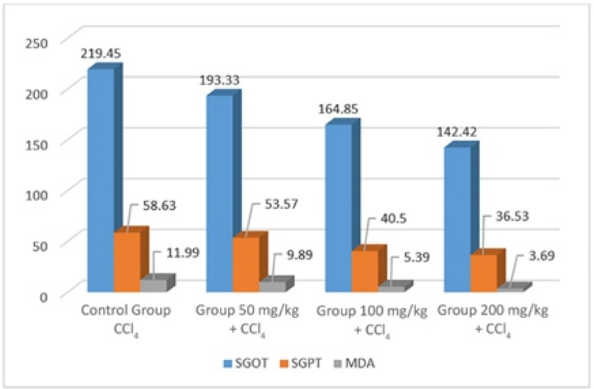

Chart 1: Hepatoprotector effect and MDA decreasing

Induction of CCl4 in this study aims to form free radicals or

hepatotoxic. In the liver endoplasmic reticulum, CCl^4 is metabolized by cytochrome P_{450} 2E1 (CYP 2E1) to trichloromethyl (Ccl_3). Trichloromethyl with oxygen will form trichloromethyl peroxyl (CCl_3O_2) which can attack the lipid of the endoplasmic reticulum membrane at a rate that exceeds the trichloromethyl free radical. Furthermore, trichloromethyl peroxyl causes lipid peroxidation thus disrupting homeostasis Ca_2+ and can eventually lead to cell death (Panjaitan & Masriani, 2014). The increase of serum levels of SGPT and SGOT enzymes were clinical parameters depicting the damage of liver cell (Gianini et al 2005), as it is seen in Table 1 in the treatment group P1 given CCl_4 was significantly different ($p < 0.05$), compared with P2, P3 and P4 goups given CCl_4 and Dewandaru fruit extract (*Eugenia uniflora L*). The effect of ethanol extract of dewandaru fruit in decreasing levels of SGOT and SGPT can be seen from the mean of SGOT and SGPT on graph. In statistical test, there was significant difference between P1 (control group) and P2 (dose 50mg/Kg b.w), P3 (dose 100mg/Kg b.w and P4 (dose 200mg/Kg b.w). On P4 (extract group of 200mg) which was given CCl4 obtained SGOT on the average of 142.42 U / L and SGPT of 36.53 U / L. Decrease happened significantly ($p < 0.05$).

MDA Levels

Giving CCI4 to a white mouse causes increasing levels of malonaldialdehid (MDA) in the liver of the mouse. According to Lilik et al. (2006) reported that there was an increase in peroxide fat in mouse liver and muscle tissue after carbon tetrachloride induction as measured using TBARS tests. Carbon tetrachloride (CCl_4) will be metabolized in the liver by cytochrome P450 enzymes and form a trichloromethyl radical which can react further by taking a hydrogen donor in PUFA in cell membranes to produce a metabolite of chloroform and fatty acid radicals which can undergo further reaction if there is oxygen. The increase of fat peroxidation is associated with antioxidant defenses caused by failure of the antioxidant mechanism to prevent free radical formation. One of the products formed during fat peroxidation is MDA (Hodgson, 2010). Malonaldialdehyde may cause chronic hepatocyte damage. This is proved from table 1 of MDA in treatment group P1 only given CCl4 and there was a significant difference ($p < 0,05$), compared to group P2, P3 and P4 given CCl4 and Dewandaru fruit extract (*Eugenia uniflora L*).

The anthocyanin content of the fruit of Dewandaru (Eugenia uniflora L) has been investigated by Einbond et al. (2004) as a very active antiradical. Antosianin is a flavonoid compound that has the ability as an antioxidant. Generally flavonoid compounds serve as primary antioxidants, chelators and scavengers against superoxide anions. The anthocyanin in the form of aglycons is more active than its glycoside form. The ability of the antioxidative anthocyanins arises from their high reactivity as a donor of hydrogen or electrons, and the ability of radicals derived polyphenols to stabilize and delocalize unpaired electrons, as well as the ability in chelating metal ion (termination reaction Fenton) (Rice-Evans et al., 1997).

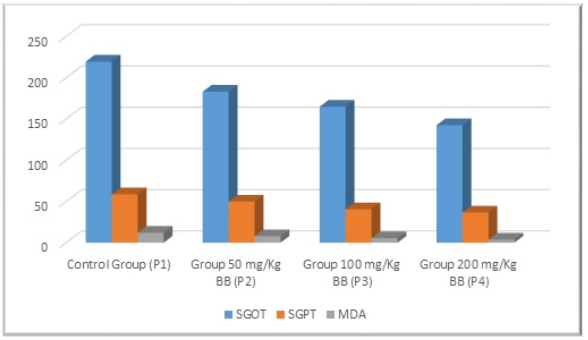

Chart 2: SGOT, SGPT, and MDA levels of all groups

In the chart, it is shown that P2 group (50mg/kg), P3 (100mg/kg) and P4 (200mg/kg) were able to significantly lower SGOT, SGPT and MDA levels ($p < 0.05$) than control P1 (CCl_4). Bioactive in lowering the three markers is suspected to be caused by the quantity of flavonoid compounds in all three doses of Dewandaru fruit which is capable in reducing or neutralizing free radicals caused by CCl_4. Antioxidants are compounds that can neutralize a free radical. According to Wang et al. (2010), that triterpenoid group compounds, flavonoids are antioxidants, that are able to reduce the amount of CCl_4 metabolites so that liver cells can be protected from damage and stability of liver cell membranes as well as its activity are maintained. Sharma and Shukla (2011) stated that the antioxidant effect of flavonoids is to improve cell regeneration process.

Natural flavonoids can protect the FUPA phospholipid membrane by donating or giving one of the hydrogen ions (H+) to the peroxy lipid radical (LOO*) so that it can stop further radical reactions, such as the following reactions: (Hamid, et al 2010)

$$HOO^* + LOOH \rightarrow H2O + LOO^*$$
$$LOO^* + FL\text{-}OH \rightarrow LOOH \text{ or } LH \text{ or } LOH + FL\text{-}O^*$$

Polyphenol compounds such as flavonoids, polyenes and heavily-containing compounds of the -OH group are multifunctional compounds which can react with free radicals as: (a) reducers, (b) free-radical arresters, (c) metal chirals, and (d) silencers the formation of singlet oxygen (Akhlaghi, et al., 2009). Dosing of 200mg/KgBB has the highest ability to decrease SGOT, SGPT and MDA. The content of phenol, flavonoid and terpenoid compounds that are high enough can be synergistic, so that it has the highest ability to lower levels of MDA.

Conclusion

Giving ethanol extract dose 50mg/kg, 100mg/kg, 200mg/kg body weigth had significantly lower effect of SGOT, SGPT, and MDA ($p < 0,05$) in CCl4 induced mice. The greatest reduction rate at doses of 200mg/kg. From the results of this study Dewandaru fruit (Eugenia uniflora L) can be recommended as natural antioxidants a hepatoprotector and prevent oxidative stress.

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