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RESEARCH ARTICLE

Syzygium aromaticum (CLOVE) EFFECT ON CATALASE ACTIVITY DUE TO CARBON TETRACHLORIDE-INDUCED OXIDATIVE STRESS IN RAT LIVER

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ABSTRACT

Background: Clove is known as antioxidant spice that used in cigarettes, spice for food/soup, and traditional medicine. It is believed that clove could protect smokers from cigarette-free radicals. Otherwise, study on clove as an antioxidant was still confused.

Objective: To reveal that clove can overcome carbon tetra chloride (CCl4) and its free radical derives

Method: This study was an experimental research, using 20 Wistar rats that were divided into 4 groups, Group 1 (CCl4 + cloves 3), group 2 (CCl4 + cloves 1), group 3 (normal control, without being offered treatment), group 4 (positive control, induced by CCl4 and followed by 100 mg alpha-tocopherol), and group 5 (negative control, only induced by CCl4). Rat livers were homogenized and followed with CAT activity measurement using spectrophotometry method of Mates.

Results: There was a significant difference in mean between the groups (p= 0,001). Further test, the Post Hoc showed that there is a significance different between group 1 and 4 (p=0.008), 1 and 5 (p=0.001), 2 and 5 (p=0.001), 3 and 5 (p=0.001), and 4 and 5 (p=0.007). Group 1 (CCl4+Clove3) has the highest catalase activity.

Conclusion: *Syzygium aromaticum* (clove) oral administration with the dose of 200 mg/kg rat body weight against 0.55 mg/kgBW CCl_4 show increased of catalase activity but did not overcome the oxidative stress.

Keywords : Clove, Carbon tetra chloride, Catalase, Liver, Vitamin E

INTRODUCTION

In the last few decades, free radicals have become a crucial problem always faced. Free radicals or Reactive Oxygen Species (ROS) are defined as compounds that have unpaired electrons in their outermost orbits. Free radicals can cause various diseases in humans, animals and so on where cellular damage can cause cancer, liver disease, atherosclerosis, coronary artery disease, and autoimmune diseases.[1] One of the most widely found ROS in the environment is carbon tetrachloride (CCl4). Carbon tetra Chloride is an exogenous compound that is often used in urban industry. CCl4 itself is a volatile compound easily dispersed in the air making easier for individuals to inhale them thus causing accumulation in the liver and heart. If CCl4 enter the liver, there is a cytochrome P450 enzyme that will convert CCl4 into highly reactive trichloromethyl (CCl3) free radical compound which will subsequently be oxidized in the liver to convert to trichloromethyl oxy (CCl3O2) can cause centrilobular necrosis.[2,3]

ROS that causes damage to cells in the body, it will be reduced by the body's defense mechanisms, one of them through the formation of body enzymes that act as antioxidants such as catalase. However, endogenous antioxidants are not too strong and little to counteract the amount of ROS in the liver after treated with CCl4. It is, therefore, necessary that exogenous natural antioxidants can help enzymatic antioxidant to eliminate free radicals.[4,5]

From various studies, it was found that cloves contain or are rich in antioxidants and Clove in Greek is Syzygium aromaticum is an original herb derived from Maluku and North Maluku commonly used by local people as ingredients and traditional medicine. Clove itself contains eugenol, eugenol acetate, caryophyllene, flavonoid, and Beta-caryophyllene which play an important role in warding off free radicals.[6-11]

Research on clove antioxidant activity has been widely studied but no studies on clove effect induced by CCl4 in mice in the changes of carbonyl, malondialdehyde, and glutathione levels (reduced glutathione) in the liver.[12-14] Therefore, the question arises whether there is an effect of clove in the induction of CCl4 will changes in catalase activity in liver. Based on these reasons, this study will observe liver organ after induction of CCl4 of catalase activity.

MATERIAL AND METHODS

This research uses experimental study in vivo with the aim to know the ability of clove effect (Syzygium aromaticum) as antioxidant induced by CCl4 in mice with the determination of catalase in the liver. This study was run in the Laboratory of Biochemistry and Molecular Biology, Faculty of the Medicine, Universitas Indonesia. The data in this research was primary data which was obtained through several procedures of laboratory work.[15]

We used 12 weeks old white Wistar rats with 150 – 200 g of body weight. Sample size chosen using Federer formula, 5 group of rats (7 each): Group 1 = CCl4 + cloves 3 (induced by CCl4 and followed water extracts of cloves for 3 days), group 2 = CCl4 + cloves 1 (induced by CCl4 and followed by water extracts for 1 day, group 3 = normal control (without being offered treatment), group 4 =positive control (induced by CCl4 and followed by 100 mg alpha-tocopherol), and group 5 = negative control (only induced by CCl4).

Prior to the research livers were taken and homogenized to obtain supernatant homogenates. Experimental stages were done prior to this research. The rats as the sources of the liver have undergone certain stages and some other preparation as follows:

Preparation of Clove extract

Clove dose that was used is 200 mg/kg body weight. Firstly, 40 g of dried clove was crushed to become smoother and mixed with 1 L of water for 5 days. Every 24 hours, the mixture was stirred with the glass stirring rod before being kept in cooler temperature as cool as 4^0 C. Finally, the concentration in the mixture of clove was 40 mg/mL.

CCl4 preparation

CCl4 dose that is used was 0,55 mg/g body weight. It was mixed into palm oil afterward based on the required dose. By having that standard, a rat that weighed 200 gr received 110 mg CCl4. The density of the CCl4 is 1,59 g/mL. This meant that there was 0,11 g in 0,069 mL CCl4 solution. In order to do so, to create a 50 mL solution to be used, 3,45 mL CCl4 was diluted in palm oil until the volume reaches 50 mL.

Treatment

The material prepared was given by using intubation syringe. The dosage was rat body weight dependent. The material was given according to the planned scheme.

Tissue extraction

The rat was killed by neck dislocation method. The liver was extracted immediately. The tissue extraction started in the thoracic region by using scissors. Furthermore, the tissues were put onto the scale to know the weight.

Homogenate Preparation

By adding phosphate buffered saline (PBS) solution 1 mL per 100 mg of preserved tissue, the tissue was crushed and blended to become smoother by using micropestle. The homogenate was mixed by using vortex and centrifuged for 10 minutes by applying 5000 rpm speed and 4°C temperature. The supernatant was taken away from the homogenate to the tube. Parafilm covered the opening of the tube and the tube was put into -84°C freezer afterward.

Protein Concentration Measurement

BSA standard solution that was used is set into 5 different concentrations ranging from 100 mg/mL to 500 mg/mL. Then, absorbance reading was conducted to compare the protein concentration the standard solutions and sample by setting the wavelength to 280 nm.

Catalase Activity Measurement

Measurement of catalase enzyme level which will use Mates method. The wavelength to be used is 210 nm. A blank 50 µL saline phosphate buffer solution of 0.05 M pH 7.0 will be used. The liver homogenate sample was piped 50 µL into the cuvette, then added 950 µL H2O2 to the blank cuvette and the sample cuvette, then mixed. Next, read the absorption in the first 30 seconds (t0) and 2 minutes later (t1) with a spectrophotometer with a wavelength of 210 nm.

RESULTS

The results of examination of catalase activity in each group are shown in table 1.

Table 1. Catalase activity (U/L)

Group Member	CCL4+clove 3	CCl4+clove1	Positive control*	Negative control**	Normal control***
1	0,0156	0,0106	0,0059	0,0061	0,0019
2	0,0102	0,0093	0,0067	0,0072	0,0008
3	0,0088	0,0096	0,0075	0,0087	0,0013
4	0,0076	0,0066	0,0087	0,0029	0,0008
5	0,0092	0,0037	0,0071	0,0006	0,0011
6	0,0058	0,0069	0,0048	0,0058	0,0003
7	0,0034	0,0057	0,0092	0,0025	0,0009
Mean	0,0087	0,0075	0,0071	0,0048	0,0010
SD	0,0038	0,0024	0,0015	0,0029	0,0005

*With Vitamin E treatment
**Only CCl_4 induced
***Not treated at all

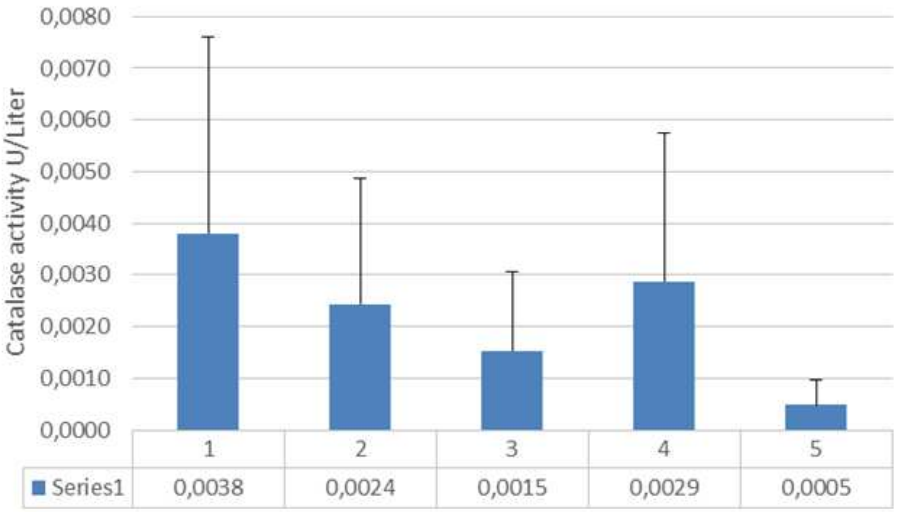

Figure 1. Liver Catalase Activity, group 1 (CCl4 + clove 3), group 2 (CCl4 + clove 1), group 3 (positive control), group 4 (negative control), and group 5 (normal control)

The Anova statistical analysis showed that there is a significant difference between the groups with the (p=0.001). Further test with the Post Hoc showed that there is a significance between group 1 and 4 (p=0.008), 1 and 5 (p=0.001), 2 and 5 (p=0.001), 3 and 5 (p=0.001), and 4 and 5 (p=0.007). From figure 1 we can see that group 1 (CCl4+Clove3) has the highest catalase activity.

DISCUSSION

Catalase is a heme-containing protein used to catalyze the conversion of hydrogen peroxide (H2O2) into water and oxygen molecules so as to protect cells from the toxic effects of hydrogen peroxide. Catalase is mainly used by the body to counteract the effect of oxidative stress.[16-19]

A certain Indonesian cigarette or more commonly known as kretek is the clove/tobacco based cigarette most Indonesians tend to smoke. According to (CDC), among some of Indonesian types of kretek, all of them contained eugenol, most of them contained eugenol only, while some of them contained eugenol and

coumarin, and a few of them contained eugenol and anethole.[8]

Between these three substances eugenol, anethole, and coumarin, Anethol and coumarin are possible carcinogens, with adverse effect exerted to the liver while based from World Health Organization (WHO), an acceptable amount of eugenol consumption is around 2,5 mg/kg body weight per day. Since consensus of the max amount of eugenol that can be in a kretek is 2 mg, assuming that the average weight of a smoker is around 50-70 kg, even if he/ she smokes 10 sticks it will still be within the acceptable margin as WHO prescribe.[7,8]

Based on the results we can see that group 1(CCl4+Clove3) has the highest catalase concentration activity. This result corresponds with the research of liver MDA concentration result run on the same rat subject that has the highest concentration on the day 3. It is assumed that catalase increased activity is a result from adjusting and compensating for the increase of MDA levels based from day 3 group and day 1 group. However, based on the carbonyl concentration from the same rat subject, the highest concentration was on the day 1 group with a lower concentration on day 3. There is a consideration that time is an important factor in how the catalase enzymes react. Based on the results there is an increase of catalase activity which was presumed to be caused by the higher MDA concentration level due to the hepatic damage from the induction of CCL4. Therefore, it can be concluded that the antioxidant effect of the Syzygium aromaticum (clove) is not proven with the dose that was determined in this study.

CONCLUSION

Syzygium aromaticum (clove) oral administration with the dose of 200 mg/kg rat body weight against 0.55 mg/kgBW CCl_4 show increased of catalase activity but did not overcome the oxidative stress.

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