

Artikel

# SGOT/SGPT levels in blood serum on rats (*Rattus norvegicus*) that CCl<sub>4</sub> induced then its treatment by ethanol extract of *Curcuma xanthorrhiza* rhizome as hepatoprotector

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**Abstract:** CCl<sub>4</sub> is a xenobiotic compound that could be entered into body by digestion and ingestion system. It trigger to produce ROS until lipid peroxydation which can increase SGOT/SGPT levels on blood serum. Both SGOT and SGPT are transaminase enzymes that activated on blood serum and they were produced in hepatocyte. If there are radicals in hepatocyte as final product of lipid peroxydation, then they are exit from cytoplasm on hepatocyte to intravenous so theirs levels is increase in blood. Producing of radicals from ROS in lipid peroxydation can be scavenged by antioxidant compounds on ethanol extract of *Curcuma xanthorrhiza* rhizome. This research are aimed to determine of SGOT/SGPT levels on blood serum in each of groups. This research used male rats that divided into five groups, that were negative control (A group), positive control (CCl<sub>4</sub> 10% induced (B group)) and therapy groups (200, 400, and 800 mg/kg BW of *Curcuma xanthorrhiza* rhizome ethanol extracts (C, D, E groups respectively)). SGOT/SGPT levels was analyzed, its result was 165/92, 264/141, 161/64, 130/59 and 135/55 U/L respectively. Based on the Tukey's HSD test ( $p = 0.1$ ), SGOT/SGPT levels in positive control was significance different ( $p < 0.1$ ) to negative control, but theirs levels in 400 and 800 mg/kg BW of *Curcuma xanthorrhiza* rhizome ethanol extract therapy groups were not significance different ( $p > 0.1$ ) to negative control. Thereby, 400 and 800 mg/kg BW of *Curcuma xanthorrhiza* rhizome ethanol extracts was more effective as hepatoprotector which known from decreasing of SGOT/SGPT levels.

**Key Word:** SGOT, SGPT, *Curcuma xanthorrhiza*, hepatoprotector

## 1. Introduction

Carbon tetrachloride (CCl<sub>4</sub>) is a xenobiotic compound, a halogenated hydrocarbon compound. It can be discovered on food, beverage and air with certain dosage. Additionally, it can enter into body by ingestion, inhalation or direct contact with skin. This compound is metabolised by cytochrome P-450 enzyme in hepatocyte endoplasm reticulum. This metabolism cause lipid peroxidation until it formed Reactive Oxygen Species (ROS) radicals which toxic and reactive, such as trichloromethyl (CCl<sub>3</sub>•) and peroxytrichloromethyl (CCl<sub>3</sub>O<sub>2</sub>•) radicals [1,2]. Presence of the radicals in hepatocyte cause the increasing of malondialdehyde (MDA) level, a biomarker of liver (hepatocyte) disorder or

oxidative stress, in other word the radicals increase oxidative stress especially in hepatocyte [3]. The oxidative stress in liver that influenced by the radical trigger transaminase (aminotransferase) enzymes, they are (SGOT) and (SGPT) enzymes in hepatocyte cytoplasm were increase. Excess of these enzymes in hepatocyte cytoplasm exit into intravenous so that these enzymes in blood serum also increase.

A bioactive on *Curcuma xanthorrhiza* rhizome ethanol extract is secondary metabolite compound. It has pharmacology effects as antioxidant and antihepatotoxic [4] to prevent and to protect oxidative stress in hepatocyte that caused by toxic radicals from xenobiotic compounds, such as CCl<sub>4</sub> [5]. As antioxidant, it could decrease the level of oxidative stress in hepatocyte effectively. It was also obstructed Reactive Oxygen Species (ROS) radical accumulation effectively by *in vitro* or it avoid production of free radicals in lipid peroxidation, as toxic neutralizer, increase a gall excretion, and prevent lipid accumulation in hepatocyte [6].

The presence of antioxidant and antihepatotoxic in *Curcuma xanthorrhiza* rhizome ethanol extract assume that it can be used as hepatoprotective drug. We assume that it will able to obstruct lipid peroxidation, so that it also obstruct the producing of Reactive Oxygen Species (ROS) radicals. Therefore, we hope it can decrease malondialdehyde (MDA) level, in other that it can decrease oxidative stress in hepatocyte. If oxidative stress in hepatocyte is decrease then transaminase (aminotransferase) enzymes, they are (SGOT) and (SGPT) enzymes in hepatocyte cytoplasm were also decrease. Based on the explanation, then it will be studied in this research about effect of *Curcuma xanthorrhiza* rhizome ethanol extract within decreasing SGOT and SGPT levels in blood serum on rats that CCl<sub>4</sub> induced.

## 2. Experimental Section

### 2.1 Experimental Animal and Research Design

This research using *Rattus norvegicus* (male, two months, body weight  $\pm$  200 g) were obtained from Animal Physiology Laboratory in Biology Department, Faculty of Science and Technology in Universitas Islam Negeri Maulana Malik Ibrahim Malang. The rats were acclimatized for a month until themselves adapt to surrounding environment. As long as the rats were acclimatized, theirs body weight must be weighed until constant ( $\pm$  200 g). Using of experimental animals in this research have obtained ethical approval from Animal Care and Use Committee Brawijaya University No: 443-KEP-UB. The experimental animals were divided into five groups, there were negative control (normal) group (A group), positive control group (they were induced by 1 mL/kg BW CCl<sub>4</sub> 10%) (B group), therapy of *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 200, 400, and 800 mg/kg BW groups (C, D, and E groups respectively). At each of group was contained four rats. Before the therapy groups (C, D, and E groups) were therapied, they were induced by 1 mL/kg BW CCl<sub>4</sub> 10% and then they were therapied. CCl<sub>4</sub> was induced on rats by intraperitoneal injection. Therapy of *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 200, 400, and 800 mg/kg BW was given to C, D, and E groups respectively once a day for two weeks.

### 2.2 Preparation of *Curcuma xanthorrhiza* rhizome ethanol extract

*Curcuma xanthorrhiza* rhizome was obtained and it have certified from UPT Materia Medica Batu Malang No: 074/104/101.8/2015. It was needed as much as 3.32 kg. Then, it was

made become *Curcuma xanthorrhiza* rhizome powder. After that, it was extracted with ethanol 96% (Sigma Aldrich) for 48 hours by maceration method until it was got extract. Furthermore, it was separated until got filtrate. It was concentrated with vaccum rotary evaporator until got crude extract of *Curcuma xanthorrhiza* rhizome then it was prepared into 200, 400, and 800 mg/kg BW *Curcuma xanthorrhiza* rhizome ethanol extract used corn oil as solvent.

### 2.3 Blood Serum Preparation

Rats in each of group were dissected then bleeding on theirs heart by syringe. To take blood serum, the blood as much as 2 mL was moved into vacutainer. It was kept at room temperature in  $\pm 45^\circ$  (slope position) until serum was separated ( $\pm 15$  minutes). The serum was moved into microtube. Then, it was centrifuged (serum reseparation from pellet) at 6000 rpm for 10 minutes to take pure serum (without impurity). Serum was could kept at  $-20^\circ\text{C}$  in refrigerator. This serum was used to assay SGOT/SGPT levels.

### 2.4 SGOT/SGPT Levels Assay

In SGOT/SGPT levels assay was used SGOT and SGPT reagent. Each of reagents was consist of the 1<sup>st</sup> reagent and the 2<sup>nd</sup> reagent. SGOT reagent (Sigma Aldrich) was gotten of the 1<sup>st</sup> reagent (Tris buffer, L-aspartate, malate dehydrogenase (MDH), and lactate dehydrogenase (LDH)) to the 2<sup>nd</sup> reagent (2-oxoglutarate and NADH) with ratio 4:1 (the 1<sup>st</sup> reagent : the 2<sup>nd</sup> reagent). SGPT reagent (Sigma Aldrich) was gotten of the 1<sup>st</sup> reagent (Tris buffer, L-alanine, and lactate dehydrogenase (LDH)) to the 2<sup>nd</sup> reagent (2-oxoglutarate and NADH) with ratio 4:1 (the 1<sup>st</sup> reagent : the 2<sup>nd</sup> reagent).

To assay SGOT levels, 100  $\mu\text{L}$  blood serum was added 1000  $\mu\text{L}$  SGOT reagent then it was mixed until homogenized by vortex at room temperature. After that, it was incubated at  $37^\circ\text{C}$  for a minute then it was also kept at room temperature for a minute, and the absorbance of the mixture was measured at  $\lambda_{\text{max}}$  (340 nm) photometrically in the 1<sup>st</sup> minute. This step was done at each minute until the 3<sup>rd</sup> minute. Next, the absorbance was converted into SGOT level.

To assay SGPT levels, 100  $\mu\text{L}$  blood serum was added 1000  $\mu\text{L}$  SGPT reagent then it was mixed until homogenized by vortex at room temperature. After that, it was incubated at  $37^\circ\text{C}$  for a minute then it was also kept at room temperature for a minute, and the absorbance of the mixture was measured at  $\lambda$  (340 nm) photometrically in the 1<sup>st</sup> minute. This step was done at each minute until the 3<sup>rd</sup> minute. Next, the absorbance was converted into SGPT level.

### 2.5 Data Analysis

Data result of SGOT/SGPT levels assay in blood serum was analyzed by One Way ANOVA Test (significance ( $p = 0.1$ )) using SPSS v.16. If there are significance result

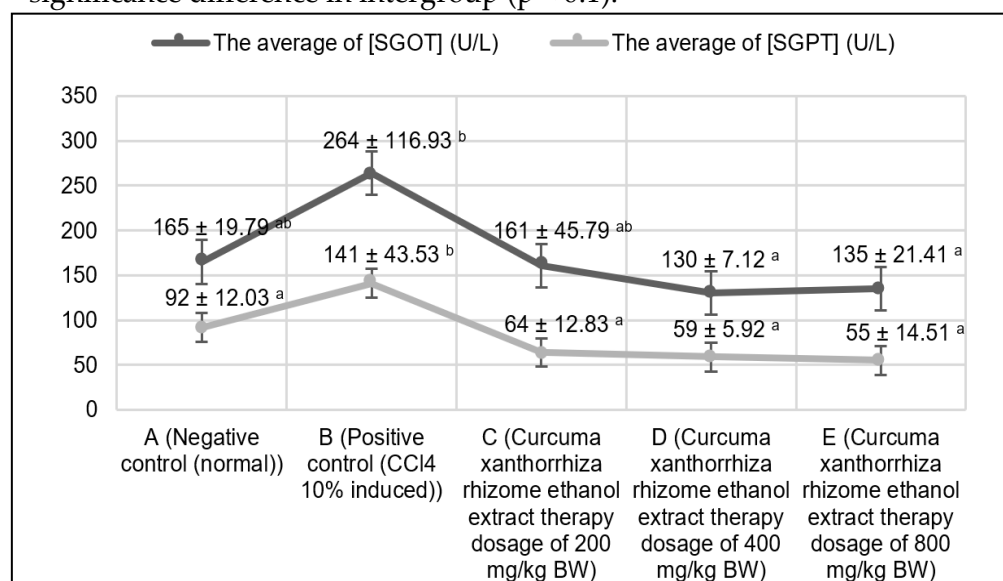
difference intergroups, then it was also reanalyzed by Tukey HSD (Post Hoc Test) (significance ( $p = 0.1$ )) using SPSS v.16.

### 3. Result and Discussion

#### 3.1 Results

Based on the Figure 1, it showed that  $\text{CCl}_4$  10% was able to increase SGOT and SGPT levels in blood serum to their levels on normal condition (negative control) from 165 U/L become to 264 U/L (on SGOT level) and 92 U/L become to 141 U/L (on SGPT level). This case showed that SGOT and SGPT levels in blood serum on positive control ( $\text{CCl}_4$  10% induced) group (B group) was significance different ( $p < 0.1$ ) to negative control (normal) group (A group). After it was therapied by *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 200 mg/kg BW (C group), 400 mg/kg BW (D group) and 800 mg/kg BW (E group), SGOT and SGPT levels was decrease become 161, 130, and 135 U/L (on SGOT level), and 64, 59, and 55 U/L (on SGPT level) respectively. The data was appropriate with Sacher and McPherson statements that hepatocyte is contain more and more SGOT than SGPT [7]. Based on the Figure 1 that after therapied by *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 200, 400 and 800 mg/kg BW respectively then SGOT and SGPT levels was decrease until their levels approach SGOT and SGPT levels in normal condition (negative control). Therefore, therapied by *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 400 and 800 mg/kg BW was more effective to decrease SGOT and SGPT levels than its therapy dosage of 200 mg/kg BW.

**Fig. 1.** SGOT/SGPT levels on blood serum in rats (*Rattus norvegicus*). <sup>a</sup> not significance different, <sup>b</sup> significance different. The difference of notation showed the presence of significance difference in intergroup ( $p < 0.1$ ).



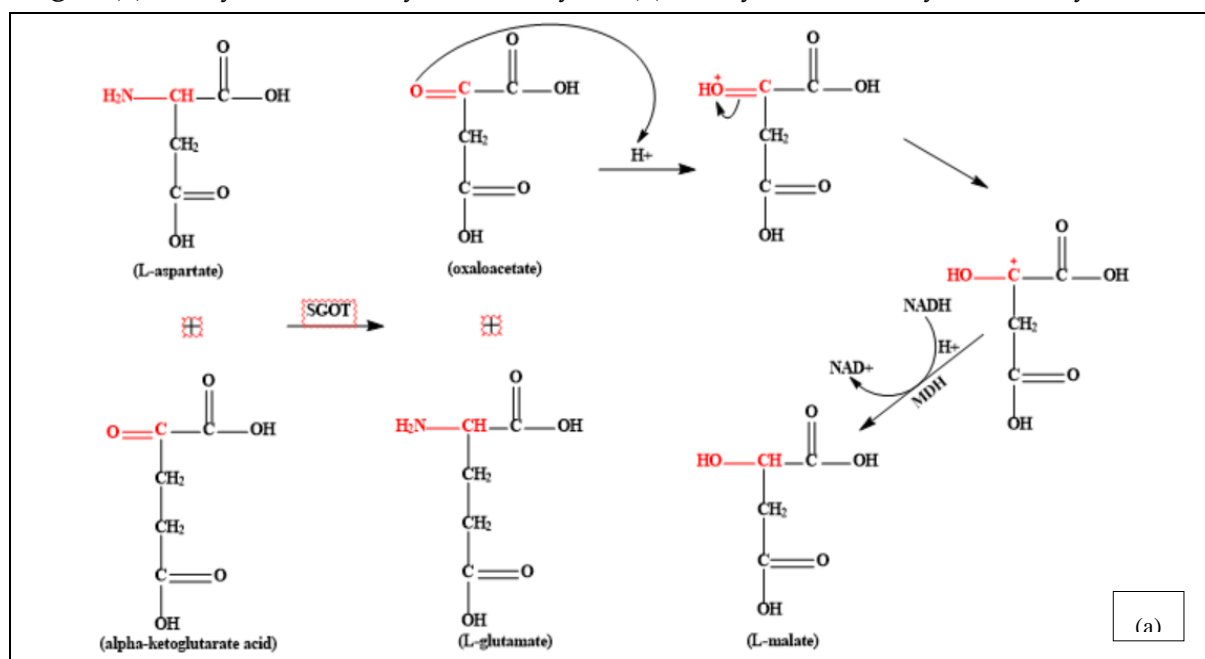
#### 3.2 Discussion

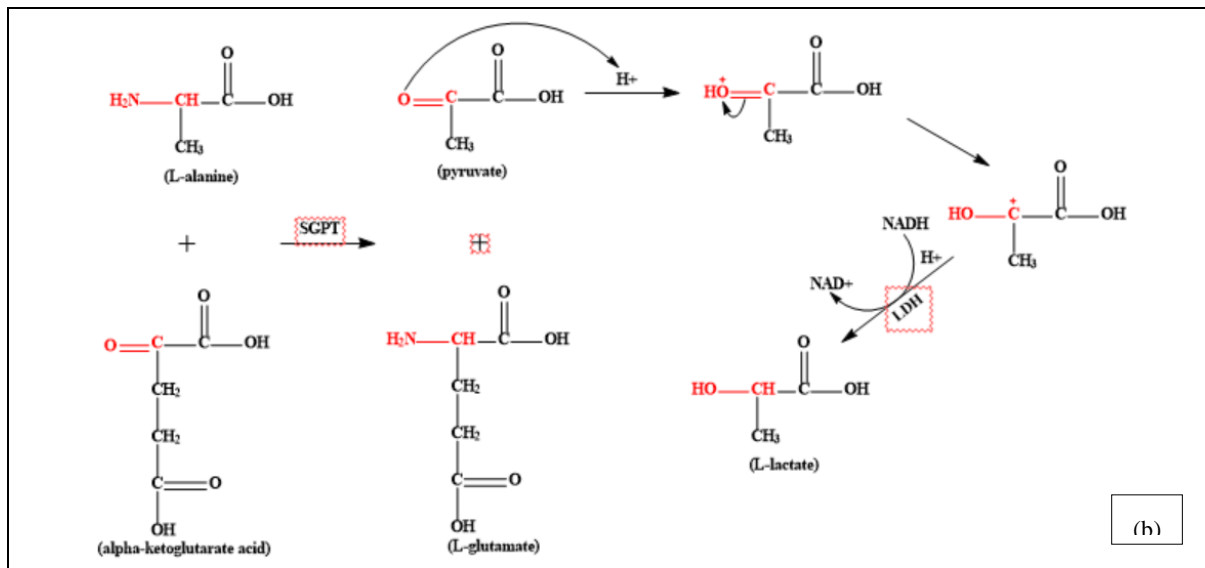
Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) are transaminase enzymes. They are classified as aminotransferase

enzyme. SGOT enzyme on body is in cytoplasm and mitochondrion of hepatocyte, whereas SGPT enzymes on body is only in hepatocyte cytoplasm [8-10]. These enzymes are used as screening enzymes or diagnostic biomarker of liver (hepatocyte) disorder [11]. If these enzymes are more increase in blood serum then liver (hepatocyte) disorder is also more increase [10, 12]. Both SGOT and SGPT levels are analyzed use enzym kinetical method by International Federation of Clinical Chemistry (IFCC) [13-14]. SGOT is one of a transaminase enzyme to catalyze transfer of amino group ( $-\text{NH}_2$ ) on L-aspartate into  $\alpha$ -ketoglutarate acid to produce L-glutamate and oxaloacetate. Then, oxaloacetate is redacted into L-malate by malate dehydrogenase (MDH) and be accompanied by converting NADH into  $\text{NAD}^+$ . Whereas, SGPT is one of a transaminase enzyme to catalyze transfer of amino group ( $-\text{NH}_2$ ) on L-alanine into  $\alpha$ -ketoglutarate acid to produce L-glutamate and pyruvate. Then, pyruvate is redacted into L-lactate by lactate dehydrogenase (LDH) and be accompanied by converting NADH into  $\text{NAD}^+$  [13,15].

Based on the result data on this research (Figure 1),  $\text{CCl}_4$  is able to increase both SGOT and SGPT levels in blood serum, it was known from the increasing of both SGOT and SGPT levels on B grup (positive control ( $\text{CCl}_4$  10% induced)) to A grup (negative control (normal)). There are significance different ( $p < 0.1$ ) between A and B groups, in other word both SGOT and SGPT levels in A and B groups have different notation. So that, we could say that  $\text{CCl}_4$ , a xenobiotic compound that induce into body can cause the increasing of both SGOT and SGPT levels in intravenous from their normal levels.

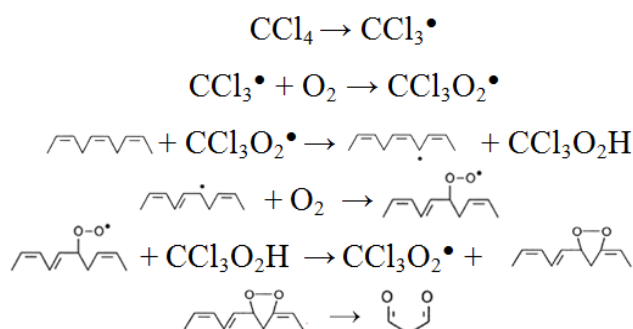
**Fig. 2.** (a) Catalytic reaction by SGOT enzyme. (b) Catalytic reaction by SGPT enzyme.





Liver is central of metabolism process in body. So, when the body was induced by a xenobiotic compound, such as CCl<sub>4</sub> then it was metabolised by cytochrome P-450 enzyme in hepatocyte endoplasm reticulum by mechanism of lipid (PUFA) peroxidation chain reaction (Figure 3). In this reaction, it produced trichloromethyl (CCl<sub>3</sub>•) radical which toxic and reactive. Then, its radical is reacting with oxygen that be triggered by Reactive Oxygen Species (ROS) formed more reactive radical is a peroxytrichloromethyl (CCl<sub>3</sub>O<sub>2</sub>•) than the previous radical [16-18]. At the formation of lipid radical, lipid peroxy radical is reacting continuously by lipid peroxidation chain reaction. This case cause that it against Polyunsaturated Fatty Acid (PUFA) in lipid bilayer membrane by getting hydrogen atom from PUFA to form hydroperoxide lipid so that stabilization achieved [2, 17-19]. Then, it formed malondialdehyde (MDA) as the final product of lipid (PUFA) peroxydation that is toxic and reactive metabolite to cell, and it cause an oxidative stress in liver tissue [20]. Oxidative stress in liver tissue that was caused by a xenobiotic compound (CCl<sub>4</sub>) is also trigger the increasing of transaminase (aminotransferase) enzymes levels, they are SGOT and SGPT in hepatocyte (liver). Because their levels were over (abnormal) in hepatocyte (liver), therefore both SGOT and SGPT enzymes in hepatocyte (liver) were exit from cytoplasm on hepatocyte (liver) into intravenous so that their levels in intravenous (blood serum) are increase [11].

**Fig. 3.** Mechanism of lipid (PUFA) peroxidation chain reaction by CCl<sub>4</sub> which entered into body form malondialdehyde (MDA).



Then they were therapied by *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 200, 400 and 800 mg/kg BW respectively once a day for two weeks. This result was both SGOT and SGPT levels was decrease until their levels approach the value of SGOT and SGPT levels (negative control, in A group). There are not significance different ( $p > 0.1$ ) between C, D, and E groups to A group, in other word both SGOT and SGPT levels in theirs groups have same notation to A group. So that, we could say that bioactive which be contained in *Curcuma xanthorrhiza* rhizome ethanol extract able to decrease transaminase (aminotransferase) enzyme levels, they are SGOT and SGPT levels until their levels was normal if body was induced by a xenobiotic compound (such as,  $\text{CCl}_4$ ). In other word, bioactive which be contained in *Curcuma xanthorrhiza* rhizome ethanol extract able as antioxidant within scavenge free radicals that be obtained as final products from mechanism of lipid (PUFA) peroxidation chain reaction).

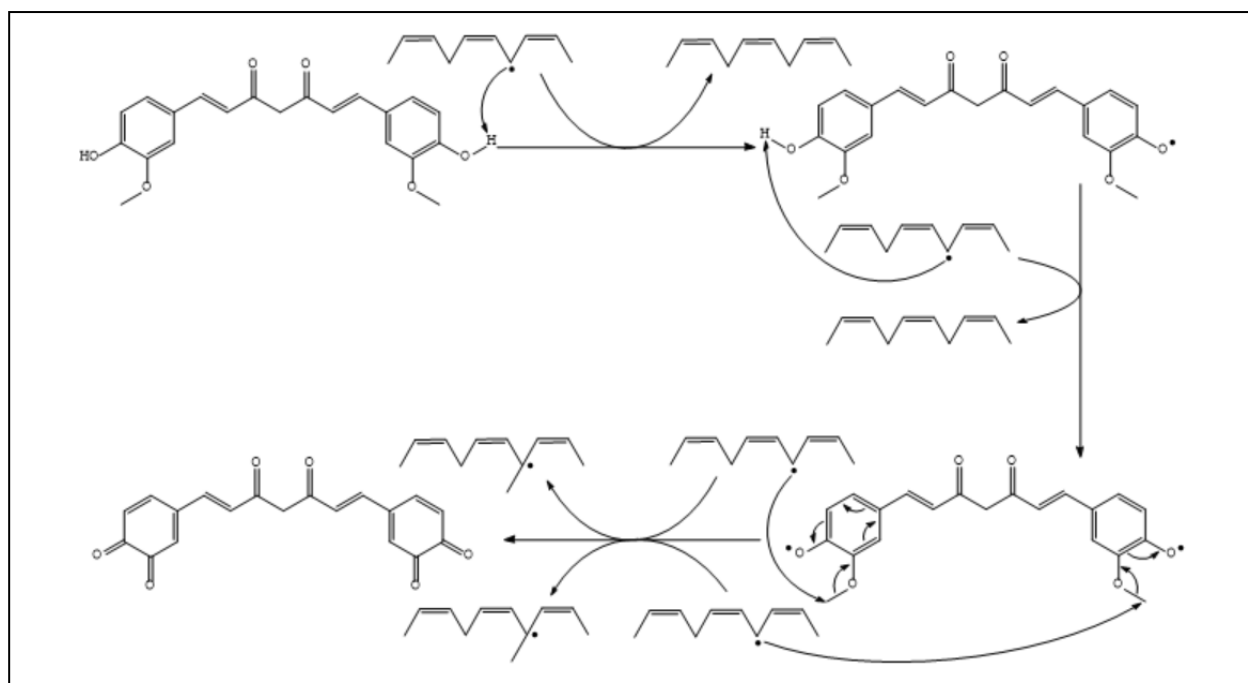
In C, D, and E groups, when the body was induced by a xenobiotic compound (such as  $\text{CCl}_4$ ) then this compound was metabolised in hepatocyte endoplasm reticulum by mechanism of lipid (PUFA) peroxidation chain reaction until it was form free radicals (oxidant) which toxic and reactive (Figure 3). If availability of the oxidant was over in hepatocyte endoplasm reticulum and it was also unballanced to availability of the antioxidant then it was caused oxidative stress in liver tissue. However, if it was therapied by *Curcuma xanthorrhiza* rhizome ethanol extract directly, then antioxidant (bioactive) that be contained in *Curcuma xanthorrhiza* rhizome ethanol extract able to scavenge free radicals (oxidant) by mechanism of free radical scavenger reaction (Figure 4).

Therapy using a bioactive on *Curcuma xanthorrhiza* rhizome ethanol extract as antioxidant [21] is effective to decrease the level of oxidative stress in liver tissue. Its antioxidant also as an antiradical to scavenge lipid radical ( $\text{R}^\bullet$ ) [22] based on the Figure 4, where it is donating its hydrogen atom to lipid radical ( $\text{R}^\bullet$ ) formed phenoxyl antioxidant by pressing the activity of cytochrome P-450 enzyme. A phenoxyl antioxidant is less reactive than lipid radical ( $\text{R}^\bullet$ ) because it stabilized by resonance effect. It is reacting continuously to result more stable compound than lipid radical ( $\text{R}^\bullet$ ) by termination reaction in mechanism of free radical scavenger reaction by antioxidant. Thereby, by this reaction then bioactive on *Curcuma xanthorrhiza* rhizome ethanol extract as antioxidant is effective to scavenge peroxidation reaction which initiated by the forming of free radicals (oxidant) or lipid radical ( $\text{R}^\bullet$ ).

Based on the whole of result in this research, reduction of oxidative stress after therapy use bioactive on *Curcuma xanthorrhiza* rhizome ethanol extract indicate that the bioactive (antioxidant) was able to scavenge free radicals (oxidant) which was caused by  $\text{CCl}_4$  (a xenobiotic compound). We could say that availability between oxidant and antioxidant was balanced in hepatocyte. As consequence, transaminase (aminotransferase) enzymes levels,

they are SGOT and SGPT in hepatocyte cytoplasm more decrease until their levels approach normal level of SGOT and SGPT. Therefore, transaminase (aminotransferase) enzymes, they are SGOT and SGPT were not exit from hepatocyte cytoplasm into intravenous, in other word both SGOT and SGPT levels in blood serum was normal again.

**Fig 4.** Mechanism of free radical scavenger reaction by antioxidant (bioactive) on ethanol extract of *Curcuma xanthorrhiza* rhizome.



#### 4. Conclusion

Bioactive on *Curcuma xanthorrhiza* rhizome ethanol extract dosage of 400 and 800 mg/kg BW as hepatoprotector, it was able to decrease both SGOT and SGPT levels in blood serum which was effected oxidative stress by CCl<sub>4</sub> induced until the transaminase (aminotransferase) enzymes levels approach normal level of SGOT and SGPT.

#### 5. Acknowledgement

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