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Chapter 11

Effect of Different Concentrations of Red Palm Olein and Different Vegetable Oils on Antioxidant Enzymes in Normal and Stressed Rat

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Additional information is available at the end of the chapter

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1. Introduction

Oxygen radicals are continuously formed in all living organisms, with deleterious effects that lead to cell injury and death. Production of oxidative species occurs under physiological conditions at a controlled rate, but it is dramatically increased in conditions of oxidative stress. Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals [1, 2]. Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals [3, 4]. Vitamin E compounds (tocopherols and tocotrienols) are well recognized for their effective inhibition of lipid oxidation in food and biological systems [5, 2]. Carotenoids can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen. In foods, carotenoids usually act as a secondary antioxidant. Beta-carotene is found in many foods that are orange in color [6].

Stress plays a significant role in the development of atherosclerotic heart disease (AHD) [7]. A stressful condition leads to the excessive production of free radicals which results in oxidative stress an imbalance in the oxidant per antioxidant system [8]. Under normal conditions, there is a natural defense system provided by several enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) which performs a vital role for detoxification of free radicals. The use of antioxidant rich food or antioxidant food supplements became immensely popular since many diseases have been associated with oxidative stress [9]. Antioxidant enzyme such as superoxide dismutase (SOD) is an important radical superoxide scavenger and it plays an important role in cell protection [10, 2]. Therefore, this CAT or SOD enzyme are very good biochemical markers of stress and their increased activity may attest to a potential for remediation [11]. Inherent

antioxidant defense systems consisting of enzymes, such as catalase (CAT), and superoxide dismutase (SOD), an antioxidant nutrients may participate in coping with oxidative stress. As antioxidant enzymes have an important role in the protection against free radical damage, a decrease in the activities or expression of these enzymes may predispose tissues to free radical damage [12].

Red palm oil (RPO) is extracted from the oil palm (*Elaeis guineenis*) fruit [13, 14]. It derives its red colour from the high content of alpha- and beta-carotenes, which can make up 0.08% (w/w) of the crude oil [13]. Red palm oil is the oil obtained before refining and the characteristic colour of RPO is due to the abundance of carotenoids (500 – 700 mg /L) in the crude oil [15, 16]. Most people are not aware of the fact that many different kinds of vitamin E occur in nature and that some forms of vitamin E are more beneficial than others. Red palm oil contains vitamin E tocotrienols, which acts as a super-antioxidant and the carotenoids in red palm oil also act as antioxidants [17].

Corn oil presents a relatively high concentration of polyunsaturated fatty acids (PUFA).Due to the high levels of unsaturation these lipids are highly susceptible to free radical oxidative reactions, giving rise to the formation of lipid peroxides. Many investigations suggest that a large number of polyunsaturated fatty acids produces more lipid peroxides and may have mutagenic activity [18, 19].

Coconut oil is a colorless to pale, brownish yellow oil [20]. It is the major sources of saturated fat apart from palm kernel. They are the only natural sources of lauric oil available to the world market. Coconut oil is the principal cholesterol-raising fat because it contains large amounts of lauric (C: 12: O) and myristic (C: 14: 0) acids [21]. Therefore the objective of this study is to investigate the effect of different concentration of red palm olein and different vegetable oils on antioxidant enzymes in normal and stressed rats.

2. Problem statement

Due to the importance of the role of antioxidants in protection against the oxidative stress which lead to many dangerous diseases such as heart diseases and cancer thus this study was done to investigate the effect of natural antioxidants particularly vitamin E and beta carotene in red palm olein on antioxidant enzymes and compared the results with four different vegetable oils in normal and stress conditions of rats.

3. Effect of different vegetable oils on antioxidant enzymes in normal and stressed rats

The evaluated red palm olein (RPO) samples consisted of carotenes (576 ppm), vitamin E (>800 ppm) and free fatty acids (0.045%) provided by Carotino SDN BHD company and palm olein (PO) (Seri Murni), corn oil (CO) and coconut oil (COC) were obtained commercially. For the first group the test diet was prepared by mixing RPO with normal commercial rat pellet to contain 5%, 10% and 15% of the red palm olein (RPO). The 5% diet was prepared by adding 5g RPO to 95g rat pellet, and mixed manually and the diets were

then left to absorb the RPO at room temperature overnight and stored at 20° C before the feeding trial was conducted. Similar process was conducted with 10%, and 15% RPO. For second group the test diet was prepared by mixing vegetable oils with normal commercial rat pellet to contain 15% of the vegetable oils. The 15% diet was prepared by adding 15g RPO, PO, CO or COC to 85g rat pellet, and mixed manually and the diets were then left to absorb the vegetable oils at room temperature overnight and stored at 20° C before the feeding trial was conducted.

Normal (N) group: Rats were maintained under standard laboratory conditions and fed with respective diet till the completion of the experiment.

Figure 1. Normal rats

Stress (S) group: Rats were restrained by placing them in individual nylon plastic bag for 3 hr/day for one week before killing. Under these conditions rat were fed with respective diet, till the completion of the experiment.

Figure 2. Stressed rats

One hundred and eighty Sprague Dawley male rats each weighing between 170-250g and approximately 80 days old were obtained from the animal house of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. They were divided into three groups.

The first group contains 78 rats were divided into 13 groups of 6 rats per group. The rats were fed *ad libitum* with commercial rat's pellet containing different concentrations of red palm olein (RPO) for 2, 4 and 8 weeks. The second group contains 66 Sprague Dawley male rats which were randomly divided into 11 groups of 6 rats per group and were treated with 15% of RPO, palm olein (PO), corn oil (CO), coconut oil (COC) and control groups for 4 and 8 weeks. The third group contains 36 Sprague Dawley male rats which were randomly divided into six groups of 6 rats per group (3 normal groups and 3 stressed groups) and were treated with 15% of RPO and PO for 4 weeks. At the end of the experiment, after 2, 4 or 8 weeks of treatment the feeding of rats was stopped and the rats were fasted for 18 hours. They were anesthetized using chloroform. The liver was removed immediately and was washed it with NaCl solution. It was stored at -80^{o}C until analyzed.

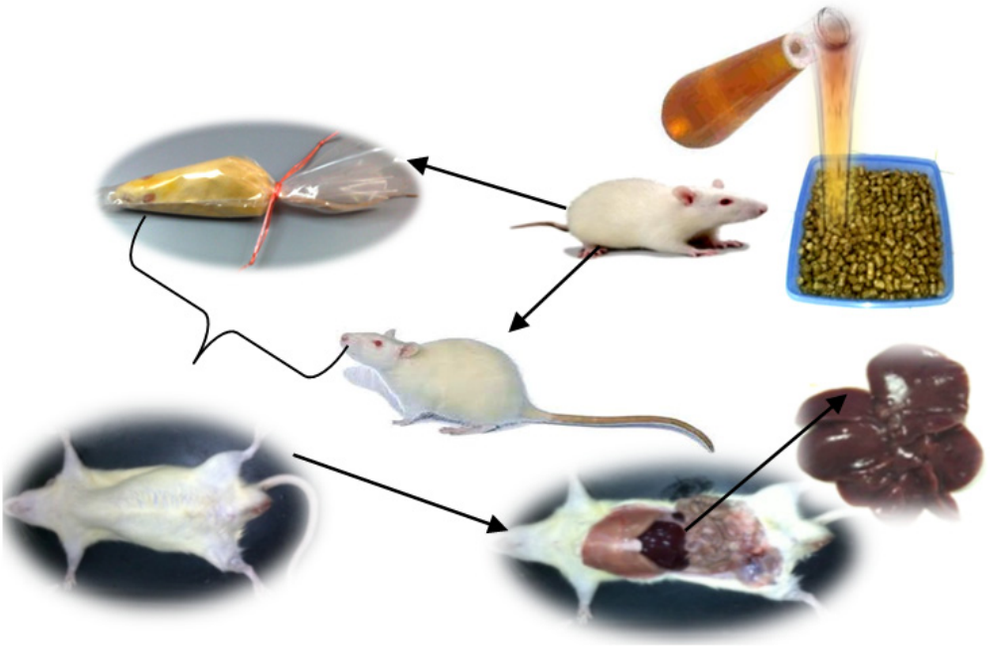

Figure 3. Procedure for collecting liver from rat

A 0.2 g sample of liver was cut to small pieces. Tissue was suspended in 2 ml of 50 mM phosphate buffer (pH 7.4), and was homogenized using a mixer at top speed for 3 min. Afterwards, the homogenate was centrifuged at 20000 g for 25 min. In this process the temperature was maintained at 4C^{0} during the homogenization process. Phosphate buffer was prepared based on Aebi's method [22]. Phosphate buffer 50 mM, PH 7.0: dissolve (a) 6.81 g KH_2PO_4, and (b) 8.90 g $Na_2HPO_4.2H_2O$ in distilled water and make up to 100 ml each mix solution (a) and (b) in proporation 1:1.5 (v/v).

Enzyme activity of catalase (EC.1.11.1.6) was determined based on Aebi's method [22]. Catalase activity was measured at 22°C by monitoring the decomposition of hydrogen peroxide. The reaction mixture consisted of 2.0 mL of the liver homogenate suspended in phosphate buffer (50 mM, pH 7.0), and 1.0 mL of hydrogen peroxide solution (30 mM). The absorbance was recorded for 2 minutes at 240 nm immediately after adding hydrogen

peroxide solution. Catalase activity was expressed as moles of hydrogen peroxide reduced/min/mg protein.

Activity superoxide dismutase (EC.1.6.4.2) was assayed based on the method of Marklund and Marklund [23]. Superoxide dismutase activity was determined at 22°C by using the pyrogallol. The reaction mixture consisted of 50 mM of cacodylic acid buffer pH 8.2, containing 1mM EDTA, 300 μl of liver homogenate, 300 μl of 0.2 mM pyrogallol. The absorbance was recorded for 3 minutes at 420 nm immediately after adding the pyrogallol solution. Superoxide dismutase activity was expressed as units of SOD/minute/mg protein.

Protein concentrations were determined based on the Lowry method [24]. To 0.1 mL of sample or standard was added 0.1 mL of 2 *N* NaOH and hydrolyze at 100°C for 10 min in boiling water bath. The hydrolysate was cooled to room temperature and added 1 mL of freshly mixed complex-forming reagent. Let the solution stand at room temperature for 10 min. After that, 0.1 mL of Folin reagent was added using a vortex mixer, and let the mixture stand at room temperature for 30–60 min. The absorbance was recorded at 750 nm. Figure 4 showed the standard curve of absorbance which was plotted as a function of initial protein concentration and used it to determine the unknown protein concentrations.

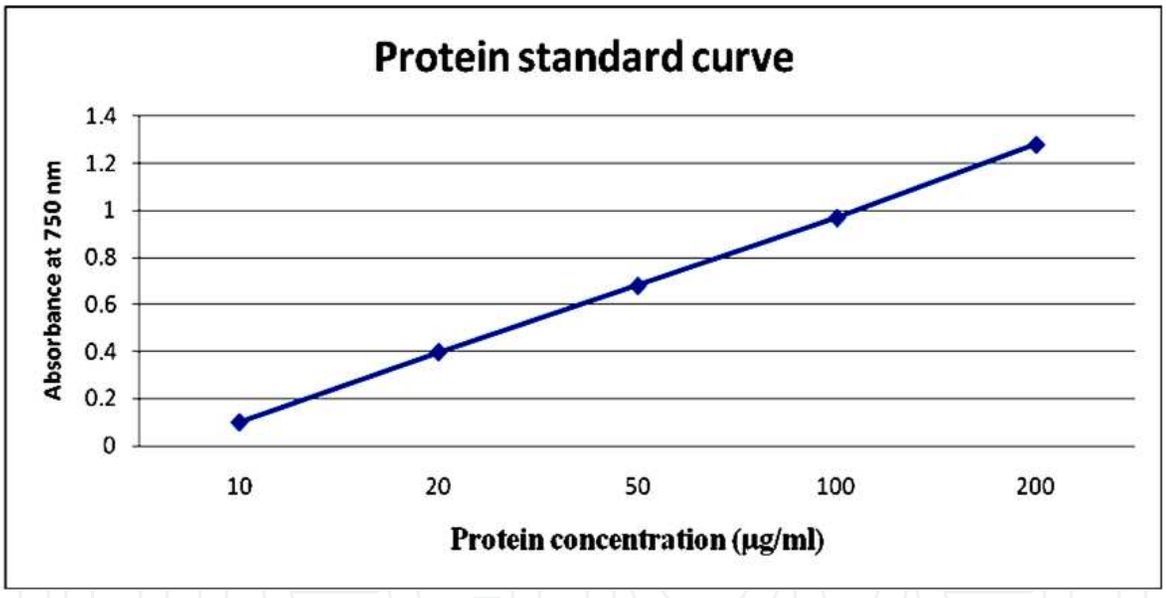

Figure 4. Protein standard curve

3.1. Effect of different concentrations of red palm olein on antioxidant enzyme of normal rat liver

Antioxidant is an important part of a cells defense against free radical damage. Antioxidant enzymes, in particular, constitute a major part of this defense [25]. It is evident from earlier work that different concentrations RPO have differential effects on the activities of antioxidant enzymes [10]. Figures 5, 6 and 7 showed the results of catalase activity at different concentrations of RPO (5%, 10% and 15%) for different times (2, 4 and 8 weeks) of treatment. After 2 weeks there was no significance difference ($p \geq 0.05$) between the control

group and 10% and 15% concentrations of RPO while at 5% there was an increased in the catalase activity. At 4 weeks there was no significance difference ($p \geq 0.05$) between the control group and different concentrations groups (5%, 10%, and 15%) of RPO. At 8 weeks there was no significance difference between the control group and 5% group while at 10% and 15% there was decreasing of the catalase activity but there was no significance difference ($p \geq 0.05$).

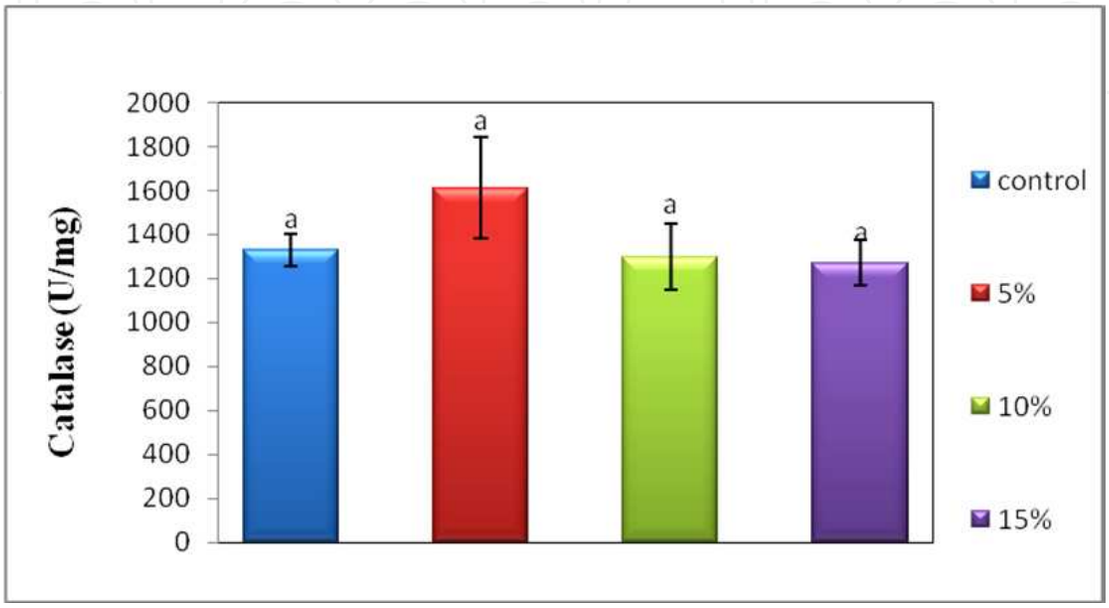

Figure 5. The catalase activity (u/mg) in liver of rats fed with different type of red palm oil (0%, 5%, 10% and 15%) for 2 weeks. Bars are mean ± SEM (n=6), no significantly different ($p>0.05$).

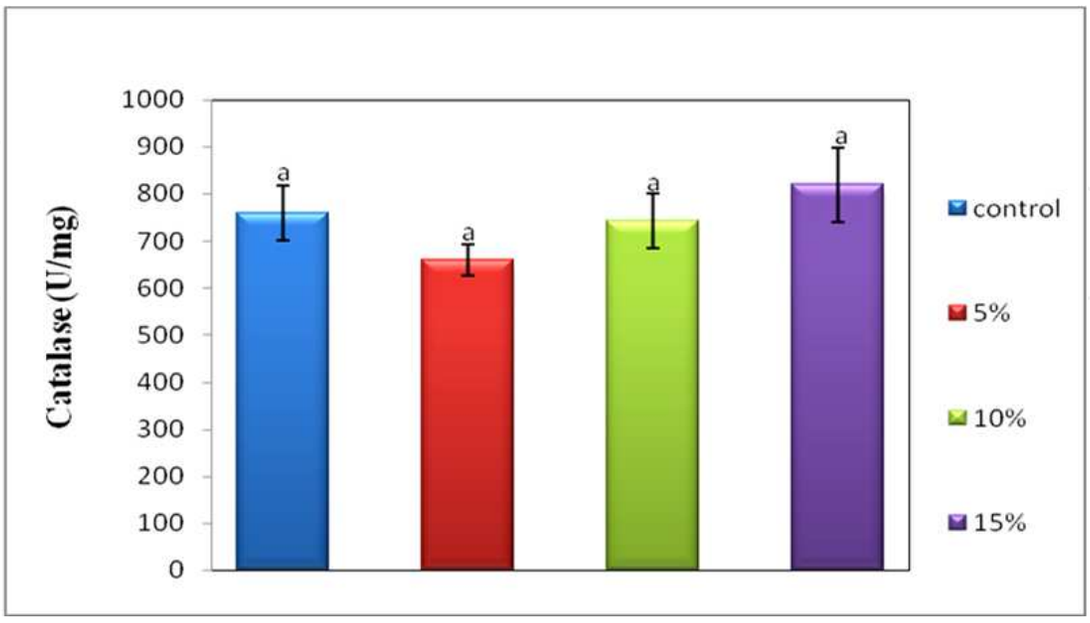

Figure 6. The catalase activity (u/mg) in liver of rats fed with different type of red palm oil (0%, 5%, 10% and 15%) for 4 weeks. Bars are mean ± SEM (n=6), no significantly different ($p>0.05$).

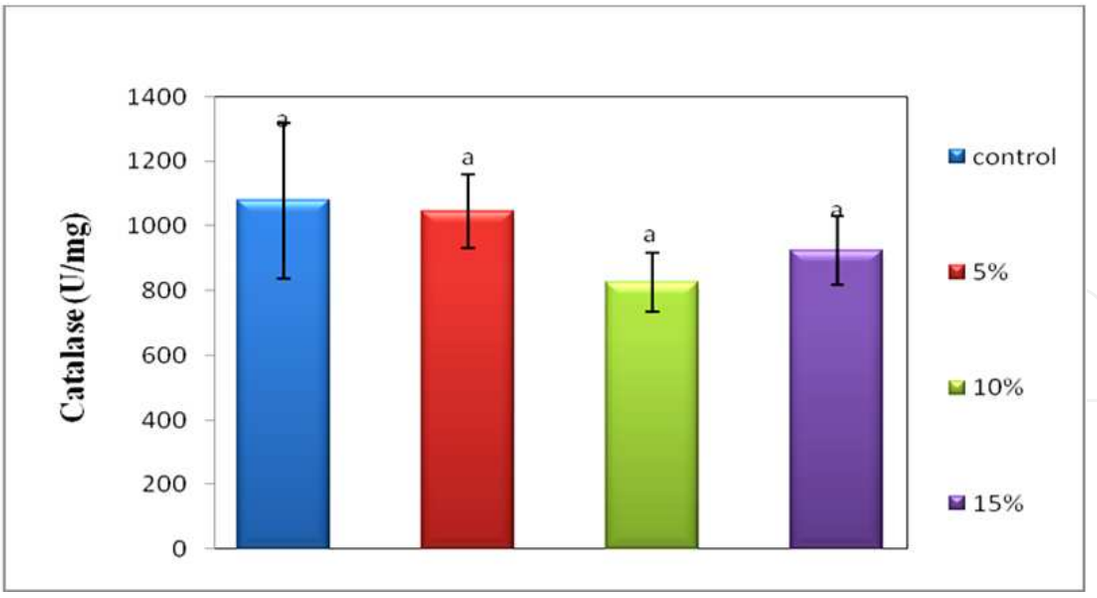

Figure 7. The catalase activity (u/mg) in liver of rats fed with different type of red palm oil (0%, 5%, 10% and 15%) for 8 weeks. Bars are mean ± SEM (n=6), no significantly different ($p>0.05$).

Figures 8, 9 and 10 showed the results of SOD activity at different concentration of RPO (5%, 10% and 15%) for different times (2w, 4w and 8w) of treatment. After 2 weeks there was an increased in SOD activity at 5% while there was a decreased in SOD activity at 10% and 15% groups. However, there were no significance differences ($p\geq0.05$) among these groups. On the contrary at 4 weeks the SOD activity increased with increasing duration of treatment in all concentrations compared to the control group. There was no significant ($p\geq0.05$) increased in SOD activity at 15% concentration of RPO.

At 8 weeks there was no significant difference ($p\geq0.05$) between the control group and all treatment groups of RPO except there was decreased in SOD activity at 15% of RPO. Therefore, after 4 weeks the activity of SOD was significantly higher ($p\leq0.05$) at 15% of RPO dietary group compared to the control group but the increase in the 10% of RPO dietary group was not statistically significant. On the other hand, there was a significantly decreased ($p<0.05$) in 15% of RPO dietary group after 2 and 8 weeks.

The results of this study showed that 15% treatment of RPO which contain β-carotene and vitamin E for 4 weeks may enhance the antioxidant enzyme (SOD) defence system. These results thus suggest that a combination of carotenoids and vitamin E (tocopherol and tocotrinol) in the RPO has an important role in the protection against free radical damage. Red palm oil contains the highest concentration of tocotrienols compared to other vegetables or plants and the tocotrienols can be 40-60 times more potent as anti-oxidant than tocopherols [26]. Tocotrienols are free radical scavenging antioxidants, however, only the α-isomer has considerable biological antioxidant activity. It is therefore not surprising that there are relatively very few studies on their antioxidative effects in oils and fats [2, 26].

Although a few of studies explicitly show the effects of vitamin E on the activities of antioxidant enzymes, there is no consensus on what might be the responses of antioxidant

enzymes to vitamin E, partly because of different feeding behavior and other ecological conditions [27].

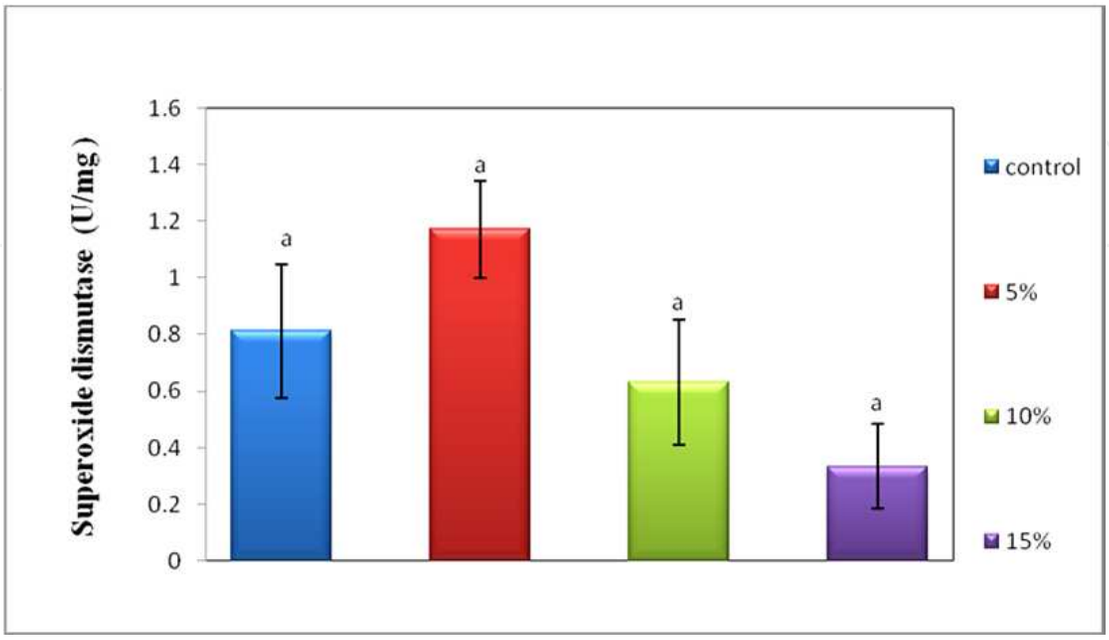

Figure 8. The superoxide dismutase (SOD) activity (u/mg) in rat liver fed with different type of red palm oil (0%, 5%, 10% and 15%) for 2 weeks. Bars are mean ± SEM (n=6), no significantly different ($p>0.05$) at all treated groups with RPO.

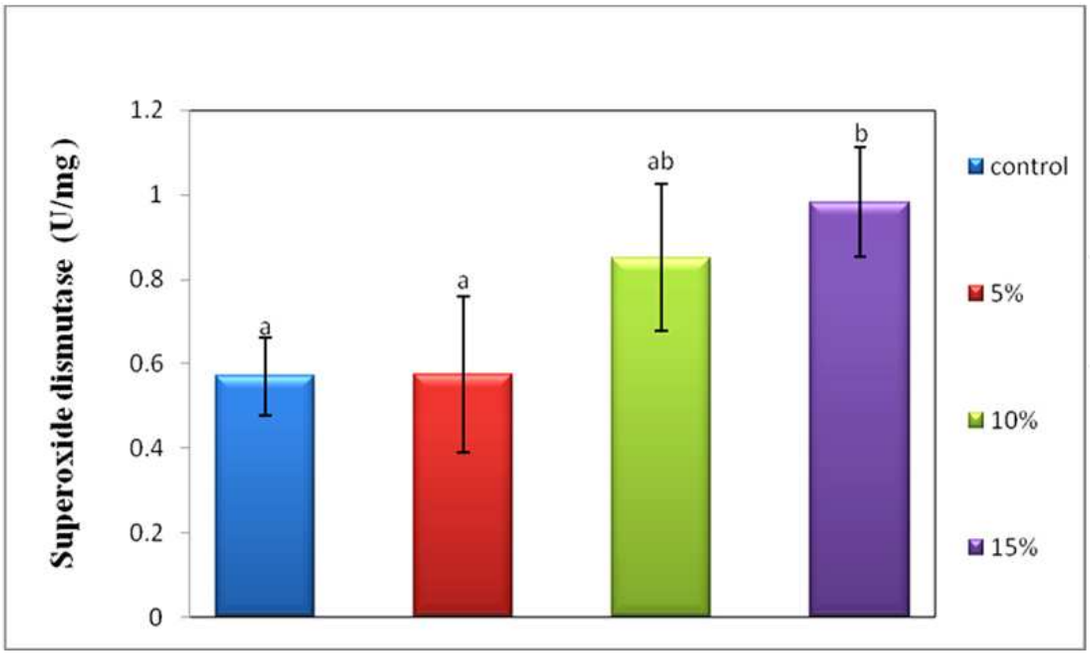

Figure 9. The superoxide dismutase (SOD) activity (u/mg) in rat liver fed with different type of red palm oil (0%, 5%, 10% and 15%) for 4 weeks. Bars are mean ± SEM (n=6), no significantly different ($p>0.05$) at 5% and 10%, significantly different ($p<0.05$) at 15%

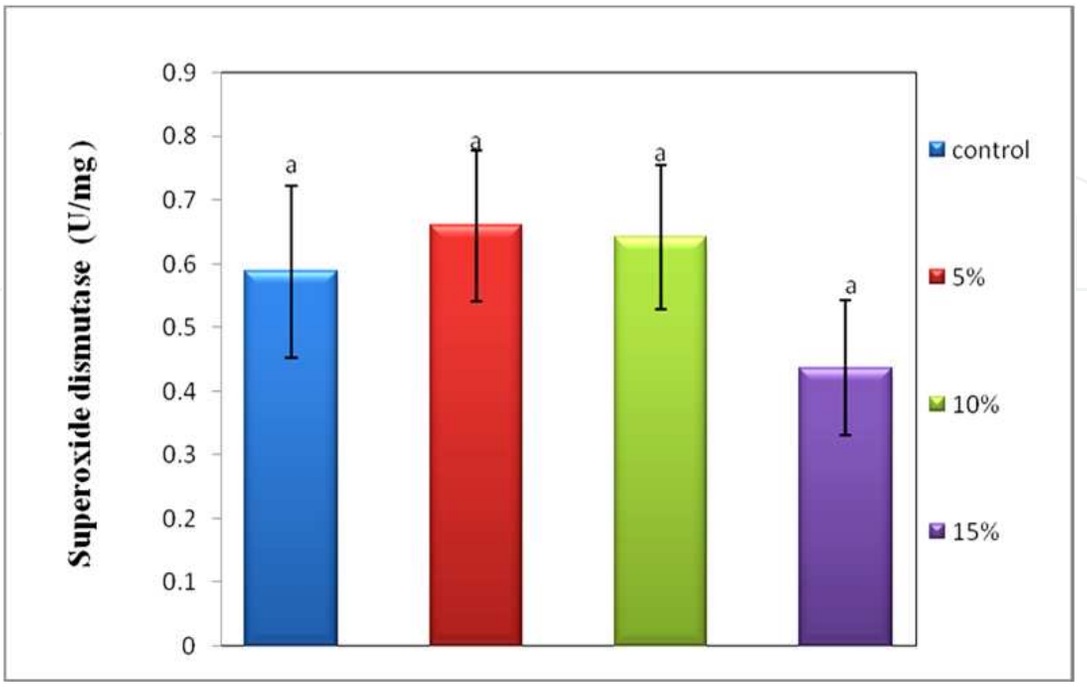

Figure 10. The superoxide dismutase (SOD) activity (u/mg) in rat liver fed with different type of red palm oil (0%, 5%, 10% and 15%) for 8 weeks. Bars are mean ± SEM (n=6), no significantly different ($p \geq 0.05$).

3.2. Effect of four different vegetable oils (RPO, PO, CO and COC) on antioxidant enzyme activity of normal rat liver

The results of CAT activity at different vegetable oils (RPO, PO, CO and COC) for different times (4 and 8 weeks) of treatment are summarized in Figures 11 and 12. After 4 weeks there was no significance different ($p \geq 0.05$) between control group and different vegetable oils treated groups while at 8 weeks there was significance decreased ($p \leq 0.05$) in PO,CO and COC groups compared to control group but the CAT liver sample was no significant different ($P \geq 0.05$) between control group and RPO group.

Several studies have illustrated that RPO is a rich cocktail of lipid-soluble antioxidants such as carotenoids (α- and β-carotene, lycopenes), vitamin E (in the form of α-, β-, δ- tocotrienols and tocopherol) [27]. Red palm oil has 17,500 mg of β-carotene per 100 g, and 28,000 mg of α-carotene per 100 g for a total of 6,140 retinol equivalents per 100 g. Thus, it has good potential for routine diets with enrichment carotenoids [28]. Red palm fruit oil (RPO) contains about 15 times more carotenes than that present in the same weight of carrots, and 44 times that of leafy vegetables [29]. Palm oil is a rich source of vitamin E, having both tocotrienols and tocopherols [30, 31].

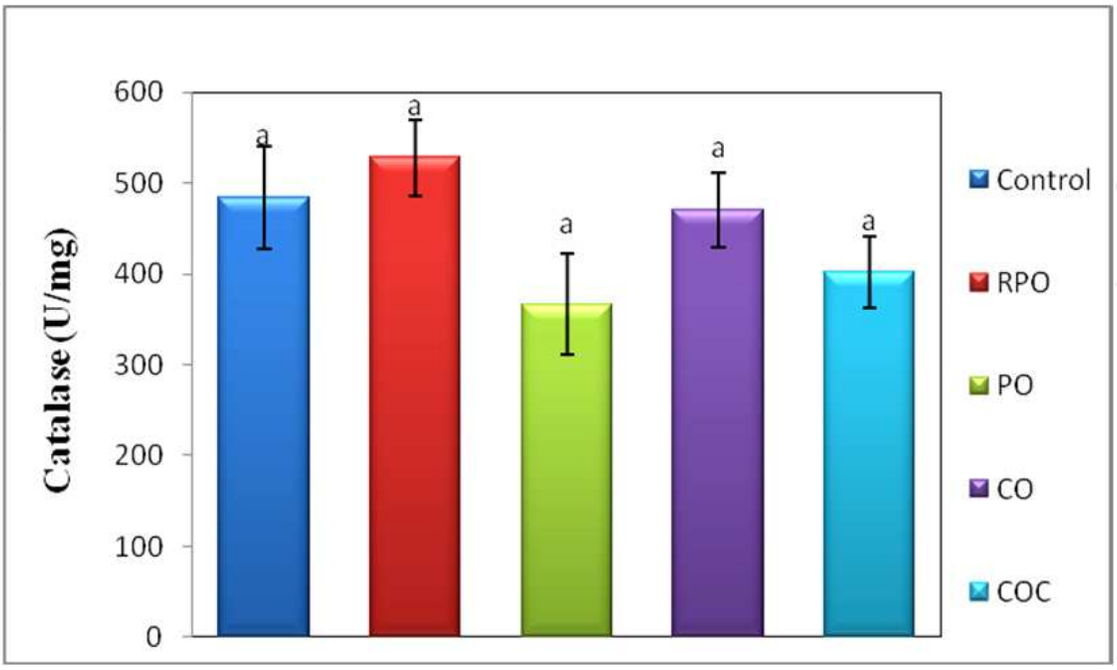

Figure 11. The catalase activity (CAT) in rat liver fed with different vegetable oils for 4 weeks. Bars are mean ± SEM (n=6), no significantly different (p≥0.05).

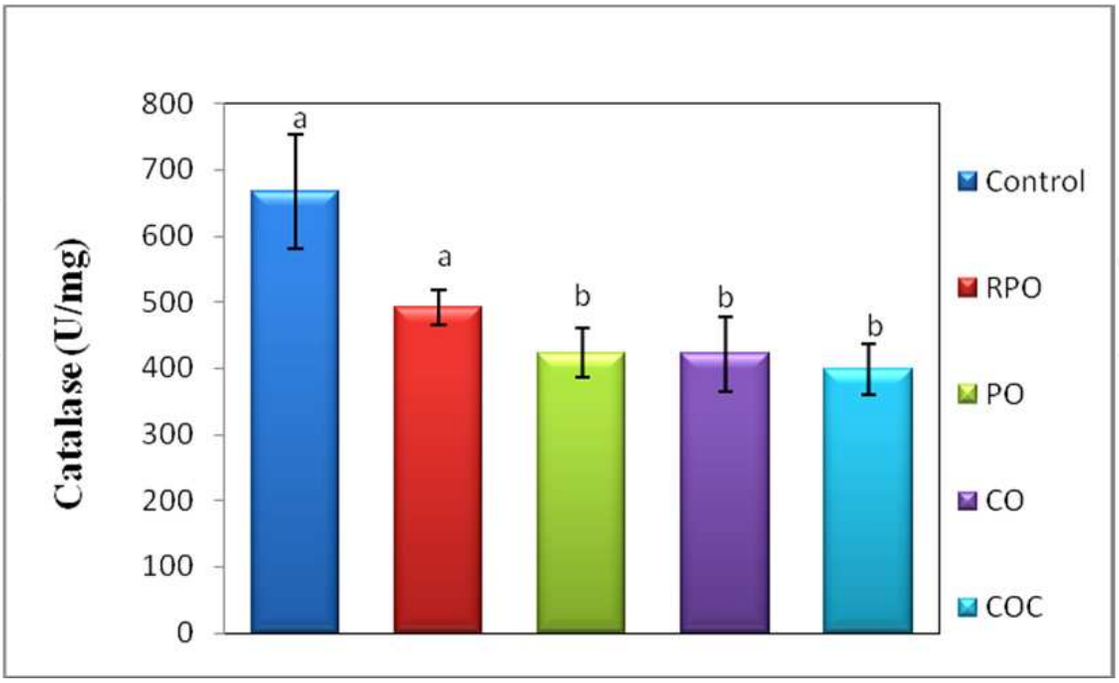

Figure 12. The catalase activity (CAT) in rat liver fed with different vegetable oils for 8 weeks. Bars are mean ± SEM (n=6), different alphabet an each bar indicate significant different (P≤0.05).

The results of SOD activity at different vegetable oils (RPO, PO, CO and COC) for different times (4 and 8 weeks) of treatment are summarized in Figures 13 and 14. After 4 and 8 weeks there was no significance different ($p \geq 0.05$) between control group and different vegetable oils treated groups.

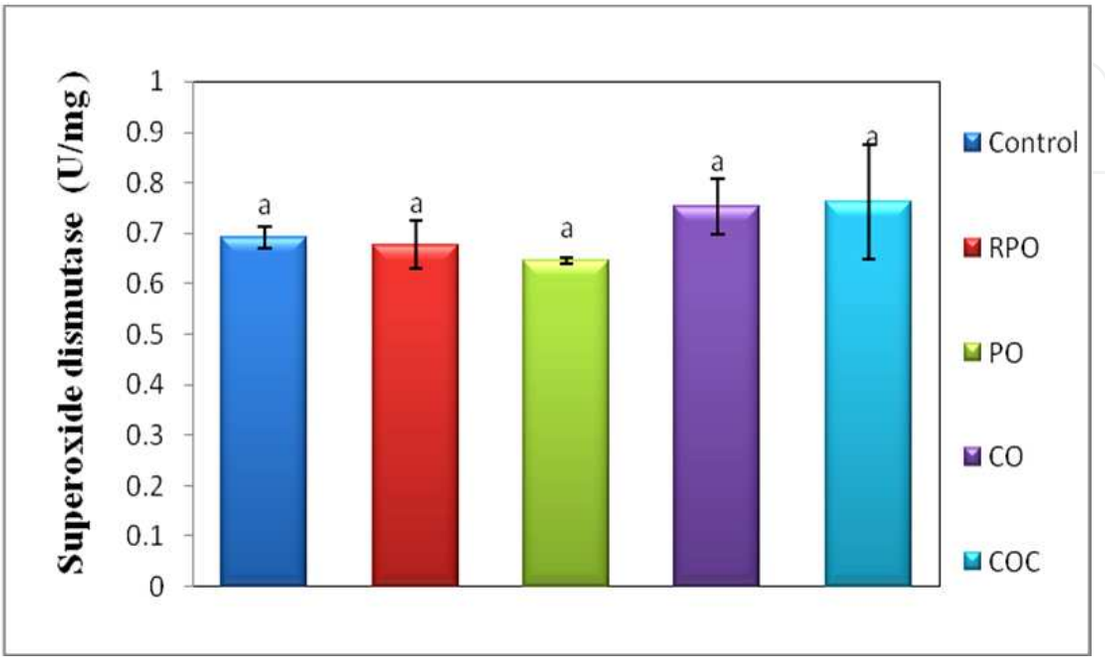

Figure 13. The superoxide dismutase (SOD) activity in rat liver fed with different vegetable oils for 4 weeks. Bars are mean ± SEM (n=6), no significantly different ($p \geq 0.05$).

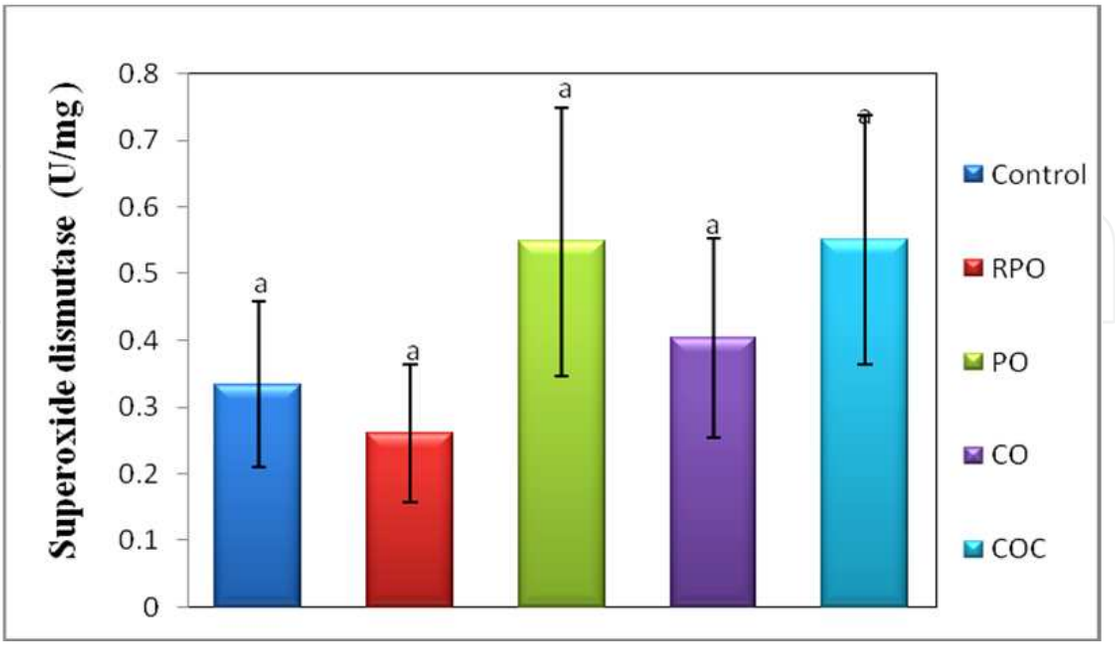

Figure 14. Mean superoxide dismutase (SOD) activity in rat liver fed with different vegetable oils for 8 weeks. Bars are mean ± SEM (n=6), no significantly different ($p \geq 0.05$).

The results from the present study, after different times, showed that under sedentary conditions, *ad libitum* feeding of RPO. There was no significant difference in level of the catalase in the control group and different concentration groups of RPO treatment. Mazlan et al. [32] reported that the catalase is the slowest of the antioxidant enzymes to respond to an increased level of free radicals. On the other hand, the CAT activity in rat liver treated with PO, CO and COC groups was decreased compared to control group.

This study finding was similar to that of Rathnagiri et al. [33] who reported that there were no statistically significant differences between control fed rats with respect to SOD activity in the corn oil. The effect of COC on antioxidant enzyme in this study was not in agreement with Anitha and Lokesh [34] who found that COC increased significantly of SOD activity in the liver but Anitha and Lokesh [34] used coconut oil with groundnut oil or olive oil instead of COC. Vitamins directly scavenge ROS and regulate the activities of antioxidant enzymes. Among them, vitamin E has been recognized as one of the most important antioxidants [27].

This probably involves their actions as antioxidants, reducing the level of free radicals and hence free radical damage. Antioxidant enzymes, such as superoxide dismutase (SOD) play a major role in removing the Reactive Oxygen Species (ROS) [11]. At this time point, it is suggested that different experimental period might lead to different result about the effect of dietary vitamin E on the activities of antioxidant enzymes [27]. In the present study, the 2 weeks period in which this experiment was carried out may be insufficient to witness any change in the activity of this enzyme.

In addition to this, Yazar and Tras [35] reported that prior induction of ROS could cause an increase intracellular SOD activity. Hence first induction of ROS may cause changes in SOD activity and then SOD activity may return to the normal level. SOD enzyme, together with CAT, protects cells against damage caused by free radicals and hydrorlipoperoxides [36]. According to Catherine et al. [37] vitamin E work synergistically to decrease the multiplication of free radicals. Vitamin E inhibits the production of lipid hydroperoxide. However, reduced SOD activities may also indicate increased lipid peroxidation end-products like acid thiobarbituric [38]. Intricately linked to lipid peroxidation are antioxidant enzymes such as SOD and catalase. As a defense against reactive free radicals, the body produces antioxidant enzymes which help to mop them up [39].

As red palm olein was shown to reduce MDA production and increase SOD in 15% group for 4 weeks, it would spare the retina from damage. This effect of palm oil may be related to the ability of β-carotene to quench free radicals and prevent tissue damage [40]. The relatively lower cholesterol level in treated rats and higher antioxidant enzyme activity could be viewed as potentially beneficial for the health of the user population in humans [41].

Presence of high amount of unsaturated fatty acids may be the reason for the low antioxidant enzyme activities of some vegetable oils fed rat since polyunsaturated fatty acids (PUFA) deteriorates the antioxidant status due to their liability to become highly oxidized. Feeding oils high in polyunsaturated fatty acids (PUFA) results and increase the oxidative stress since PUFA are highly susceptible to peroxidation than monounsaturated and saturated fatty acid [42].

(A) Stressed control group

(B) Stressed red palm olein group

(C) Stressed palm olein group

Figure 15. (A) Stressed control group, (B) Stressed red palm olein group, (C) Stressed palm olein group

3.3. Effect of red palm olein and palm olein on antioxidant enzymes in stressed rat liver

Figure 16 shows the results of CAT activity in liver samples of normal and stressed rats that were treated with 15% of RPO and PO for 4 weeks of treatment. After 4 weeks, there was no significant difference ($P \geq 0.05$) between control group and 15% RPO and PO normal groups whereas there was significant decreased ($P \leq 0.05$) between control group and 15% RPO stressed group and there was significantly higher ($P \leq 0.05$) in 15% PO stressed group than

the control group. This study finding were similar to that of Benson and Kshama [7] who reported that the CAT activity in RPO group has shown significant decrease compared to PO and RPO groups under stress conditions.

Many recent studies emphasize the important role of reactive oxygen species (ROS) in the pathogenesis of various liver diseases. Stress known to increase oxidative stress in the major organs including the liver [42].

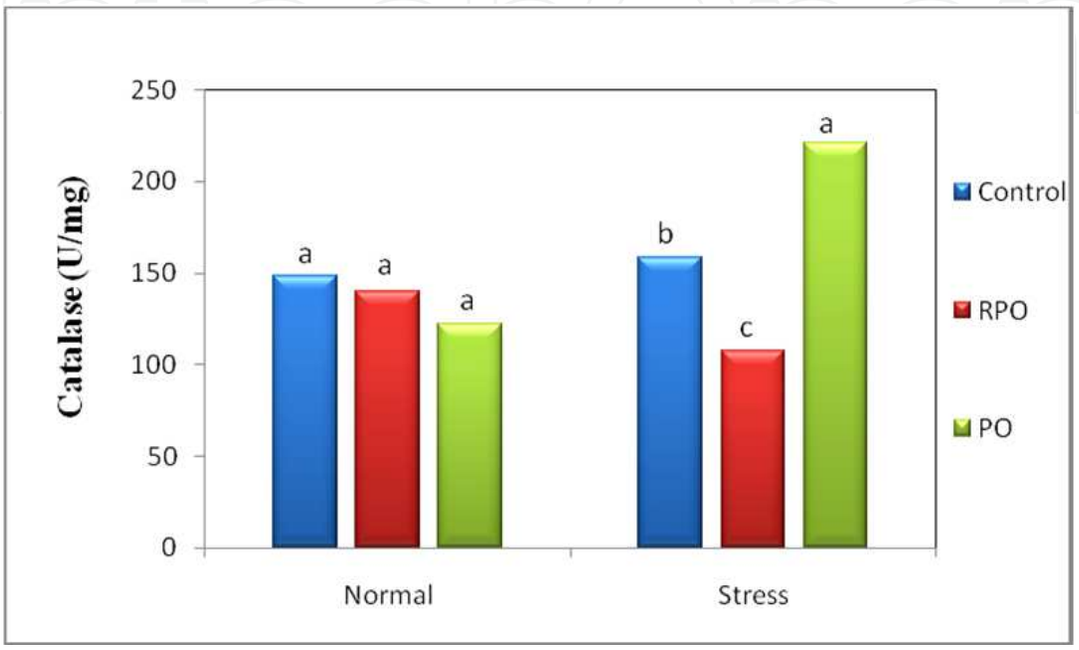

Figure 16. The catalase (CAT) activity in normal and stressed rats fed with red palm olein and palm olein for 4 weeks. Bars are mean ±SEM (n=6), different alphabet an each bar indicate significant different (P≤0.05).

Figure 17 shows the results of SOD activity in liver samples of normal and stressed rats that were treated with 15% of RPO and PO for 4 weeks of treatment. After 4 weeks, there was significantly lower (P≤0.05) in 15% RPO and PO normal and stressed groups than the control group. Vitamin E is a major antioxidant vitamins found in the cell and can prevent cell damage through its activity as a free radical chain breaker [43]. Free radicals have been implicated in the etiology of large number of major diseases. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels [43].

β-Carotene has received considerable attention in recent times as a putative chain-breaking biological antioxidant and its ability to interact with free radicals such as peroxyl radicals and to scavenge and quench singlet oxygen is well documented [44]. Defense mechanisms against free radical-induced oxidative damage include the catalytic removal of free radicals and reactive species by factors such as catalase (CAT), superoxide dismutase (SOD) and reduction of free radicals by electron donors, Such as vitamine E (tocopherol and tocotrienol) [45].

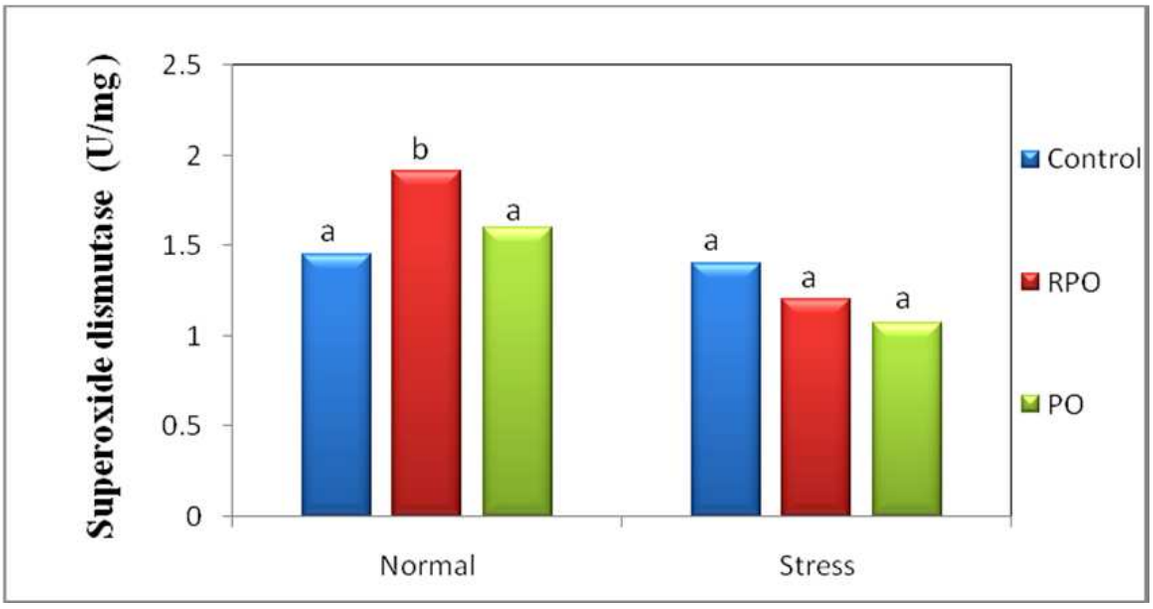

Figure 17. The superoxide dismutase (SOD) in normal and stressed rats fed with red palm olein and palm olein for 4 weeks. Bars are mean ±SEM (n=6), different alphabet an each bar indicate significant different (P≤0.05).

4. Conclusion

In conclusion, palm oil may offer some protection to liver of the treated rats by reducing free radicals damage, as well as increasing SOD. The present study shows no significant difference in level of catalase in control group and different concentration groups of RPO treatment but after 4 weeks 15% of RPO was enhanced the SOD activity level in rat liver. It can be concluded that the effect of different concentrations of RPO appear to depend on the different period of treatment. The current study shows no significant difference in level of catalase in control group and RPO group but the treated rat liver with PO, CO and COC groups were the lowest and it were significantly lower than control group. After 4 weeks of treatment, 15% of RPO enhances the SOD activity level in rat liver.These results could be due to the high content of vitamin E (tocopherols and tocotrienols) and β-carotene in red palm olein. Treatment with 15% RPO and PO diets did not affect the CAT activity after 4 weeks of treatment under normal condition while there was decreased in CAT activity with RPO and increased with PO under stress conditions. Additionally, the results in RPO group showed that higher SOD activity compared to PO and control groups under normal conditions while there were no significant difference (P≤0.05) in SOD between the control group and treated groups under stress conditions.

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