

Full Length Research Paper

Effect of different concentrations of red palm olein on antioxidant enzymes activity of rat liver

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The objective of this study was to evaluate the effect of different concentrations of red palm olein (RPO) on antioxidant enzymes activity of rat liver. Seventy eight (78) rats were randomly divided into thirteen (13) groups of 6 rats per group and treated with different concentrations of RPO (5, 10 and 15%) for 2, 4 and 8 weeks. Rats in the control group were given normal rat pellet only, while those in treated groups, 5, 10 and 15% of additional RPO were given. After 2, 4 and 8 weeks of treatment, the catalase (CAT) activity results showed that there was no significant difference ($P \geq 0.05$) between the control group and the treated groups. The mean value of the catalase activity after 2 weeks in control group for 5, 10, and 15% were 1328, 1612, 1298 and 1270 U/mg, respectively. For superoxide dismutase (SOD), there was no significant difference ($P \geq 0.05$) between the control group and treated groups after 2 and 8 weeks of treatment but there was significant difference ($P \leq 0.05$) in the 15% RPO group after 4 weeks.

Keywords: Red palm olein, catalase, superoxide dismutase, vitamin E.

INTRODUCTION

Palm oil is the only vegetable oil available in the world market in appreciable quantities that are rich in tocotrienols and have been reported to be natural inhibitors of cholesterol synthesis (Edem, 2002; Radhika et al., 2003). The tocopherols and tocotrienols promote an antithrombotic state by reducing platelet aggregation and modulating prostanoid synthesis, and the tocopherols are important minor components of oils and fats because of their antioxidant properties (Che et al., 2005). Red palm oil (RPO) is extracted from the oil palm (*Elaeis guineensis*) fruit and the red colour is derived from the high content of alpha- and beta-carotenes, which can make up 0.08% (w/w) of the crude oil (Monica et al., 2006). 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) (Spinnler, 2003; Oguntibeju et al., 2010). The first step is the formation of a lipid radical by the abstraction of a hydrogen atom by an

initiating radical and the most susceptible substrates for this radical attack in biomembranes are the polyunsaturated fatty acids (PUFAs) containing two or more double bonds (Kevin et al., 1984). Antioxidant enzymes such as superoxide dismutase (SOD), an important radical superoxide scavenger and play an important role in cell protection (Zullyt et al, 2007). Therefore, these enzymes are very good biochemical markers of stress and their increased activity may attest to a potential for remediation (Malgorzata and Edward, 2003; Edem and Akpanabiatu, 2006). Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2005). Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (David, 2004). Hydrogen peroxide is a harmful by-product of many normal metabolic processes to prevent damage; it must be quickly converted into other less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen

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peroxide into less reactive gaseous oxygen and water molecules (Geatani et al., 1996; Ohkawa et al., 1979). Superoxide dismutase (SOD, EC 1.15.1.1) is a class of enzymes that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (McCord and Fridovich, 1988; Anuradah and Chatterjee, 1988). SOD is an enzyme that is found ubiquitously in oxygen metabolising organisms. The substrate of superoxide dismutase is the superoxide radical anion which is generated by the transfer of one electron to molecular oxygen. It is responsible both for direct damage of biological macromolecules and for generating other reactive oxygen species (ROS). SODs keep the concentration of superoxide radicals in low limits and therefore play an important role in the defense against oxidative stress (Ilan et al., 2005). SOD catalyzes the dismutation of two superoxide radicals to O₂ and H₂O₂ (Yazar and Tras, 2001). Therefore, the objective of this study was to investigate the effect of different concentrations of red palm olein (RPO) on antioxidant enzymes of rat liver fed with RPO until 8 weeks of growth.

MATERIALS AND METHODS

The following instruments were used in this study: (i) High-speed homogenizer (DI18 basic, IKA, Germany); (ii) Centrifuge (Eppendorf 5810 R, Germany); (iii) UV-Visible spectrophotometer (Hitachi U-1800 single, Germany).

Sodium hydrogen phosphate (Na₂HPO₄), potassium dihydrogen phosphate (KH₂PO₄), EDTA (Ethylenediaminetetraacetic acid), sodium carbonate (Na₂CO₃), anhydrous copper sulphate (CuSO₄ 5H₂O) and Folin reagent were obtained from Sigma (USA). Hydrogen peroxide (H₂O₂), sodium potassium tartrate, NaOH, cacodylic acid ((CH₃)₂AsO₂H) and pyrogallol (C₆H₆O₃) were from Merck (Germany). Oil samples evaluated consist of carotino[®] red palm olein provided by carotino SDN BHD company. The test diet was prepared by mixing RPO with normal commercial rat pellet to contain 5, 10 and 15% of the RPO. The 5% diet was prepared by adding 5 g RPO to 95 g 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.

Seventy eight (78) Sprague Dawley male rats each weighing between 170 and 250 g and approximately 80 days old were obtained from the animal house of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The rats were fed *ad libitum* with commercial rat's food containing 5, 10 and 15% red palm oil. 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 h. They were anesthetized using chloroform. The liver was removed immediately and was washed with NaCl solution. It was stored at -8°C until analyzed.

Sample preparation

A 0.2 g sample of liver was cut into small pieces by scissors. Tissue was suspended in 2 ml of 50 mM phosphate buffer (pH 7.4) containing 1% triton, 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 main-

tained at 4°C during the homogenization process.

Determination of catalase (CAT)

Enzyme activity catalase (EC.1.11.1.6) was determined based on Aebi's method (1984). 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 min at 240 nm immediately after adding hydrogen peroxide solution. Catalase activity was expressed as moles of hydrogen peroxide reduced/min/mg protein.

Determination of SOD

Activity of superoxide dismutase (EC.1.6.4.2) was assayed based on the method of Marklund and Marklund (1974). Superoxide dismutase activity was determined at 22°C by using the pyrogallol. The reaction mixture consisted of 50 mM of Tris-cacodylic acid buffer at pH 8.2, containing 1 mM EDTA, 300 µl of liver homogenate and 300 µl of 0.2 mM pyrogallol. The absorbance was recorded for 3 min at 420 nm immediately after adding the pyrogallol solution. Superoxide dismutase activity was expressed as units of SOD/minute/mg protein.

Statistical analysis

Results were expressed as mean values ± SEM (n = 6). Means of six samples were compared by analysis of variance (ANOVA). Significant differences between means were determined by Tukey's least different significant difference (P≤0.05). The software used was MINITAB[®] (14.20).

RESULTS

Figure 1 shows the results of CAT 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 significant difference (P≥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 significant difference (P≥0.05) between the control group and different concentration groups (5, 10 and 15%) of RPO. At 8 weeks, there was no significant difference between the control group and 5% group, while at 10 and 15% there was decrease of the catalase activity and there was no significant difference (p≥0.05).

Figure 2 shows the results of SOD activity at different concentration of RPO (5, 10 and 15%) for different times (2, 4 and 8 weeks) of treatment. After 2 weeks, there was an increased in SOD activity at 5% as compared to the control group, while there was a decrease in SOD activity at 10 and 15% groups when compared to 5% RPO group. However, there was no significant difference (P≥0.05) among these groups. On the contrary, at 4 weeks, the SOD activity increased with increasing duration

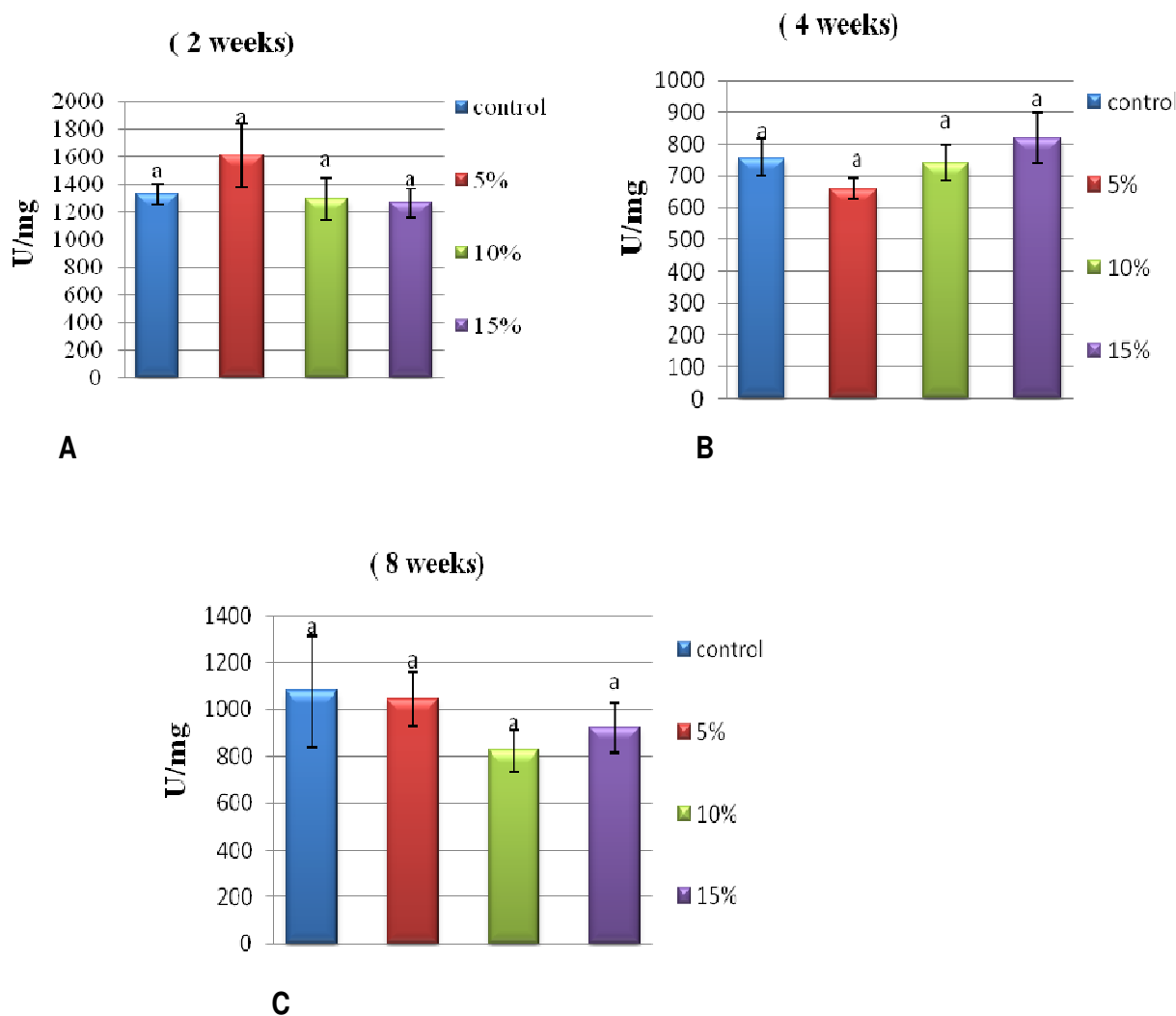


Figure 1. Mean (n = 6) of CAT in rat liver sample was at **A** (2 weeks), **B** (4 weeks) and **C** (8 weeks). There was no significant different ($P \geq 0.05$) between control group and rat liver treated with different concentrations of red palm olein (RPO).

duration of treatment in all concentrations as compared to the control group. There was no significant ($P \geq 0.05$) increase in SOD activity at 15% concentration of RPO. At 8 weeks, there was no significant difference ($P \geq 0.05$) between the control group and all the treatment groups of RPO, except that there was decrease in SOD activity at 15% of RPO.

DISCUSSION

Antioxidants form an important part of a cell defence against free radical damage. Antioxidant enzymes, in particular, constitute a major part of this defence (Arunabh et al., 2003; Edem, 2009). It is evident from earlier work that different concentrations of RPO have differential effects on the activities of antioxidant enzymes (Zullyt et al., 2007).

The results from this study, after 2, 4 and 8 weeks, showed that under sedentary conditions, *ad libitum* feeding of RPO showed no significant difference in the level of the catalase in the control group and different concentration groups (5, 10, and 15%) of RPO treatment. Mazlan et al. (2002) reported that the catalase is the slowest of the earlier mentioned antioxidant enzymes to respond to an increased level of free radicals. Therefore, after 4 weeks, the activity of SOD was significantly increased ($P \leq 0.05$) in 15% of RPO dietary group as compared to the control group but the increase in the 10% of RPO dietary group was not statistically significant. On the other hand, the decrease in 15% of RPO after 2 and 8 weeks was not statistically significant.

Results from the antioxidant enzyme determinations showed that basal SOD level were higher in 15% RPO group after 4 weeks of treatment, confirming the earlier reports of Mazlan et al. (2002) that showed that supple-

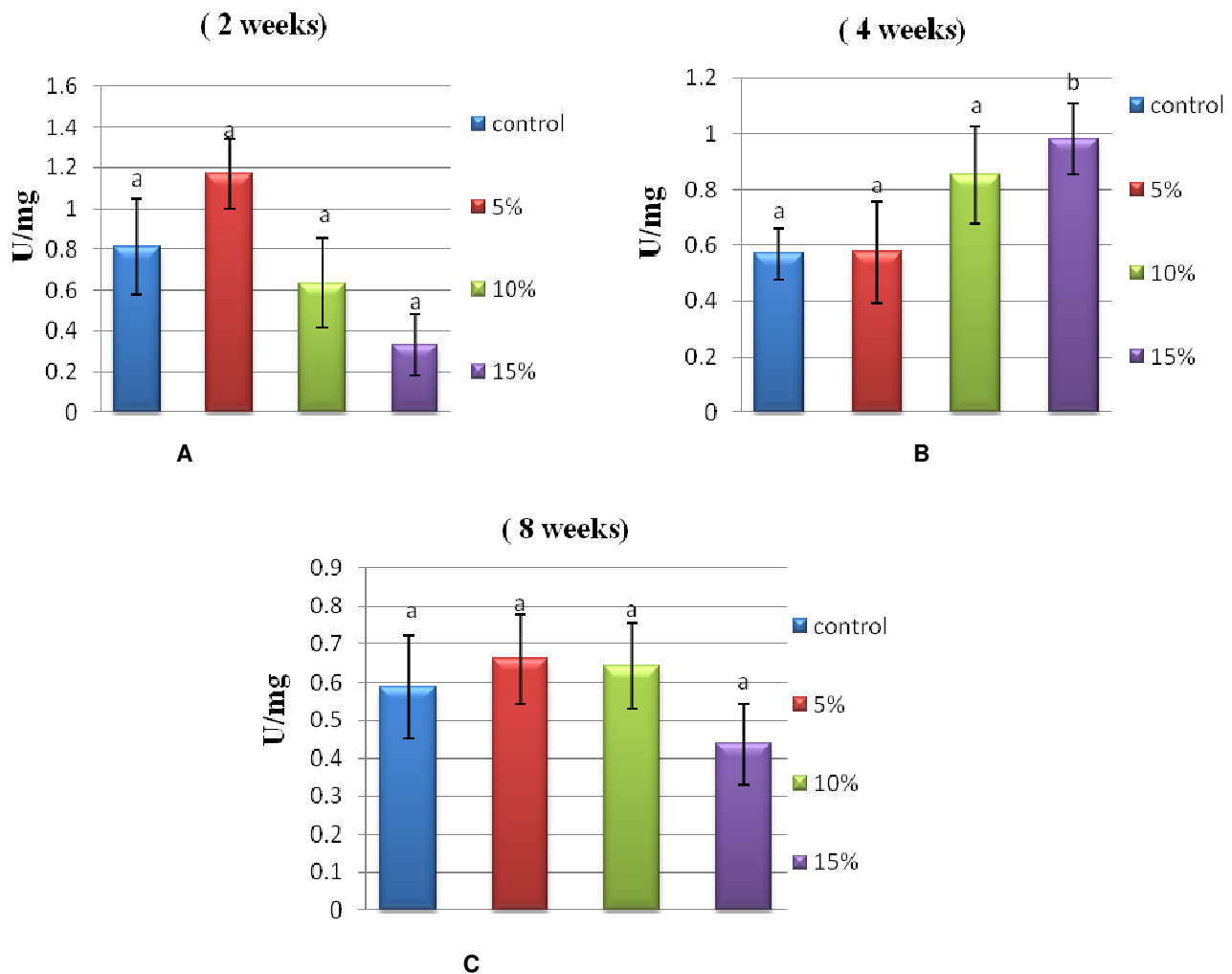


Figure 2. Mean ($n = 6$) of SOD rat liver samples at **A** (2 weeks), **B** (4 weeks) and **C** (8 weeks). Different alphabets within the same period indicate significant different ($P \leq 0.05$).

mentation with α -tocopherol increased the level of SOD. This enzyme scavenges free radicals and prevents oxidative damage. The results of this study showed that 15% of RPO which contain β -carotene and vitamin E for 4 weeks may enhance the antioxidant enzyme (SOD) defence system but recent study observed that SOD level decreased in 15% after 8 weeks of treatment.

Antioxidant enzymes, such as SOD play a major role in removing the ROS (Malgorzata and Edward, 2003). Similar to the reported case of Mazlan et al. (2002), this study found that two weeks period in which this experiment was carried out may be insufficient to observe any change in the activity of this enzyme. However, this study found that SOD activity level decreased with increase of the period to 8 weeks of 15% RPO treatment. In addition to this, Yazar and Tras (2001) 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 hydro or lipoperoxides (Suleyman et al., 2007; Surapaneni and Vishnu, 2009). These results thus, suggest that a combination of carotenoids and vitamin E (tocopherol and tocotrienol) in the RPO has an important role in the protection against free radical damage. Red palm oil contains the highest concentration of tocotrienols when compared with other vegetables or plants and the tocotrienols can be 40 to 60 times more potent as antioxidant than tocopherols (Jacques et al., 2008). Inherent antioxidant defense systems consisting of enzymes, such as CAT and SOD, and 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 (Jaya et al., 1998).

Conclusion

This study shows that there was no significant difference in the level of catalase in the control group and different concentrations of the groups of RPO treatment, but after 4 weeks at 15%, RPO enhanced the SOD activity level in liver rat. It can be concluded that the effect of different concentrations of RPO appear to depend on the different period of treatment.

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