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Effect of Four Different Vegetable Oils (Red Palm Olein, Palm Olein, Corn Oil, Coconut Oil) on Antioxidant Enzymes Activity of Rat Liver

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Abstract: The objective of the study was to evaluate the effect of four different vegetable oils [Red Palm Olein (RPO), Palm Olein (PO), Corn Oil (CO), Coconut Oil (COC)] on antioxidant enzymes activity of rat liver. Sixty six Sprague Dawley male rats which were randomly divided into eleven groups of 6 rats per group and were treated with 15% of RPO, PO, CO and COC for 4 and 8 weeks. Rats in the control group were given normal rat pellet only while in treated groups, 15% of additional different vegetable oils were given. After 4 weeks of treatment the Catalase (CAT) activity results showed that there was no significance difference ($p \geq 0.05$) between the control group and treated groups while after 8 weeks of treatment showed that there was no significant different ($p \geq 0.05$) between control group and RPO group but the treated rat liver with PO, CO and COC groups were the lowest and it were significantly lower ($p \geq 0.05$) than control group. For Superoxide Dismutase (SOD) there was no significance difference ($p \geq 0.05$) between the control group and treated groups of vegetable oils after 4 and 8 weeks of treatment. Thus the study indicated that there was no significant ($p \geq 0.05$) effect on antioxidant enzyme (superoxide dismutase) but there was significant effect ($p \geq 0.05$) on catalase in rat liver.

Key words: Vegetable oils, catalase, red palm olein, superoxide dismutase, Vitamin E

INTRODUCTION

Reactive Oxygen Species (ROS), such as superoxide ions, hypochlorous acid and Hydrogen Peroxide (H_2O_2), are produced to kill organisms. However, if these noxious oxygen derivatives are not controlled by antioxidant defence systems, oxidative stress occurs. Oxidative stress has been related to many pathophysiological states (Dongwu, 2008). Thus, protecting against oxidative damage to cells. Besides providing high energy density in the diet, β-carotene is the most abundant carotenoids which can be converted to vitamin A; which is an important in the visual process. In addition, it is an antioxidant that destroys singlet oxygen and free radicals (Edem, 2002). Reactive Oxygen Species (ROS) are highly reactive and in the absence of any protective mechamism they can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Gulen *et al*., 2008). Vegetable oil is very common, affordable and used by majority of people across the globe especially in the tropics. Its use as antidote to prevent some oxidative stress related diseases and a complication is advocated (Ogugua and Ikejiaku, 2005). Vegetable oils in particular are natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acid contain about 14 to 20 carbon atoms with different degrees of unsaturation (Aluyor and Ori-Jesu, 2008). Palm oil contains approximately an equal amount of saturated and unsaturated fatty acids. Amongst the former, palmitic and stearic acid account for 45 and 5% of the total fatty acids, respectively. Palm oil has a wide range of applications and it is commonly fractionated into olein and stearin (Khosla, 2006). Red palm oil is also a rich source of vitamin E which is about 559 to 1000 ppm. Vitamin E acts as a potent antioxidant serving to protect cellular membranes from free radical-catalyzed lipid peroxidation (Edem, 2002). Coconut oil is commercially a major source of lauric acid (Gregorio, 2005). Coconut oil contains approximately 90% saturated fats. Saturated fats are known to contribute to Coronary Artery Disease (CAD) (Sabitha *et al*., 2009). Corn oil provides essential fatty acids, mostly linoleic acid. Linoleic acid is necessary

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for the integrity of the skin, cell membranes and the immune system and for synthesis of eicosanoids. Eicosanoids are necessary for reproductive, cardiovascular, renal, gastrointestinal functions and resistance to disease and it is highly effective for lowering serum cholesterol, primarily low-density-lipoprotein cholesterol (Cedomila *et al*., 2001). Antioxidant enzymes such as Superoxide Dismutase (SOD) an important radical superoxide scavenger and play an important role in cell protection (Rodriguez *et al*., 2007). Therefore, these enzymes are very good biochemical markers of stress and their increased activity may attest to a potential for remediation (Kopyra and Gwozdz, 2003). Therefore, the objective of this study was to investigate the effect of four different vegetable oils (RPO, PO, CO and COC) on antioxidant enzymes activity of rat liver fed until 8 weeks of growth.

MATERIALS AND METHODS

Instruments: 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 (Hitachj U-1800 single, germany).

Chemicals: Sodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), EDTA (Ethylenediaminetetraacetic acid), sodium carbonate (Na_2CO_3), anhydrouse copper sulphate ($CuSO_4$ $5H_2O$), Folin reagent were obtained from Sigma (USA). Hydrogen pyroxide (H_2O_2), sodium potassium tartrate, NaOH, cacodylic acid ($(CH_3)_2$ AsO_2H) and Pyrogallol ($C_6H_6O_3$) were from Merck (Germany).

Animals: Sixty six Sprague Dawley male rats each weighing between 170-250 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 15% different vegetable oils (RPO, PO, CO and COC). They were randomly divided into eleven groups of 6 rats per group and were treated with 15% of RPO, PO, CO and COC for 4 and 8 weeks. At the end of the experiment, after 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 it with NaCl solution. It was stored at -80°C until analyzed. The experiment was conducted on May 2010 to July 2010.

Experimental diets: The evaluated Red Palm Olein (RPO) samples consist of carotenes (576 ppm), Vitamine 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. 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 85 g 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.

Sample preparation: 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 4°C during the homogenization process.

Preparation of phosphate buffer: Phosphate buffer was prepared and determined based on method of Aebi (1984). Determination of Catalas (CAT) Activity: Catalase activity was determined based on Aebi (1984).

Determination of Superoxide Dismutase (SOD) activity: Superoxide dismutase activity of rat liver was determined based on the method of Marklund and Marklund (1974).

Determination of protein concentrations: Protein Concentrations was determined based on the Lowry method (Waterborg, 2009).

Statistical analysis: Results were expressed as Mean±SEM (n = 6). Means of six samples were compared by analysis of variance (one-way Anova). Significant differences between means were determined by Tukey's least different significant difference ($p \geq 0.05$). The software used was MINITAB® (14.20).

RESULTS

The results of CAT activity at different vegetable oils (RPO, PO, CO and COC) for four weeks of treatment are summarized in Fig. 1. The results showed that there was no significance different ($p \geq 0.05$) between control group and different vegetable oils treated groups. The results of CAT activity at different vegetable oils (RPO, PO, CO and COC) for eight weeks of treatment are summarized in

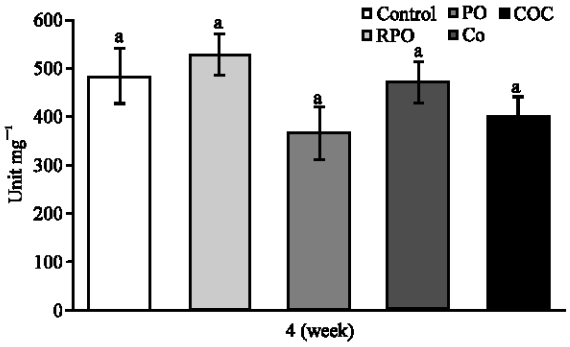

Fig. 1: Mean (n = 6) catalase activity of rat liver sample of the control group and treated groups after 4 weeks of treatment. Alphabet on each histogram indicates no significant different (p≥0.05). RPO: Red palm olein, PO: Palm olein, CO: Corn oil and COC: Coconut oil. [Catalase activity] [Unit mg^{-1}]

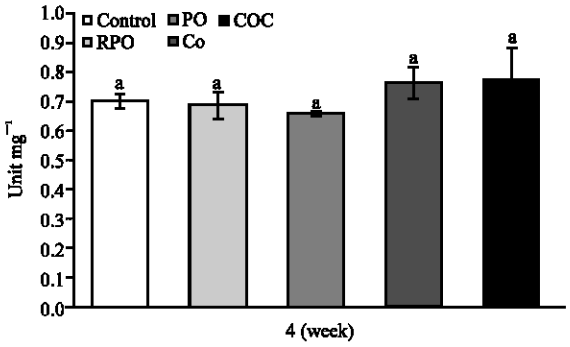

Fig. 3: Mean (n = 6) superoxide dismutase activity of rat liver sample of the control group and treated groups after 4 weeks of treatment. Alphabet on each histogram indicates no significant different (p≥0.05). RPO: Red palm olein, PO: Palm olein, CO: Corn oil and COC: Coconut oil. [Superoxide dismutase activity] [Unit mg^{-1}]

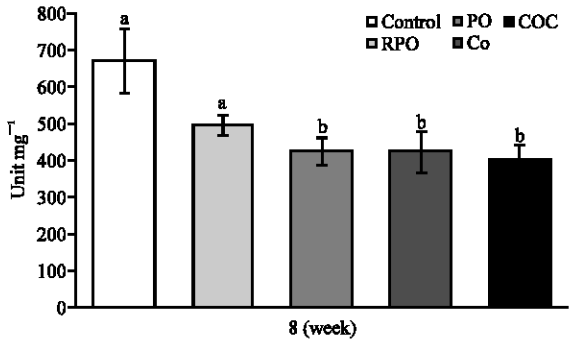

Fig. 2: Mean (n = 6) catalase activity of rat liver sample of the control group and treated groups after 8 weeks of treatment. Different alphabet on each histogram indicates significant different (p≥0.05). RPO: Red palm olein, PO: Palm olein, CO: Corn oil and COC: Coconut oil. [Catalase activity] [Unit mg^{-1}]

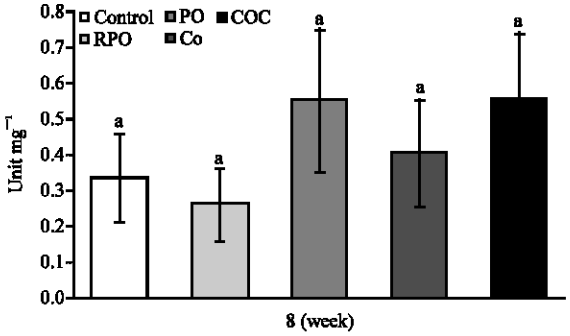

Fig. 4: Mean (n = 6) superoxide dismutase activity of rat liver sample of the control group and treated groups after 8 weeks of treatment. Alphabet on each histogram indicates no significant different (p≥0.05). RPO: Red palm olein, PO: Palm olein, CO: Corn oil and COC: Coconut oil. [Superoxide dismutase activity] [Unit mg^{-1}]

Fig. 2. It showed that there was significance decreased (p≥0.05) in PO, CO and COC groups compared to control group but the CAT liver sample was no significant different (p≥0.05) between control group and RPO group.

The results of SOD activity at different vegetable oils (RPO, PO, CO and COC) for four weeks of treatment are summarized in Fig. 3 and the results of SOD activity at different vegetable oils (RPO, PO, CO and COC) for four weeks of treatment are summarized in Fig. 4. After four and eight weeks there was no significance different (p≥0.05) between control group and different vegetable oils (RPO, PO, CO and COC) treated groups.

DISCUSSION

The results from the present study, after different times, showed that under sedentary conditions, *ad libitum* feeding of RPO no significant difference in level of the catalase in the control group and different concentration groups of RPO treatment. It is evident from earlier work that different concentrations RPO have differential effects on the activities of antioxidant enzymes (Rodriguez *et al*., 2007). Musalmah *et al*. (2002) 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 decreased compared to the control group. The results of this study showed that antioxidants (Vitamin E and β-carotene) in RPO were higher than in other vegetable oils used in this study. These antioxidants directly scavenge ROS and regulate the activities of antioxidant enzymes. Among them, Vitamin E has been recognized as one of the most important antioxidants (Fu *et al*., 2007). In the present study, the SOD activity level decreased with increase the period of RPO treatment at 8 weeks. In addition, 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. Superoxide dismutase enzyme, together with CAT, protects cells against damage caused by free radicals and hydrolipoperoxides (Kozat *et al*., 2007).

These probably involve 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) (Kopyra and Gwozdz, 2003). 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 (Fu *et al*., 2007).

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 with other vegetables or plants and the tocotrienols can be 40-60 times more potent as anti-oxidant than tocopherols (Van Rooyen *et al*., 2008). 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 (Christine *et al*., 2010). Although few 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 (Fu *et al*., 2007).

Comparing antioxidant activities from this study and other published data is difficult. The difference in CAT and SOD values among different studies is due to several factors. These factors include (1) period of treatment, (2) type of vegetable oils, (3) the concentration of oils used and (4) type, weight or age of rats. The different studies compared here used different vegetable oils for treatment and this may be one of the reasons in the variation of the data. In this study, 15% vegetable oils (RPO, PO, CO and COC) were used while Benson *et al*. (2010) used 10% of groundnut oil, palm oil and coconut oil. Furthermore, it is very important when compared between results to clarify the experiment condition (i.e Time, age or weight) which are not always provided by authors (Stratil *et al*., 2006).

CONCLUSION

The present study shows no significant difference in the level of catalase in the control group compared to the RPO group. However, the treated rat liver with PO, CO and COC groups have significantly ($p=0.05$) lower catalase activity than the control group and after 4 weeks of treatment with 15% of RPO. Thus this enhances the SOD activity level in rat liver.

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