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Palm Oil Fat Diet Consumption and its Effects on Serum Liver Enzymes and Microscopic Changes in Experimental Rats

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Abstract: The present study aimed to observe the effect of consumption of heated palm oil especially with regard to liver histology and enzymes such as alanine transaminase (ALT) and alkaline phosphatase (ALP). We divided forty female Spraque-Dawley rats into four groups (I to IV). The group I was administered with 2% cholesterol diet. The groups II, III and IV were administered with 2% cholesterol diet fortified with 15% weight/weight (w/w) fresh palm oil (FPO), heated once palm oil (1HPO) and heated five times palm oil (5HPO), respectively for a period of 16 weeks. Blood for liver enzymes were drawn and analyzed prior to and at the end of the study. At the end of the study the animals were sacrificed and the liver tissue was examined histologically. The histological specimens were stained with haematoxylin and eosin. Fresh, heated once and heated five times palm oil diet caused significant increase in serum ALT compared to their respective baseline values. No significant difference in the ALT levels among groups fed with oil was observed. The increase in serum ALP was only observed with heated once and five times palm oil. Histologically, palm oil rich diet causes liver inflammation and microsteatosis but not necrosis. The hepatic histological changes were not affected by heating. High fat diet cause inflammation, microsteatosis and altered serum ALT and ALP in the liver. The histological changes and altered liver enzymes were not affected by heating except for serum ALP.

Key words: Heated, palm oil, liver, damage, histology, inflammation

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) includes steatosis (fatty change), non-alcoholic steatohepatitis (NASH) and cirrhosis in the absence of alcohol abuse. NAFLD is widely associated with insulin resistance and one of the features of the metabolic syndrome (Elizabeth *et al*., 2008). Less commonly, NAFLD may be due to secondary causes such as drug like corticosteroid, rapid weight loss or metabolic diseases such as lipodystrophy or dysbetalipoproteinemia, obesity and diabetes mellitus (Quentin and Robert, 2006). Oxidative stress and lipid peroxidation lead to many progressive liver diseases including steatohepatitis. The presence of biological markers of oxidative stress was reported in both human and animals with steatohepatitis (Sanyal *et al*., 2002; George *et al*., 2003). The increase in NAFLD is most likely due to the increasing prevalence of obesity, type II diabetes and the metabolic syndrome in the general population. It is estimated that more than half of all patients with obesity have some form of NAFLD. It was estimated at least 25% of patients with NAFLD have NASH with or without hepatic fibrosis (Clark, 2003; Ruhl and Everhart, 2003, 2004).

The underlying mechanism of hepatic triglyceride accumulation in NAFLD is not well understood. Increase caloric intake with less energy expenditure leads to an increase in the plasma long chains fatty acids (LCFAs) concentration in the portal system. Such an increase in the LCFAs in portal system which in turn increases hepatic uptake leading to overproduction of hepatic triglycides (TG) and steatosis (Browning *et al*., 2004; Bardbury and Berk, 2004). Hepatic clearance of LCFAs is only possible by esterification to TG and exports as very low- density lipoprotein (VLDL) or by β oxidation. While, lipid transfer to apolipoprotein B100 is the rate limiting step in VLDL formation. Therefore, abetalipoproteinemia predisposed to steatosis (Letteron *et al*., 2003; Bardbury and Berk, 2004). Besides failure of VLDL synthesis and TG export, high blood glucose and insulin activates *de novo* long chains fatty acids (LCFAs) synthesis within hepatocytes (Dentin *et al*., 2005). Thus, insulin resistance may play important roles in NAFLD. One of the most common forms of NAFLD is simple hepatic steatosis which is largely reversible. Hepatic lipid accumulation does not necessary result in hepatocellular injury. Hepatocellular oxidation of LCFAs in both mitochondria and extra-mitochondrial sites which generate oxygen reactive oxygen species may contribute to hepatocellular injury as the reactive oxygen species may trigger the release

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of cytokines in particular TNF-α, TNF-β and interleukin (IL) 6. These cytokines then initiate mitochondrial dysfunction (Robertson *et al*., 2001).

Majority of the patients with NALFD remain asymptomatic although they may suffer from abdominal discomfort and hepatomegaly. Liver enzymes may be normal in majority of the cases. Thus, liver function tests are insensitive for the detection of NAFLD. In addition, wide spectrum of histological changes may be present among patients with NALFD with normal ALT levels. Therefore, liver function test alone is reliable test to rule out the presence of advanced liver disease. The changes in liver enzymes if present are generally modest and restricted to alanine amino transaminase (ALT) and aspartate amino transaminase (AST).

Previous studies showed that excessive intake of fatty meal and lipid peroxidation may contribute to the occurrence of fatty liver (steatosis). Much of the fat consumed in our diet are exposed to heat encountered during processing and also in the preparation of food during any cooking. In many parts of the world including Malaysia, the practice of using repeatedly heated oil was rampant (Azman *et al*., 2012). In deep-frying, fat especially polyunsaturated fat (PUFA) undergo complex series of chemical reactions lead to generation of degradation products, like hydro peroxide and aldehydes which are reactive oxygen species (ROS). The fried food absorbs this heated oil together with ROS thus it becomes part of our diet. Reactive oxygen species (ROS) has been linked to pathological effects associated with oxidative stress such as cardiovascular, liver, kidney disease and cancer (Chun Yi *et al*., 2014; Siti Khadijah *et al*., 2009; Xin Fang *et al*., 2009; Dobarganes and Marquez-Ruiz, 2003; Dutta and Dutta, 2003; Totani and Ojiri, 2007). Several studies also showed the harmful effect of oxidized dietary fats on humans and experimental animals. These include cardiovascular changes such as hypertension and atheroclerosis (Xin Fang *et al*., 2009; Adam *et al*., 2009). While Owu *et al*. (1998) and Jaarin *et al*. (2010) reported that Fresh and heated palm and soy oil increase ALT, respectively. Polavarapu *et al*. (1998) demonstrated that lipid peroxidation and high corn oil diet cause liver injury. The presnt study was undertaken to observe the effect of high fresh or heated palm oil diet on liver enzymes and microscopic changes.

MATERIALS AND METHODS

Ethical approval was obtained for the study. We divided forty female rats of Sprague-Dawley species (200-250 g) equally into four groups. The rats were given the following prescribed course of food: Group I- fed with 2% cholesterol diet as the control (without any oil); Group II, III and IV with 2% cholesterol diet fortified with 15% weight/weight fresh palm oil (FPO), or heated once palm oil (I-HPO) or heated 5 times palm oil (5H-PO), respectively for 24 weeks. The animals were kept in stainless steel cages (temperature of 27±2°C) and were quarantined for a two-week period before the start of experiment with different diets. All the tests and control animals had free access to food and tap water for 24 weeks. The serum ALT and ALP were taken at baseline and at the end of 24 weeks of study.

The rats were sacrificed and their liver was removed and weighed. A portion of each lobe of the liver was formalin fixed and the sections were stained with haematoxylin-eosin. Special stains were done in a few cases with Oil Red O for lipid and Periodic Acid Schiff (PAS) with and without diastases. Histological examination was carried out using light microscopy.

Assessment of liver microscopic changes: The liver pathology score was calculated as described by Polavarapu *et al*. (1998) Steatosis (the percentage of liver cells containing fat): 1+, (less than 25% of cells containing fat); 2+,(26 to 50%); 3+,(51 to 75%) and 4+(more than 75%).

Inflammation and necrosis: 1+,(one focus/lobule); 2+(Two or more foci/lobule).The total liver pathology score was calculated by adding the scores from each of the parameters.

Source and preparation of heated oil diets: The vegetable oils used were palm oil which was purchased from Lam Soon Edible Oils, Malaysia. The oils were used fresh, heated once or heated five times (as described by an earlier protocol by Owu *et al*. (1998). The heating process involved using 2500 ml of the vegetable oil to fry 1 kg of 'keropok lekor' (fish-flavored chips) in a metal wok. The temperature of the heated oil reached about 180°C and the cooking process lasted about 10 min. To heat the oil 5 times, the oil was cooled for 5 hours, then the whole frying process repeated with a fresh batch of 'keropok lekor'. Standard rat pellets were obtained from Gold Coin (Malaysia). Fifteen percent (15%) weight/weight of the respective oils were mixed with ground rat pellets. The pellets were reformed, dried in an oven at 70-90°C and used.

Determination of serum ALT: Blood sample was analyzed by colorimetric assay technique using Cobas Integra 800 Roche Diagnostic. The action of ALT is to catalyse the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase to form L-lactate and NAD. The rate of the NADH oxidation was directly proportional to the catalytic ALT activity. It was determined by measuring the decrease in absorbance at 340 nm.

Determination of serum ALP: The enzyme was determined using the colorimetric assay on Cobas Integra 800 in accordance with IFCC recommendation.

In the presence of magnesium and zinc ion, p-nitrophenyl phosphate was cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released was directly proportional to the catalytic ALP activity. It was determined by measuring the increase in absorbance at 409 nm.

Data analysis: The data was presented as the Mean±SEM. Normally distributed data were analyzed using parametric tests, i.e., Student's t-test and ANOVA. Data which were not normally distributed were analyzed using non-parametric test, i.e., the Kruskal-Wallis, Mann-Whitney and Wilcoxon Signed Rank tests. A value of $p<0.05$ was considered to be significant. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software.

RESULTS

Semiquantitative analysis of microscopic appearance of liver tissue: In the Fig. 1 there was a significant increase in microvesicular steatosis and inflammation in palm oil fed group compared to the control (*$p<0.05$). However; there was no significant different in microvesicular steatosis and inflammation among the palm oil fed diet. There was no significant difference in macrosteatosis and necrosis among the study groups.

Microscopic appearance of the liver: In the Fig. 2, minimum macrosteatosis, inflammation and necrosis were observed in the control group. However, two or more foci of inflammation and necrosis were seen in all group fed with fresh and heated palm oil. Microvesicular steatosis were noted in perivenular, midzonal and periportal areas of liver parenchyma in palm oil fed diet groups. Touton giant cells were seen in all palm oil fed groups. No Touton giant cell was seen in the control group.

Effect of fresh and heated palm oil on serum ALT: In the Fig. 3 there was no change in serum ALT in the control group. In contrast fresh, heated once and heated 5 times palm oil rich diet caused a significant increase in serum ALT compared to their respective baseline values. However, there was no significant difference in serum ALT among the palm oil fed groups. Thus, it appeared that the effect of palm oil rich diet on serum ALT was not affected by heating process.

Effect of fresh and heated palm oil on serum ALP: In the Fig. 4 there was no change in serum ALP in the control group. Fresh palm oil did not increase serum ALP. However, heated once and heated five times palm oil caused a significant increase in serum ALP compared to control and FPO group. However, there was no significant different in serum ALP between 1HPO and 5HPO. It appeared that heated palm oil increased serum ALP.

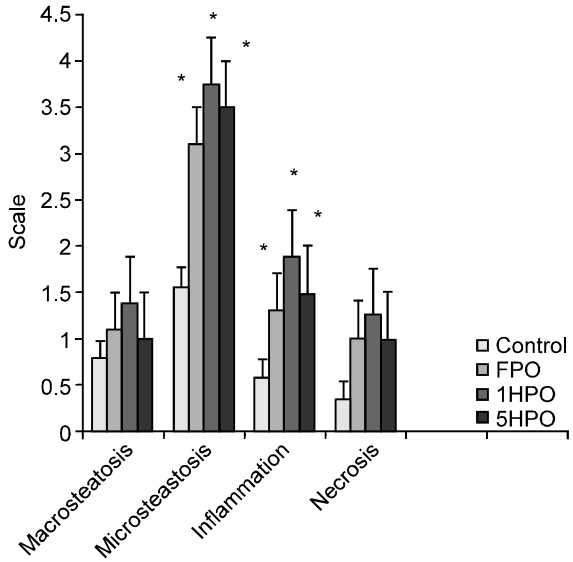

Fig. 1: Semiquantitative analysis of histological changes with palm oil

DISCUSSION

In the present study, we observed that fresh and heated palm oil rich diet caused liver microsteatosis and inflammation. However, palm oil rich diet did not cause significant necrosis and macrosteatosis. This finding was in contrast to an earlier finding reported by Kamsiah *et al.* (2010) which demonstrated that soy oil rich diet not only caused liver inflammation and microsteatosis but necrosis. The reason for the discrepancy in this finding was unclear. Palm oil is monosaturated oil while soy oil is polyunsaturated oil. It may suggest that polyunsaturated oil is more detrimental to liver compared to monosatuarted oil as it easily undergoes lipid peroxidation which may be detrimental to hepatocytes. Robertson *et al.* (2001) suggested that reactive oxygen species may contribute to hepatocellular injury as it may trigger the release of cytokines in particular TNF-α, TNF-β and interleukin (IL) 6. These cytokines eventually initiate mitochondrial dysfunction. This finding again supported that diet that high in fatty acids content causes fatty liver. The exact mechanism on how diet with high fatty acid (FA) content caused steatosis was not clearly understood. Overproduction of triglyceride secondary to excessive influx of FA into the liver has been suggested to play an important role (Browning *et al.*, 2004; Bardbury and Berk, 2004). Reduction in apolipoprotein B biosynthesis which in turn impaired transportation of TG out from the liver may be responsible for the occurrence of steatosis (Letteron *et al.*, 2003; Bardbury and Berk, 2004). Measurement of lipid metabolism in the liver such as apo lipoprotein B, phospholipid and lecithin acyl transferase are advisable in future studies in order to ascertain this suggestion.

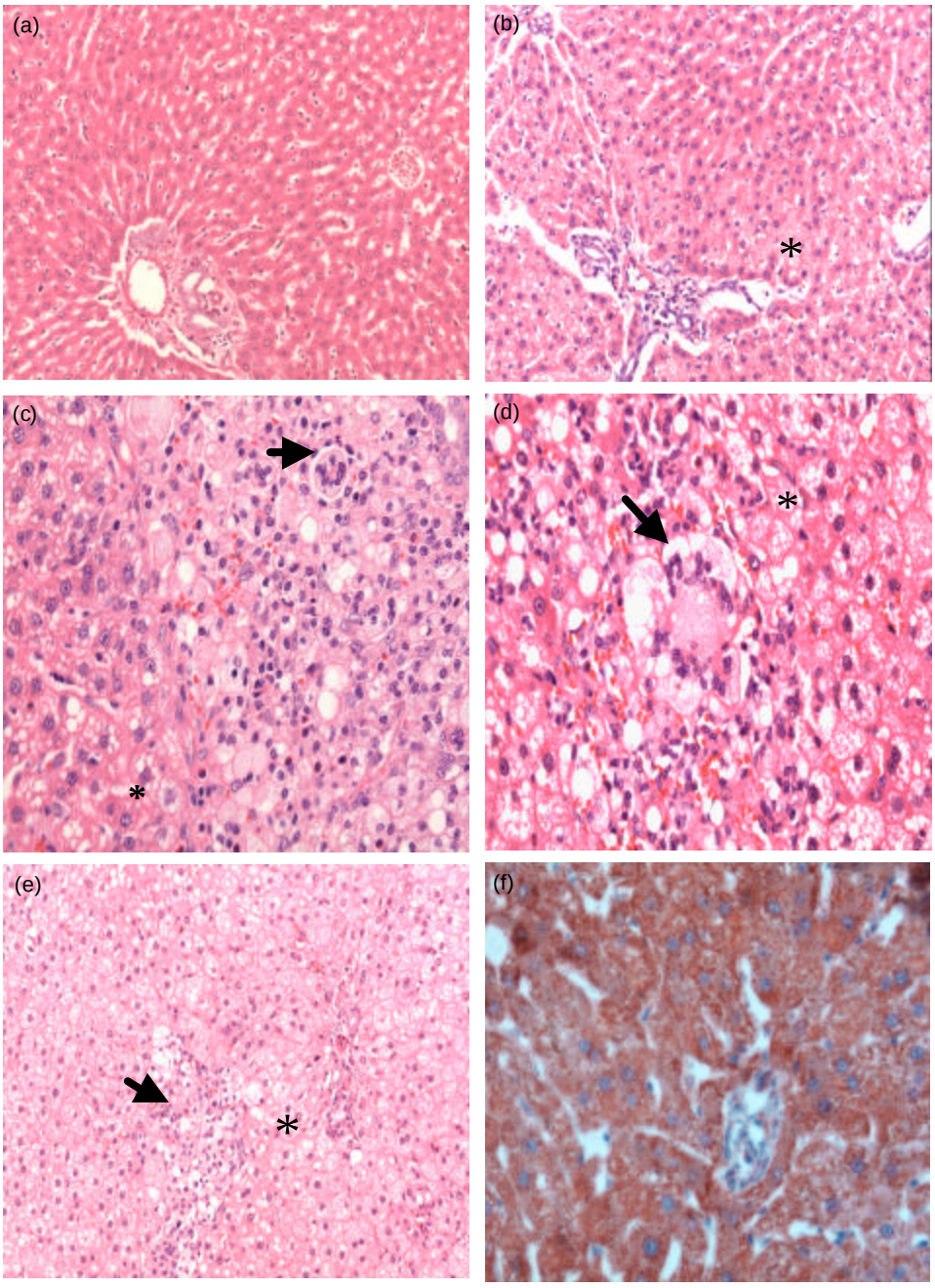

Fig. 2: Cross sections of the liver rats for normal, fresh palm oil (FPO), heated once (1HPO) and heated 5 times palm oil (5HPO): A-Normal liver of rat (control) [Haematoxylin and Eosin (H and E), magnification x 100]; B-Liver section of FPO shows microvesicular steatosis (*) (H and E, x 200); C-Liver pathology of rat fed 1FPO shows microvesicular steatosis (*), inflammation and Touton-type giant cells (➔) [H and E, x 200]; D-Liver pathology of rat fed 5HPO shows microvesicular steatosis (*), inflammation and an abnormal Touton-type giant cell (➔) [H and E, x 200]; E- Liver pathology of rat fed 5HPO shows microvesicular steatosis (*) and inflammation(➔) [H and E, x 100]; F-Liver section of rat fed 1HPO shows microvesicular steatosis stained red in colour (Oil Red O, x 200)

All groups including control were fed with a 2% cholesterol diet. Since, there was no significant change in the liver enzymes in the control group; this finding suggests that high cholesterol diet alone did not alter

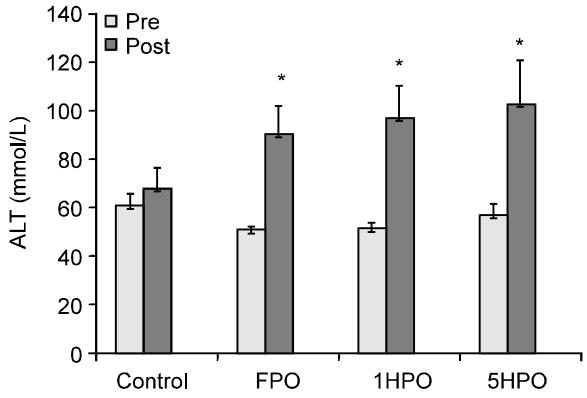

Fig. 3: Effect of Fresh and heated palm oil on serum ALT

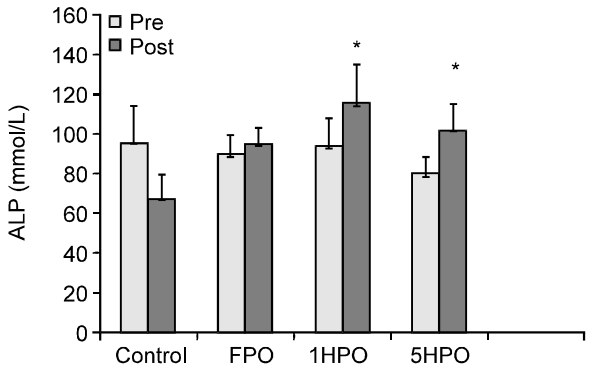

Fig. 4: Effect of fresh and heated palm oil on serum ALP

serum liver enzymes. Therefore, palm oil rich diet was responsible for the increase in serum ALT. The effect of palm oil diet on serum ALT in this study was not affected by heating process. The effect of palm oil on serum ALT in this study could be compared to an earlier study by Owu *et al.* (1998) and Kamsiah *et al.* (2010) who reported that fresh and heated palm oil and soy oil increase ALT, respectively. In this study, heated palm oil appears to be more detrimental on serum ALP as the changes in serum ALP was only observed in the group fed with heated once and heated five times palm oil. The effect of palm oil in serum ALP in the present study was in contrast to the finding of Kamsiah *et al.* (2010) which reported that both fresh and heated soy oil increase serum ALP. The reason for the differences in the results was unclear. However, it appears that soy oil has more detrimental effect on serum ALP compared to palm oil. The reason for the increase in ALT and ALP with high fat diet is not known; we believe that lipid peroxidation may be responsible for the inflammatory changes in the liver and hence induced enzymes changes.

It is not known whether inflammation, or increase in serum ALT, ALP with fresh and heated palm oil, respectively may lead to hepatotoxicity and liver failure. Further studies are advised in order to observe the effect and understand the possible mechanism.

Conclusion: Palm oil rich diet fresh or heated cause inflammation, microsteatosis and altered serum ALT and ALP in the liver. The histological changes and altered liver enzymes were not affected by heating except for serum ALT.

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