

Effect of Rutacea Plant Extract (ADD-X) on Inflammatory Biomarkers, Cardiac LDL, Troponin T and Histological Changes in Ovariectomized Rats Fed with Heated Palm Oil

Kumeshini Sukalingam¹, Kamsiah Jaarin², Qodriyah Haji Mohd Saad², Suhaila Mohamed³, Faizah Othman^{1*}

¹Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

²Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

³Department of Food Science, Universiti Putra Malaysia, Serdang, Malaysia.

Available Online: 10th August, 2016

ABSTRACT

Palm oil is chief vegetable oil usually consumed worldwide. The consumption of repeatedly heated palm oil produces detrimental effect that attributes to the development of cardiovascular diseases. This study was aimed to determine the effects of ADD-X and repeatedly heated palm oil mixed with 2% cholesterol diet on cardiac, inflammatory biomarkers and histological changes in ovariectomized rats. Based on the administration of food, animals were divided into 7 groups with 6 rats each. All groups of the animals were ovariectomized except sham after anesthetized. The study was conducted for 6 months. Group I- Sham control fed with Normal rat chow (Sham); Group II- fed with Normal rat chow [Ovx (n)]; Group III- fed with 2% cholesterol chow [Ovx (c)]; Group IV- fed with 2% cholesterol chow mixed with five times heated palm oil (5HPO); Group V- fed with 2% cholesterol chow mixed with five times heated palm oil and ADD-X extract (5HPO-X); Group VI- fed with 2% cholesterol chow mixed with ten times heated palm oil (10HPO); Group VII fed with 2% cholesterol chow mixed with ten times heated palm oil and ADD-X extract (10HPO-X). The rats were sacrificed at the end of the study; blood was collected for cardiac and inflammatory biomarkers. The cardiac tissues of all groups were obtained for biochemical parameters and processed for histopathological examinations. Five times (5HPO) and ten times heated (10HPO) palm oil caused a significant ($p < 0.05$) increase in the level of cardiac lactate dehydrogenase (LDH), troponin (Trp), serum C-reactive protein (CRP), Tumour necrosis factor- α (TNF- α), free fatty acids and triglycerides with increase in cardiac weight compared to control. The changes in the biochemical parameters and cardiac weight were significantly ($p < 0.05$) attenuated by ADD-X supplementation to the heated oil. The histological sections of the heart showed presence of cardiac necrosis in the 5HPO and 10HPO. These histological changes were reduced with ADD-X supplementation. The consumption of repeatedly heated palm oil increases LDL, troponin, CRP, TNF α , triglyceride, FFA, heart weight and cause cardiac necrosis in the post menopausal rat models. Those changes were attenuated by ADDX supplementation.

Keywords: ADD-X; Heated palm oil; cardiac and inflammatory markers; post menopausal rat models.

INTRODUCTION

Palm oil is the most extensively produced vegetable oil in the world. It can be thermally oxidized to augment its palatability, composition changes and deteriorative effect¹. Repeatedly heated palm oil undergoes a sequence of chemical reactions causing alteration the fatty acid composition. Ingestion of the thermally oxidized oil has been reported as concomitant cytotoxic by-products, which are detrimental to cells, tissues and organs². Chronic consumption of dietary fats and oils influence the lipid metabolism and ultimately enhance the possibilities of cardiovascular diseases (CVD). Studies reported that heated vegetable oils increased risk of CVD such as hypertension³⁻⁵ and atherosclerosis⁶. Study postulated that the thermal oxidation of heated oil together with destruction of vitamin E⁷ were the attributed factors for heated oil-induced of CVD. Thermal oxidation and antioxidant

deficiency generate free radicals that might cause vascular inflammation^{8,9} that predisposed to vascular dysfunction leading to both hypertension and atherosclerosis. ADD-X is a plant crude extracts prepared from the Rutaceae family rich in polyphenols. It has been proven to have antioxidants properties and prevent oil absorption into the food¹⁰ (Mohamed and Nor, 2013). The present study was undertaken to see if ADD-X supplementation could reduced inflammation and cardiac protective effect.

MATERIALS AND METHODS

Chemicals

The ADD-X was obtained from Faculty of Medicine, University Putra Malaysia (UPM), Malaysia. Palm oil used for this study was purchased from local manufacturer Organic Gain Sdn Bhd (Bangi, Selangor, Malaysia). For the inflammatory and cardiac markers studies, the ELISA

kits for Troponin, TNF- α and CRP were purchased from ELab Science, USA. LDH activity assay kit was purchased from BioVision, USA. The cardiac biochemical parameters kits such as TGL and FFA were purchased from BioAssay System, USA. Two percent cholesterol diet was obtained from Next Gene Scientific Sdn Bhd, Singapore. All other chemicals were of analytical grade.

Animals

This study was performed after obtaining the ethical approval from the Universiti Kebangsaan Malaysia Animal Ethics Committees. Thirty adult female Sprague-Dawley rats (weighing 250-300g) were used for the present study. All the rats had free access to drinking water and fed on the cholesterol chow. The animals were allowed to acclimatize for 1 week before the experiment was performed. Throughout the study, the rats were housed one per cage, kept under controlled environmental conditions (12-hour cycle- light / dark) and provided free access to food and water *ad libitum*.

Experimental design

Based on the administration of food, animals were divided into seven groups with six rats each. All groups were ovariectomized except sham after being anesthetized with ketamine hydrochloride and xylazine at respective doses of 50 and 10 mg/kg b.w/i.p once before the ovariectomy procedure. Duration of the feeding was 6 months.

Group I: Sham control fed with Normal rat chow (Sham).

Group II: fed with Normal rat chow [Ovx (n)]

Group III: fed with 2% cholesterol chow [Ovx (c)]

Group IV: fed with 2% cholesterol chow mixed with five times heated palm oil (5HPO)

Group V: fed with 2% cholesterol chow mixed with five times heated palm oil and ADD-X extract (5HPO-X)

Group VI: fed with 2% cholesterol chow mixed with ten times heated palm oil (10HPO)

Group VII fed with 2% cholesterol chow mixed with ten times heated palm oil and ADD-X extract (10HPO-X)

Collection of blood and cardiac tissues

At the end of the study, i.e. at 24th week following HPO and ADD-X extract treatment, the rats were sacrificed with overdose of diethyl ether. Each rat was placed in a clean, dry place before blood and heart were taken out. Blood was drawn in heparinized tubes, and the serum was separated by centrifugation. The cardiac tissues were quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and cardiac tissue weights were measured. Half of the tissues were then homogenized in 0.1M Tris-HCl buffer, at pH 7.4. The homogenate was used for the estimations of cardiac lipid profile, cardiac biomarker and inflammatory markers.

Preparation of palm oil diet

Palm oil (PO) used for this study was purchased from local manufacturer Organic Gain Sdn Bhd (Bangi, Selangor, Malaysia). Palm oil was used in five times heated or ten times heated according to the earlier protocol explained by Owu et al.¹¹ with little modifications. Before the frying process, ADD-X extract was prepared with a proportion of 1:10 of ADD-X extract to palm oil and added to the palm oil. In brief, 2.5 L of palm oil was heated up to 180°C in a steel container and employed to deep-fry of 1.5kg of sweet

potatoes. The heating process lasted for 15 min. The heated oil was then kept to chill at room temperature for 4 hours. The same protocol was applied in the once heated palm oil group (1HPO). Pre-cooled hot oil was again used to deep-fry one more new batch of 1kg sweet potatoes. Frying process was continuously carried out without addition, any fresh oil to reimburse for oil losses. The similar heating method was repeated in that order of four and nine times, to make up the five times heated palm oil (5HPO) and ten times heated palm oil (10HPO) respectively. The diets were prepared twice a week. The ratio of cholesterol chow to the oil was 100:15. The mixture was then dried at 70°C in an oven for 4 hours. Each 1000g of food prepared would consist of 850g of rat chow and 150g of heated oil. The heated oil consisted of 135g of palm oil either fresh or repeatedly heated palm oil and 15g of ADD-X extract.

Biochemical parameters

Assay of LDH

Serum LDH level was determined using a commercially available ELISA kit by the method of Hanson et al.¹² with little modifications. The standards and samples were prepared following the manufacturer's instruction. The intensity of coloured product (NADH) was measured using Thermo Scientific micro plate reader (USA) at 450 nm. OD 450 nm was measured to read A1 and incubated at 37°C for 30 minutes. Following the incubation OD 450 nm was measured again to read A2. The standard curve was generated using the A2 reading after subtracting the blank reading. Values of NADH at A1 and A2 were calculated from the standard curve and the LDH activity calculation is as follows as specified in the manufacturer's instruction:

$$\text{LDH activity} = \frac{B}{(T2 - T1)} \times \text{Sample dilution}$$

$$= \frac{\frac{\text{nmol}}{\text{min}}}{\text{ml}} - \frac{\text{mU}}{\text{ml}}$$

B was the NADH amount that was generated between T1 and T2 (Value A2 – Value A1) in nmol.

T1 was the time of first reading (A1) in min.

T2 was the time of second reading (A2) in min.

V was the pre-treated sample volume added into the reaction well (in ml).

Unit definition: One unit LDH was the amount of enzyme that catalyzed the conversion of lactate to pyruvate to generate 1.0 μmol to NADH per minute at 37°C.

Assay of Troponin

Cardiac troponin level was determined using a commercially available ELISA kit by the method of Amran et al.¹³ with little modifications. An amount of 100 μL of Standard, Blank, or Sample was placed in a well. Solutions were then added to the bottom of the micro ELISA plate well. It was gently mixed. The plate was covered and incubated for 90 minutes at 37°C. The liquid was removed from each well, and immediately added to 100 μL of Biotinylated Detection Ab working solution to each well. The plate was gently tapped to ensure thorough mix and incubated for 1 hour at 37°C. Each well aspirated and washed by buffer, and the process was

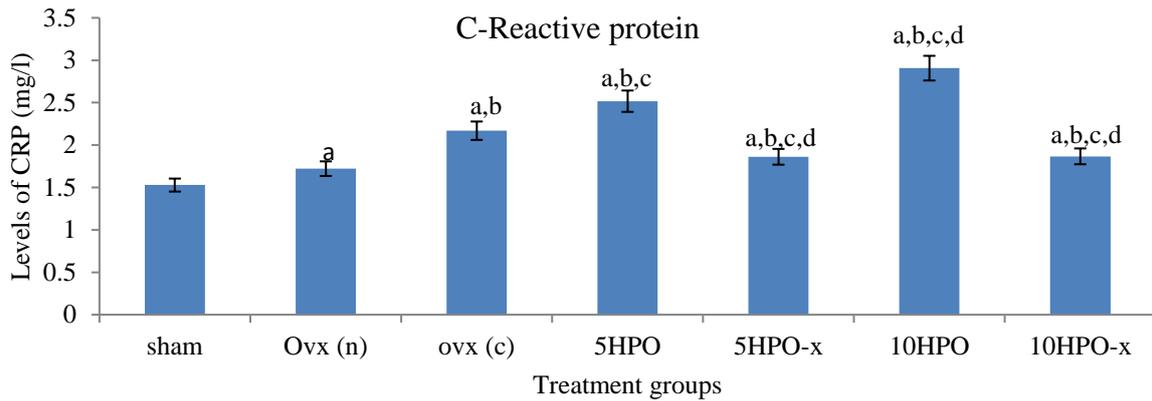


Figure 1: Effect of ADD-X and repeatedly heated palm oil on the CRP level. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

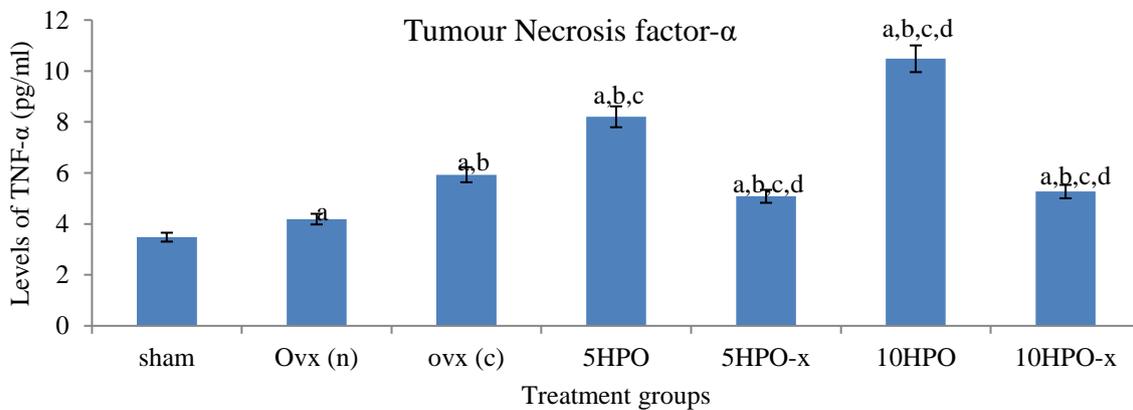


Figure 2: Effect of ADD-X and repeatedly heated palm oil on the TNF-α levels. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

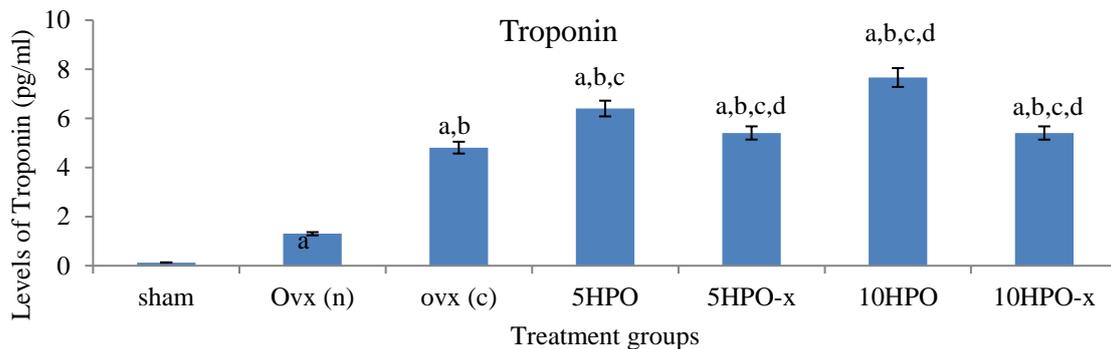


Figure 3: Effect of ADD-X and repeatedly heated palm oil on the Troponin level. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

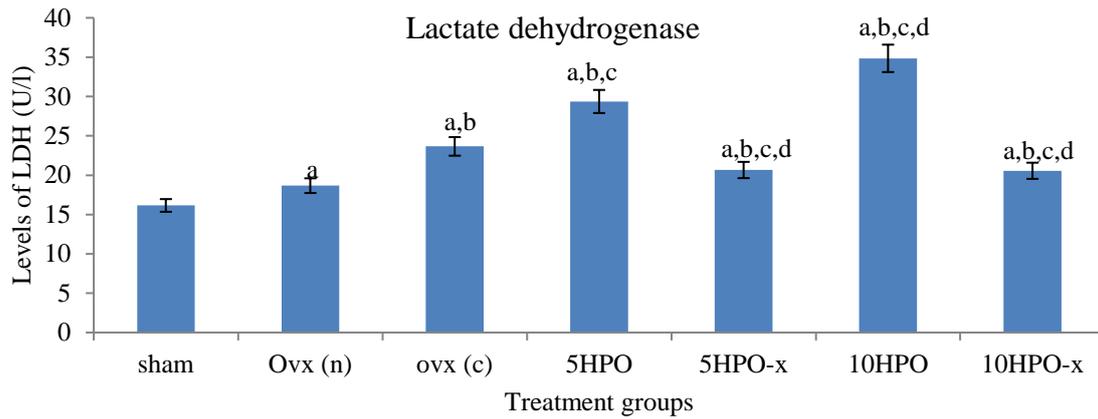


Figure 4: Effect of ADD-X and repeatedly heated palm oil on the LDH level. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

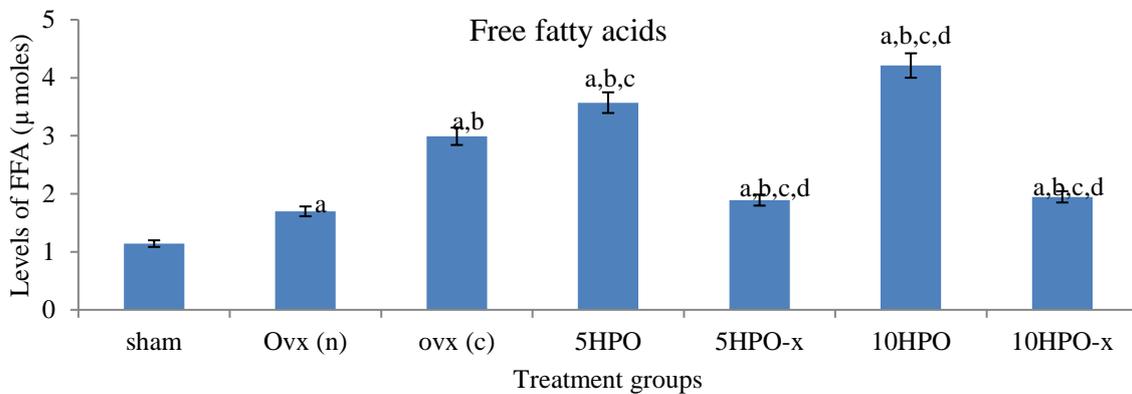


Figure 5: Effect of ADD-X and repeatedly heated palm oil on the cardiac FFA level. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

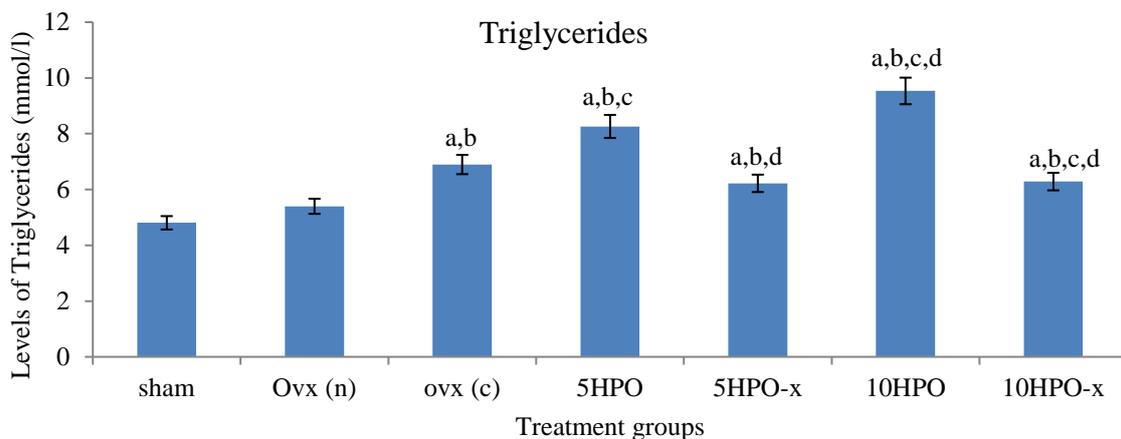


Figure 6: Effect of ADD-X and repeatedly heated palm oil on the cardiac triglycerides level. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

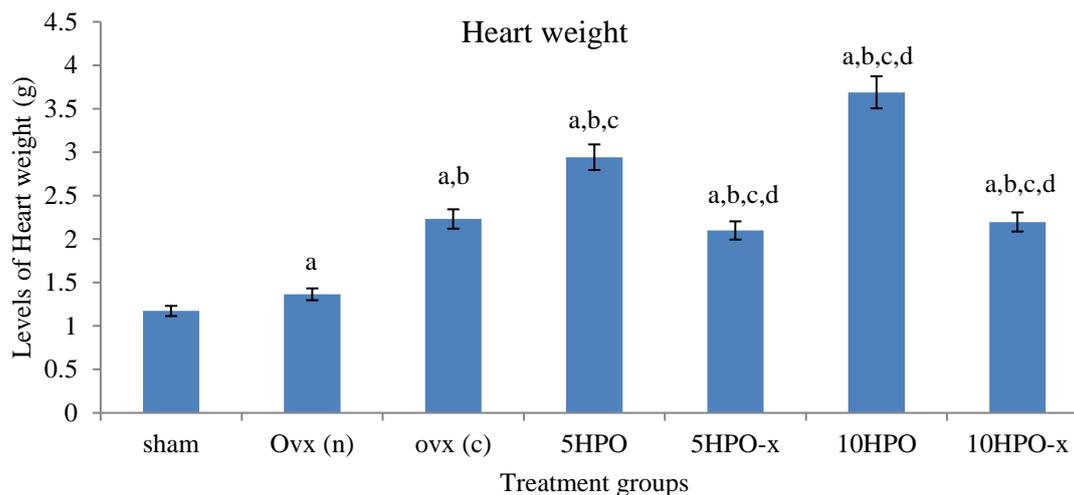


Figure 7: Effect of ADD-X and repeatedly heated palm oil on heart weight. Cholesterol diet (Chol-C), palm oil heated 5 times (5HPO), ADD-X with palm oil heated 5 times (5HPOX), palm oil heated 10 times (10HPO), ADD-X with palm oil heated 10 times (10HPOX). The bars represent mean, and error bars, SD, with $n = 6$ in each group. $p < 0.05$ indicates significant difference compared to ^asham, ^bOvx (n), ^cOvx (c), ^d5HPO and ^e5HPO-x groups respectively.

repeated three times. Complete removal of liquid at each step is essential. After the last wash, the remaining Wash Buffer was removed by aspiration or decantation. The plate was inverted and patted it against thick clean absorbent paper. An amount of 100 μ L of HRP Conjugate working solution was added to each well and covered with the plate sealer and incubated for 30 minutes at 37°C. The washing process was repeated for five times and 90 μ L of Substrate Solution was added to each well, covered and incubated for about 15 minutes at 37°C. The plate was protected from light and 50 μ L of Stop Solution was added to each well. When the color turned yellow the optical density of each well was determined at once, using a micro-plate reader set to 450 nm

Assay of CRP

Serum CRP level was determined using a commercially available ELISA kit by the method of Amran et al.¹³ with little modifications. The intensity of coloured product was measured using a Thermo scientific micro plate reader (USA) at 450 nm absorbance. Values of samples were calculated by the software provided with the micro plate reader and compared to the standard curve generated.

Assay of TNF- α

Serum TNF- α level was determined using a commercially available ELISA kit by the method of Amran et al.¹³ with little modifications. An amount of 100 μ L of standard or sample was added to each well and incubated for 90 minutes at 37°C. After that the liquid was removed and 100 μ L of Biotinylated Detection Ab was added and further incubated for another hour at 37°C. This was then aspirated and washed 3 times. An amount of 100 μ L of HRP Conjugate was further and incubated for another 30 minutes at 37°C. This was then aspirated and washed 5 times. An amount of 90 μ L of Substrate Reagent was further added and incubated for 15 minutes at 37°C. Finally, the 50 μ L Stop Solution was added and read at 450nm immediately.

Assay of cardiac TGL

The Cardiac triglycerides level was determined using a commercially available ELISA kit by the method of Zhao and Erdman¹⁴ with little modifications. 10mL of diluted standards and samples were added into wells of a clear 96-well plate. Working Reagent was prepared for each well, by mixing 100 μ L Assay Buffer, 2 μ L Enzyme Mix, 5 μ L Lipase, 1 μ L ATP and 1 μ L Dye Reagent in a clean tube. 100 μ L of Working Reagent was transferred into standards and sample wells. The plate was tapped to mix and incubated 30 min at room temperature, and finally the optical density was read at 570nm.

Assay of cardiac FFA

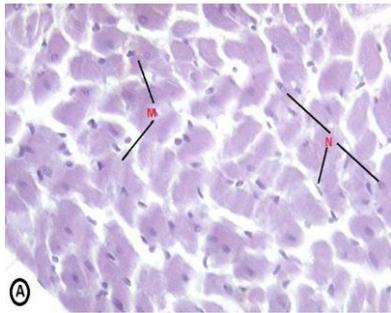
The Cardiac free fatty acids level was determined using a commercially available ELISA kit by the method of Jelinek et al.¹⁵ with little modifications. 10mL diluted standards and samples were added into wells of a clear 96-well plate. Working Reagent was prepared by mixing, 90 μ L Assay Buffer, 1 μ L Enzyme A, 1 μ L Enzyme B, 1 μ L CoSubstrate and 1 μ L Dye Reagent in each well. An amount of 90 μ L of Working Reagent was added to each well. The plate was tapped to mix and incubated 30 min at room temperature, and finally the optical density was read at 570nm.

Cardiac tissue Preparation

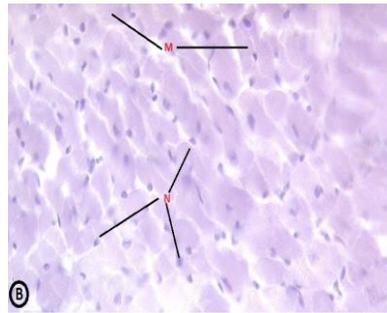
The specimens were sectioned longitudinally, and fixed heart tissues were embedded with paraffin using tissue-embedding center (LEICA EG 1160, Wetzlar, Germany). Tissues were sliced (MICROM HM 340E, Walldorf, Germany) and stained with hematoxylin and eosin (H&E) for light microscopy (BX-40; Olympus, Tokyo, Japan). Histological examination was carried out at magnifications of 20X and 100X.

Statistical analysis

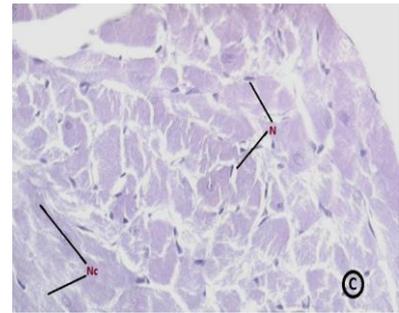
The results for the BP, lipid peroxidation and antioxidant enzyme levels were presented as percentages of the baseline values. All data analyses were conducted using SPSS version 22. The normality of the data was determined by Kolmogorov–Smirnov test. The peroxide



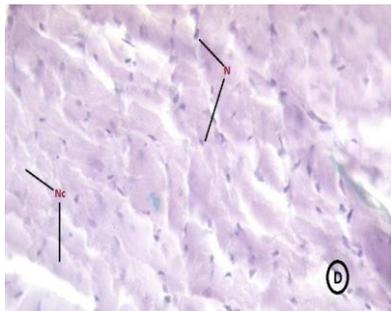
Photomicrograph A represents sham under H & E stain (20X). N- nucleus; M- cardiac muscle



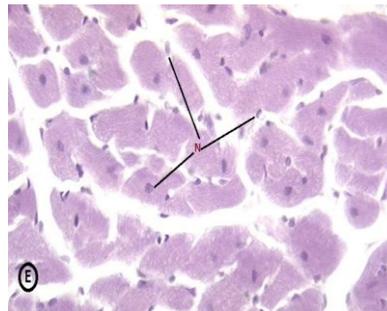
Photomicrograph B represents Ovx (n) under H & E stain (20X). N- nucleus; M- cardiac muscle



Photomicrograph c represents Ovx (c) under H & E stain (20X). N- nucleus; Nc- Necrotic cell



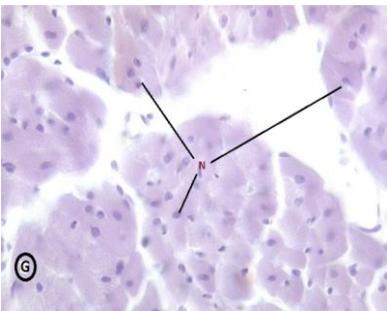
Photomicrograph c represents 5HPO under H & E stain (20X). N- nucleus; Nc- Necrotic cell- eosinophilic cytoplasm, condensed shrunken nuclei.



Photomicrograph c represents 5HPOx under H & E stain (20X). N- nucleus



Photomicrograph c represents 10HPO under H & E stain (20X). Nc- Necrotic cell



Photomicrograph c represents 10HPOx under H & E stain (20X). N- nucleus;

Figure 8: Photomicrograph represents longitudinal section of cardiac tissue in sham, reheated palm oil and ADD-X treated groups under H & E stain (20X).

values among the edible oil groups were compared using one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Differences post-hoc test for differences between pairs of means when applicable. To analyze the differences in the BP activities and the levels of lipid peroxidation among the experimental groups, the Kruskal-Wallis and Mann-Whitney tests were performed. Statistical significance was defined as $P < 0.05$. Data are expressed as means (SD).

RESULTS

Inflammatory markers

The repeatedly heated palm oil caused a significant increase ($p < 0.05$) in the C - reactive protein (CRP) and Tumor necrosis factor α (TNF- α). The level of CRP and TNF- α were significantly reduced with ADD-X supplementation (Figure 1 and 2).

Cardio biomarkers

The repeatedly heated palm oil caused a significant increase ($p < 0.05$) in cardiac lactate dehydrogenase (LDH) and troponin levels. The level of LDH and troponin levels significantly reduced with ADD-X supplementation (Figure 3 and 4)

Cardiac of free fatty acids (FFA) and triglycerides (TG)

The reheated palm oil groups cause a significant increase ($p < 0.05$) in cardiac tissue free fatty acids (FFA) (Figure 5) and triglycerides (TG) (Figure 6) levels at the end of study. The cardiac level of FFA and TG were markedly attenuated with ADD-X.

Changes in heart weight

There was a significant increase ($p < 0.05$) in heart weight in groups fed with repeatedly heated palm oil compared to the control, ADD-X treated groups and sham groups as shown in Figure 7.

Microscopic Changes in Cardiac Tissue

Large, rounded nuclei were centrally located in the cardiac muscle fiber in the control group. A similar pattern was found in the group fed with Ovx (N). Presence of necrotic cells in cardiac tissues of the groups fed with 5HPO and 10HPO were noted. Necrotic cells could be identified by the eosinophilic characteristic that stained bright pink compared to the surrounding normal cells. This was due to the degeneration of the structural protein in cytoplasm that led to the formation of a homogeneous mass. Furthermore, necrotic cells had smaller nuclei compared to the normal nuclei. Pyknosis or nuclei condensation could be observed as a result of the coagulation of chromatin in nuclei, forming a basophilic area following hematoxylin staining. Necroses were noted and more severe in cardiac tissue of the groups fed with 10HPO compared to the 5HPO group. However, there were no significant changes in cardiac histology for control and group fed with ADD-X.

DISCUSSION

Inflammations have been implicated in pathogenesis of atherosclerosis and have been exposed to be connected with an increase in inflammatory biomarkers such as C-reactive protein (CRP), and Tumour Necrosis Factor- α ^{13,16,17}. These inflammatory biomarkers have been identified as high risk factors for CVD such as hypertension and atherosclerosis^{18,19}. In the present study, the increased expression of CRP and TNF- α were seen in 5HPO and 10HPO treated groups. This finding suggests that heated palm oil cause inflammation that predispose to CVD. This finding was comparable the finding of Ng et al.¹⁷ that reported heated vegetable oil cause vascular inflammation. ADD-X supplementation attenuates the increase in CRP and TNF α suggests ADD-X has anti-inflammatory effect. ADDX is rich in polyphenols which may be responsible for the anti-inflammatory effect. The anti-inflammatory effect of polyphenols was reported by Li et al.²⁰ and Aziz et al.²¹. The protective effect of antioxidant such as polyphenol on CVD was already reported before^{22,23}. Elevation in the troponin and lactate dehydrogenase (LDH) activity also indicates the disturbance in the normal cardiac functions²⁴. In the present study, the levels of troponin and LDH activity were increased in 5HPO and 10HPO treated groups, which may suggest that reheated palm oil might have caused cardiac injury. Our finding was in line with Hamsi et al.²⁵ which reported that heated virgin coconut oil increase LDH and troponin which might indicate cardiac injury. Again the reduction in LDH and troponin by ADD-X suggests polyphenols rich antioxidant has protective effect on the heart. These cardiac injuries may be due to inflammatory reactions caused by the ROS and it was generated by the thermal oxidation of repeatedly heating oil, and this caused the endothelial cell injury in heart²⁶. In the present study, histological study of heart showed necrosis in cardiac tissue of 5HPO and 10HPO groups with the latter showing more severity. This finding was similar to Leong et al.²⁷ confirmed necrotic cells and myolysis in cardiac tissues. Histopathology changes of this study was supported by earlier investigation by Shastry et al.²⁸ and Farag Radwan et al.²⁹ which showed the broad fatty vacuolation present

in the cardiac muscle fibres and packed myocardial and vacuolation in papillary muscles respectively. The histological changes of the cardiac tissues appear to be preserved by ADDX. Polyphenols were reported to preserve cardiac tissue²⁰. Cardiac toxicity of heated palm oil was also reported by Edem³⁰ who, suggested the cardiac toxicity was due to lipid peroxidation. Our previous finding showed that heated oil increased TBARS and peroxide value suggest s heated oil increased lipid peroxidation which was reduced by ADD-X administration³¹. The possible mechanism on how heated oil cause cardiac toxicity was unclear. Cardiac tissue is provided with great numbers of mitochondria for blood circulation in the body. We believe that lipid peroxidation induced by heated oil cause mitochondrial damage as the mitochondria is the important site for oxidative phosphorylation and ATP formation. Ozawa³² reported that free radicals may cause cell death. Cell deterioration is caused by biochemical abnormalities and structural changes. Prolonged cell deterioration cause cell necrosis³³ as shown in the present study. Despite the difference in the degree of heating process, the food intake of the treatment groups were high and higher intake of fat diet (2% cholesterol) was associated with increase in their heart weight. In this study, we found that the biochemical and histological changes was associated with increase in cardiac's weight. This finding was in agreement with an earlier study reporting that in rats fed with oxidized palm oil, an increase in organ weights including the heart was found³⁴. We are not sure of the mechanism behind this finding. We postulated that the increased in cardiac weight was likely due to the increased in food intake and weight gain. The increased in food intake lead to accumulation of TG and FFA in cardiac tissue. The increase in cardiac weight was lower with ADD-X supplementation as ADD-X reduced food intake and weight gain, therefore it caused less FFA and TG accumulation in the cardiac tissue.

CONCLUSION

The findings of this study suggest that the consumption of reheated palm oil caused inflammation which produce detrimental effect on cardiac tissues, in an estrogen-deficient state. The consumption of ADD-X with palm oil restores these markers enzymes and protects the cardiac tissues from the necrotic effects by its antioxidant potential. ADD-X appears as stable antioxidant even though it has been treated at high temperature; therefore, it may be use as suitable additive to prevent heated-oil-induced cardiac changes.

ACKNOWLEDGMENTS

This study was supported by a grant from the KPM Ministry of Higher Education. There are no conflicts of interest regarding each of the authors involved in this research work.

REFERENCES

1. Falade AO, Oboh G, Ademiluyi AO, Odubanjo OV. Consumption of thermally oxidized palm oil diets alters biochemical indices in rats. Beni-Suef University

- Journal of Basic and Applied Sciences 2015; 4: 150-156
2. Choe E, Min DB. Chemistry and reactions of deep-fat frying oils. *Journal of Food Science* 2007; 72 (5): R77-R86.
 3. Jaarin K, Mustaffa MR, Leong XF. The effect of heated vegetable oils on blood pressure in rats. *Clinics* 2011; 66(12): 2125-2132.
 4. Ng CY, Kamisah Y, Faizah O, Jaarin K. The role of repeatedly heated soybean oil in the development of hypertension in rats: association with vascular inflammation. *Int J Exp Pathol* 2012; 93(5): 377-387.
 5. Leong XF, Ng CY, Jaarin K. Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. *BioMed Research International* 2015, Article ID 528757, 11 pages <http://dx.doi.org/10.1155/2015/528757>
 6. Adam SK, Soelaiman IN, Nor Aini Umar, Norhayati Mokhtar, Norazlina Mohamed, Kamsiah Jaarin. Effects of Repeatedly Heated Palm Oil on Serum Lipid Profile, Lipid Peroxidation and Homocysteine Levels in a Post-Menopausal Rat Model. *McGill Journal of Medicine* 2008; 11(2): 145-151.
 7. Adam SK, Das S, Othman F, Jaarin K. Fresh soy oil protects against vascular changes in an estrogen-deficient rat model: an electron microscopy study. *Clinics* 2009; 64(11): 1113-1119.
 8. Ng CY, Leong XF, Masbah N, Adam SK, Kamisah Y, Jaarin, K. Heated vegetable oils and cardiovascular disease risk factors. *Vascular Pharmacology* 2014; 61: 1-9
 9. Leong XF, Ng CY, Jaarin K, Mustafa MR. Effects of Repeated Heating of Cooking Oils on Antioxidant Content and Endothelial Function. *Austin J Pharmacol Ther.* 2015; 3(2):1068
 10. Mohamed S, Nor FM. Cooking oil composition with additive to reduce oil absorption, United States Patent. 2013; 8,425-969 B2.
 11. Owu DU, Osim EE, Ebong PE. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta Tropica* 1998; 69: 65-73.
 12. Hanson DG, Juhn SK, Giebink GS, Paparella MM. Lactate dehydrogenase as a measure of inflammation in experimental otitis media. *Archives of Otolaryngology* 1978; 104(6): 333-335.
 13. Amran AA, Zakaria Z, Faizah O, Das S, Hesham AMM. Changes in the vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and c-reactive protein following administration of aqueous extract of piper sarmentosum on experimental rabbits fed with cholesterol diet. *Lipids Health Dis* 2011; 10: 2.
 14. Zhao JR, Erdman JW. Phytic acid in health and disease. *Crit Rev Food Sci Nutr* 1995; 35(6): 495-508
 15. Jelinek D, Castillo JJ, Richardson LM, Luo L, Heidenreich RA, Garver WS. The Niemann-Pick C1 Gene Is Downregulated in Livers of C57BL/6J Mice by Dietary Fatty Acids, but Not Dietary Cholesterol, through Feedback Inhibition of the SREBP Pathway. *J Nutr.* 2012; 142(11):1935-1942. doi: 10.3945/jn.112.162818
 16. Ng CY, Kamisah Y, Faizah O, Jaarin K. The role of repeatedly heated soybean oil in the development of hypertension in rats: association with vascular inflammation. *Int J Exp Pathol.* 2012; 93: 377-387.
 17. Ng CY, Kamisah Y, Faizah O, Jubri Z, Qodriyah HMS, Jaarin K. Involvement of Inflammation and Adverse Vascular Remodelling in the Blood Pressure Raising Effect of Repeatedly Heated Palm Oil in Rats. *Int J Vascul Med Volume* 2012; Article ID 404025, 10 pages doi:10.1155/2012/404025
 18. Sesso HD, Buring JE, Rifai N. C-reactive protein and the risk of developing hypertension. *JAMA* 2003; 290: 2945-2951.
 19. Pai JK, Pischon T, Ma J. Inflammatory markers and the risk of coronary heart disease in men and women. *NEJM* 2004; 351: 2599-2610.
 20. Li, S-H, Zhao P, Tian HP, Chen LH, Cui LQ. Effect of Grape Polyphenols on Blood Pressure: A Meta-Analysis of Randomized Controlled Trials. *PLoS ONE* 2015; 10 (9): e0137665.
 21. Aziz NUA, Othman F, Yusof K, Jaarin K, Das, S. Effect of curcumin on the cardiovascular system of ovariectomized experimental rats fed with high cholesterol and heated palm oil diet. *British Journal of Biomedical Science* 2012; 1(1): 63-73.
 22. Diebolt M, Bucher B, Andriantsitohaina R. Wine Polyphenols Decrease Blood Pressure, Improve NO Vasodilatation, and Induce Gene Expression. *Hypertension*, 2001; 38: 159-165.
 23. Jaarin K, Masbah Norliana, Yusof Kamisah, Mohammad Nursyafiza and Hj Mohd Saad Qodriyah. Potential role of virgin coconut oil in reducing cardiovascular risk factors. *Experimental and Clinical Cardiology* 2014; 20(8): 3399-3410
 24. Risenfors M, Hartford M, Dellborg M, Luepker R, Hjalmarsson K, Swedberg K. Effect of early intravenous rt-PA on infarct size estimated from serum enzyme activity: results from the TEAHAT study. *J Intern Med Suppl* 1991; 734:11-18.
 25. Hamsi MA, Faizah Othman, Srijit Das, Yusof Kamisah, Zar Chi Thent, Haji Mohd Saad Qodriyah, Zaiton Zakaria, Adel Emran, Kogilavani Subermaniam, Kamsiah Jaarin. Effect of consumption of fresh and heated virgin coconut oil on the blood pressure and inflammatory biomarkers: An experimental study in Sprague Dawley rats. *Alex J Med* (2014); <http://dx.doi.org/10.1016/j.ajme.2014.02.002>.
 26. Leong XF, Salimon J, Mustafa MR, Jaarin K. Effect of Repeatedly Heated Palm Olein on Blood Pressure-Regulating Enzymes Activity and Lipid Peroxidation in Rats. *Malays J Med Sci.* 2012; 19(1): 20-29.
 27. Leong XF, Aishah A, Nor Aini U, Das S, Jaarin K. Heated palm oil causes rise in blood pressure and cardiac changes in heart muscle in experimental rats. *Arc Med Res* 2008; 39(6): 567-572
 28. Shastry CS, Narendrakumar Ambalal P, Himonshu J. Evaluation of effect of reused edible oil on vital organs

- of wister rats. Nitte University of Health Science 2011; 1: 10-15.
29. Farag Radwan S, Abdel-Latif, Mohamed S. Effect of non-fried and fried oils of varied fatty acid composition on rat organs. *Agrand Biol J North Am* 2010; 1 (4): 501-509.
30. Edem DO. Palm oil: biochemical, physiological, nutritional, haematological, and toxicological aspects: a review. *Plant Foods Human Nutrition* 2002; 57:319-41.
31. Sukalingam K, Faizah Othman, Saad QHM, Mohamed S, Jaarin K. Consumption of ADD-X and repeatedly heated palm oil on the blood pressure and oxidative stress markers in ovariectomized rats. *Int J Pharmacol.* 2016; (Article in Press)
32. Ozawa T. Mitochondria in cellular aging and degeneration. In: Cadenas E, Packer L, eds. *Understanding the Process of Aging: The Roles of Mitochondria, Free Radicals and Antioxidants*. New York: Marcel Dekker;1999; 265-292.
33. Chandrasoma P, Taylor CR. *Concise Pathology: Cell Degeneration and Necrosis*. Singapore: McGraw-Hill; 2001.
34. Osim EE, Owu DU, Isong EU, Umoh IB. Influence of chronic consumption of thermooxidized and fresh palm oil diets on basal metabolic rate, body weight and morphology of tissue in rats. *Discov Innov* 1994; 6: 389-396.