International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.514.522

Research Article

Consumption of ADD-X and Repeatedly Heated Palm Oil on the Blood Pressure and Oxidative Stress Markers in Ovarectmized Rats

[1]Kumeshini Sukalingam, [2]Kamsiah Jaarin, [2]Qodriyah Haji Mohd Saad, [3]Suhaila Mohamed and [1]Faizah Othman

[1]Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[2]Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[3]Department of Food Science, Universiti Putra Malaysia, Serdang, Malaysia

Abstract

Background and Objective: Oxidative stress is connected with the pathogenesis of cardiovascular diseases. A series of deep-fat frying in dietary cooking oil produce more amount of reactive oxygen species. In this study, repeatedly heated palm oil and ADD-X were studied for its effect on the Blood Pressure (BP), lipid peroxidation and antioxidant parameters. **Methodology:** Thirty female Sprague-Dawley rats were divided into 5 groups with 6 rats each. All groups of the animals were ovariectomized after anesthetized. Group 1 was fed 2% cholesterol chow (Chol-C), groups 2 and 4, respectively fed 2% cholesterol chow along with 5 and 10 times heated palm oil (5 HPO and 10 HPO). Groups 3 and 5 were respectively given 2% cholesterol chow with 5 HPO and 10 HPO along with ADD-X. Duration of the feeding was 6 months. Systolic, diastolic and mean BP were monitored 4 weeks intervals using a non-invasive method. At the end of the study, blood was collected for oxidative stress and antioxidant parameters. **Results:** The BP was elevated significantly ($p<0.05$) in 10 HPO and 5 HPO groups, respectively, where the 10 HPO group viewing higher values. The Increased in BP was significantly lower in ADD-X treated groups. The level of thiobarbituric acid reactive substances (TBARS) was significantly increased in 5 HPO and 10 HPO treated animals and decrease antioxidant parameters compared to Chol-C animals. Consumption of ADD-X reduces TBARS and increase antioxidant enzymes. **Conclusion:** Repeatedly heated palm oil increases BP, lipid peroxidation and decrease antioxidant enzymes. Supplementation of ADD-X reversed the BP and reduce oxidative stress biomarkers in the post menopausal rat models.

Key words: ADD-X, heated palm oil, blood pressure, oxidative stress, antioxidant level

Received: February 20, 2016 Accepted: March 17, 2016 Published: June 15, 2016

Citation: Kumeshini Sukalingam, Kamsiah Jaarin, Qodriyah Haji Mohd Saad, Suhaila Mohamed and Faizah Othman, 2016. Consumption of ADD-X and repeatedly heated palm oil on the blood pressure and oxidative stress markers in ovarectmized rats. Int. J. Pharmacol., 12: 514-522.

Corresponding Author: Faizah Othman, Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In this quick-paced society, frying remains as one of the well-liked methods in food preparation. Utilization of ready-made deep-fried food is high, especially in developing countries. Highly oxidized fatty acids are consumed through intake of these fried foods. Edible vegetable oil is the chief element in these fried food stuffs. Therefore, the cost of the oil becomes the most important factor to be considered in terms of economy. As a result, vegetable oil is often to be repeatedly heated to ensure cost effectiveness. The oil is thus reused until it is discarded and replaced with fresh oil[1,2].

Palm oil is the chief oil among the globally produced oil and other fat market, which is proposed to remain the prominent fat source[3] through 2016. It suggests superior resistance to high temperature oxidation during frying as well as usual antioxidants from the vitamin E cluster, the tocotrienols[4]. Hence, the usage of oil is repeatedly used to reduce the expenditure of food preparation. When frying oil is heated at high temperatures, the oil undergoes various changes of organoleptic properties, nutritional alteration along with the formation of hydroperoxides and aldehydes[5]. These noxious stuffs are engrossed with the food and ultimately enters into the body and may have a detrimental effect on health[1].

Studies have shown that the consumption of repeatedly heated palm and soybean oils drastically increased the blood pressure in experimental animals[2,6]. Moreover, Leong *et al*.[2] also reported that consumption of repeatedly heated frying oils is linked with increased menace of hypertension. The usage of reheated frying oil gives rise to unfavour health risks such as atherosclerosis[7,8], disturbance of endothelial function and alterations in genetic materials[9,10]. Many study data recommended that chronic consumption of reheated cooking oils enhances the risk of CVD and cancer[1,6]. The occurrence of CVD is higher in men relatively to women of the same age group[11]. However, after menopause, the risk increases in women due to deficiency of estrogen level. The estrogen was shown to be cardioprotective in ovariectomized female rats[12].

The generation of reactive oxygen species during the reheated frying process may possibly injure membrane lipids through lipid peroxidation. Malondialdehyde (MDA), one of the end products of secondary oxidation of poly unsaturated fatty acids has been revealed to be of biochemical implications[13], which causes endothelial damage, vascular inflammation and cell membrane injury[14]. Oxidative degradation of heated palm oil was proven to produce higher peroxide values[15-17] and increased in plasma MDA level[17]. Many of the antioxidant additives available in the retail market are made up of synthetic chemicals, which help to scavenge these free radicals. Plants possess abundant biophenolic composites, which are antioxidant agents based on their redox properties or that permitted them to act as reducing agents.

The ADD-X is a crude extracts obtain from the plant parts of the Rutaceae family to be incorporated into a cooking oil for reducing oil adsorption into fried food composition[18]. The additive stuffs proved to employ as a natural fat antioxidant, inhibitor of oil absorption due to the presence of health promoting composition that makes the fried food less dangerous to health. The present study aimed to determine the effect of ADD-X and reheated palm oil on blood pressure and antioxidant biomarkers. To the best of our facts, this might be the initial study of its kind. The study further suggested that ADD-X is a stable antioxidant even though it has been treated at high temperature, may have no detrimental effect on BP and interrelated oxidative biomarker enzymes. Therefore, it may be consumed as frying cooked oil in near future.

MATERIALS AND METHODS

Chemicals: The ADD-X was acquired from Faculty of Medicine, University Putra Malaysia (UPM), Malaysia. The SOD, CAT, TBARS and reduced GSH assay kits were purchased from the Cayman chemical company, 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 250-300 g) were used for the present study. All the rats had free access to drink water and feed 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 h cycle-light/dark) and provided free access to food and water *ad libitum*.

Experimental design: Animals were divided into 5 groups with 6 rats each. All groups of the animals were ovariectomized after anesthetized of ketamine hydrochloride and xylazine at respective doses of 50 and 10 mg kg^{-1} b.wt., i.p. of once before the ovariectomy protocol. Group 1 was fed 2% cholesterol chow (Chol-C), while groups 2 and 4, respectively fed 2% cholesterol chow added along with 5 and 10 times heated palm oil (5 HPO and 10 HPO).

Groups 3 and 5 were respectively given 2% cholesterol chow added along with ADD-X and 5 and 10 times heated palm oil (5 HPOX and 10 HPOX). All groups of the rats were treated daily for 6 months. The BP was measured at baseline and at intervals of 4-24 weeks using a non-invasive method. After the treatment, the blood was drawn through the retro-orbital sinus superior to the treatment and at the end of the study. The blood was then centrifuged and obtains serum for the biochemical analysis.

Preparation of palm oil diet: Palm Oil (PO) used for this study was purchased from local manufacturer Organic Gain Sdn Bhd (Bangi, Selangor, Malaysia). It was used in 5 times heated or 10 times heated according to the earlier procedure explained by Owu *et al.*[19] with little modifications. In brief, 2.5 L of palm oil was heated up to 180°C in a steel container and employed to deep-fry of 1.5 kg of sweet potatoes. The ADD-X extract with the proportion of 1:10 of ADD-X extract to palm oil were prepared. The heating process lasted for 15 min. The heated oil was then kept to chill at room temperature for 4 h. The same protocol was applied in the once heated palm oil group (1 HPO). Pre-cooled hot oil was again used to deep-fry one more new batch of 1 kg 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 4 and 9 times, 5 times heated palm oil (5 HPO) and 10 times heated palm oil (10 HPO) were obtained. The diets were prepared two times in a week. The ratio of cholesterol chow of the oil was 100:15. Then the mixture was dehydrated at 70°C in an oven for 4 h. The 1000 g of food will consist of 850 g of rat chow and 150 g of heated oil. The heated oil was consisting of 135 g of palm oil either fresh or repeatedly heated palm oil and 15 g of ADD-X extract.

Measurement of Peroxide Value (PV): The peroxide content of palm oil and ADD-X was measured using standard titration method (Official method Cd 8-53) as per the American Oil Chemists Society[20]. Normally this technique needed 5 g of the oil that was mixed with 30 mL of acetic acid 20 mL of chloroform in a conical flask. Then, the solution was mixed with a gentle shaking followed by added 0.5 mL of saturated potassium iodide 30 mL of distilled water and a few drops of 10% starch solution. The sample was titrated against 0.01 N sodium thiosulphate solutions taken in the burette, which were previously standardized by using potassium dichromate and potassium iodide. The titration was persisting until the disappearance of blue colour. Peroxide values were then defined by the divergence in volume of sodium thiosulphate solution used for oil samples and blank (without oil), divided by its normality. The rate of peroxidation in the oil sample was expressed as milliequivalents of active oxygen per kilogram.

Measurement of blood pressure: Blood pressure was measured using the non-invasive tail-cuff method with sphygmomanometer technique using CODA data at the baseline and at the end of the experiment. The rats were anesthetized by inhalation of diethyl ether before the measurement was taken[14]. The readings were measured thrice one after the other and the mean was then taken as an ultimate reading for systolic BP[14]. The experiment was continued for 24 weeks. The systolic, diastolic and mean blood pressure was determined monthly.

Biochemical parameters

Assay of catalase (CAT): The serum CAT level was estimated by the following method of Aebi[21] with little modifications. The blood was mixed with 50 mM of phosphate buffer (pH 7.4) and kept at 4°C. Then, it was centrifuged at 3,000 rpm for 10 min. About 50 µL of supernatant was added to a cuvette that had 2 mL of 50 mM phosphate buffer. Added 1 mL of 30 mM hydrogen peroxide and changes in absorbance were measured for 30 sec at 240 nm at an interval of 15 sec. Catalase activity was assayed as units/mg protein.

Assay of superoxide dismutase (SOD): The serum SOD level was estimated by the following method of Kakkar *et al.*[22] with little modifications. The blood was mixed with 0.25 M tris sucrose buffer and kept at 4°C. Then, it was centrifuged at 10,000×g rpm for 15 min. Supernatant was then fractionated by 50% ammonium sulphate and dialyzed overnight. An aliquot of the supernatant (100 µL) was added to sodium pyrophosphate buffer (pH 8.3) followed by the addition of 0.1 mL of 186 µM phenazine methosulphate, 0.3 mL of 300 mM nitroblue tetrazolium and 0.2 mL of 780 µM NADH. The reaction mixture was incubated for 90 sec at 30°C and stopped the reaction by adding 1 mL of glacial acetic acid. About 4 mL of n-butanol was then added and centrifuged at 3,000 rpm for 10 min. The absorbance was measured at 560 nm. The SOD activity is expressed as units/mg protein as compared to the standard.

Assay of reduced glutathione (GSH): Serum reduced GSH level was estimated by the following method of Griffith[23] with little modifications. Centrifuged the blood at 200 rpm for

10 min at 4°C. Pipetted out the top yellow serum layer without disturbing the white buffy layer. Removed the white buffy layer (leukocytes) and discard. Lysed the erythrocytes in 4 times its volume of ice-cold water. Centrifuge at 10,000 rpm for 15 min at 4°C. Collected the supernatant and stored on ice. The serum was carried to be deproteinated before assay. Quantification of GSSG is accomplished by first derivatizing GSH with 2-vinylpyridine. Prepare a 1 M solution of 2-vinylpyridine in ethanol by mixing 108 µL of 2-vinylpyridine and 892 µL of ethanol. Add 10 µL of the 2-vinylpyridine solution per milliliter of sample from step 4 of deproteination of the sample. Mix well on a vortex mixer and incubate at room temperature for about 60 min and assay the sample. This procedure can derivatize up to 1 mM GSH. More concentrated samples should be diluted with MES Buffer before derivatization. Read the plate at 414 nm after 25 min. Reduced GSH activity is expressed as units/mg protein as compared to the standard.

Assay of thiobarbituric acid reactive substances (TBARS): Lipid peroxidation was measured in serum in form of MDA using thiobarbituric acid. The level of serum TBARS was estimated following a method of Ohkawa *et al.*[24]. The blood was centrifuged using with 10% trichloroacetic acid at 4°C. About 0.2 mL homogenate was pipetted out into a clean test tube and added 0.3 mL of 8% sodium dodecyl sulphate and 2 mL of 30% acetic acid, 2 mL of 1% TBA and volume was made up to 6 mL using Double Distilled Water (DDW). The test tubes were then heated at 95°C for an hour in a boiling water bath. After cooling, added 1 mL of DDW and 5 mL of n-butanol: Pyridine (in the ratio of 15:1) mixture of the test tubes and it was centrifuged at 5000 rpm for 10 min. The absorbance of developed colour was measured at 540 nm using photometry. Commercially available tetraethoxypropane was used as a standard. The TBARS was assayed as nmol/mg protein.

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

Peroxide values: The peroxide values measured in the oils are shown in Fig. 1. The values in the 1, 5 and 10 times heated palm and ADD-X were significantly elevated ($p<0.005$) compared to the fresh oil, respectively. There was a significant increase ($p<0.05$) in the peroxide index for 1 HPO (3-fold increase), 5 HPO (5-fold increase), 10 HPO (6-fold increase) compared with the value of fresh oil. Addition of ADD-X reduced peroxide values of 5 HPO and 10 HPO significantly ($p<0.05$) compared to respective reheated palm oils groups.

Blood pressure: By the end of the study, there was an increase in systolic, diastolic and mean blood pressure in 5 HPO and 10 HPO compared to respective control. Rats fed 5 HPOX or 10 HPOX showed a significant decrease ($p<0.05$) in systolic,

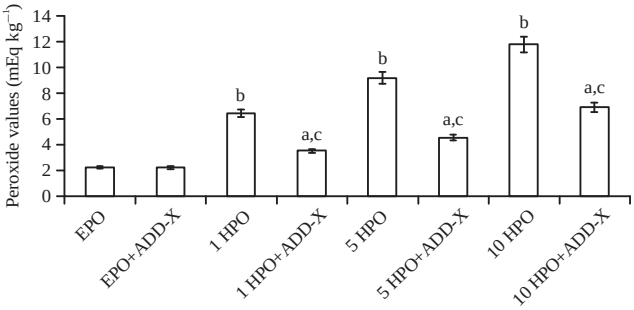

Fig. 1: Peroxide values (mEq kg^{-1}) in the fresh and heated palm and ADD-X. Bars represent Mean±SEM (n = 6). Horizontal line indicates a maximum allowable peroxide value for edible oils according to the American Oil Chemists Society[20], [a]$p<0.005$ compared to 1 HPO, 5 HPO and 10 HPO, [b]$p<0.05$ compared to FPO and [c]$p<0.05$ compared to FPO

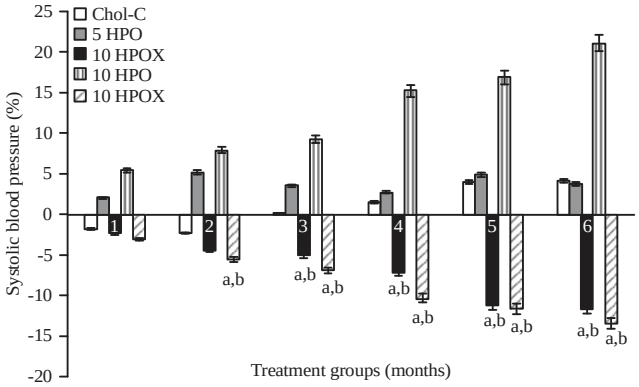

Fig. 2: Changes in the systolic BP level. Cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]p<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

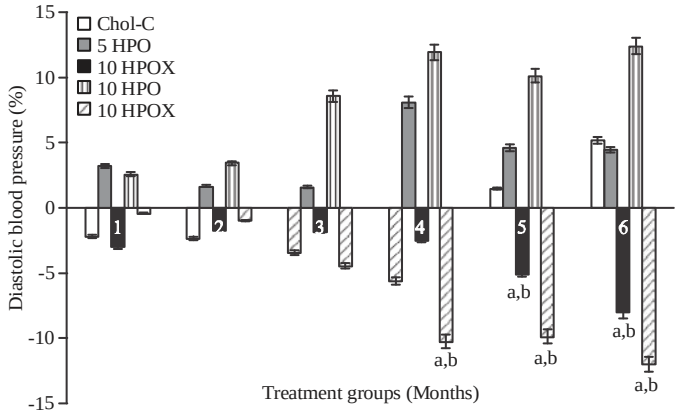

Fig. 3: Changes in the diastolic BP level. Cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]p<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

diastolic and mean blood pressure compared to the 5 HPO and 10 HPO groups. Nevertheless, the results found that the rats fed with 10 HPOX showed significantly lower systolic, diastolic and mean blood pressure at the final month compared to all experimental groups (Fig. 2-4).

Changes in the SOD level: 5 HPO and 10 HPO groups reduced the CAT levels by 10 and 23%, respectively (Fig. 5). The ADD-X caused 18 and 20% elevation in SOD levels (p<0.05) in 5 HPOX and 10 HPOX treated group, respectively compared to control.

Changes in the CAT level: The changes in the serum CAT level exhibited in Fig. 6. The 5 HPO and 10 HPO groups reduced the CAT levels by 9 and 26%, respectively. In contrast, ADD-X caused 25 and 28% increased in the activities of CAT levels (p<0.05) for rats fed with 5 HPOX and 10 HPOX, respectively compared to normal control.

Changes in the reduced GSH level: The 5 HPO and 10 HPO groups reduced the GSH levels by 22 and 33%, respectively (Fig. 7). There were 28 and 32% enhanced activities of GSH levels (p<0.05) for rats fed with 5 HPOX and 10 HPOX, respectively compared to control.

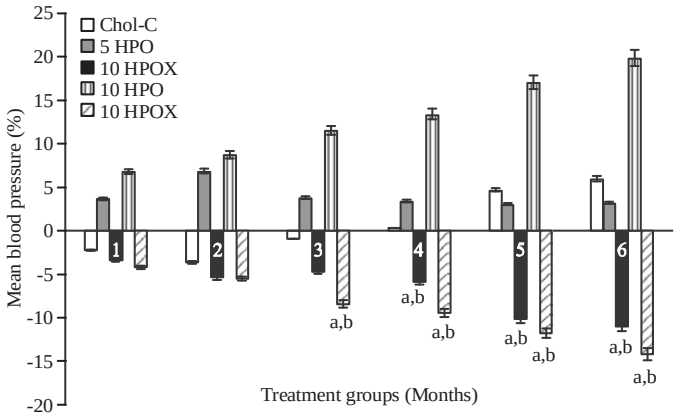

Fig. 4: Changes in the mean BP level. Cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]p<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

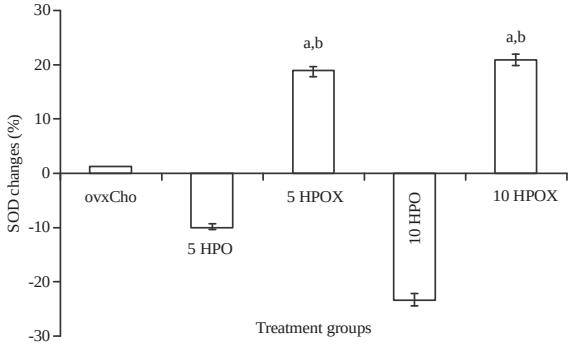

Fig. 5: Percentage of change in the superoxide dismutase (SOD) level after 6 months of feeding with the cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]p<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

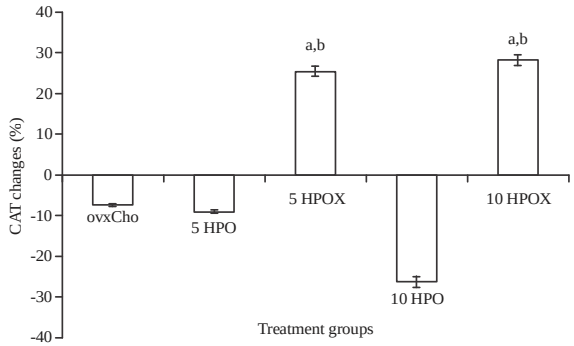

Fig. 6: Percentage of change in the catalase (CAT) level after 6 months of feeding with the cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

Changes in the TBARS level: The changes in the serum TBARS level exhibited in Fig. 8. The 5 HPO and 10 HPO groups increased the TBARS levels by 2 and 30%, respectively. There were 21.7 and 19.2% decreased activities of TBARS levels (p<0.05) for rats fed with 5 HPOX and 10 HPOX, respectively compared to normal control rats.

DISCUSSION

In the present study, the peroxide values were increased by repeated heating of palm oil. This result may indicate repeated heating increased lipid peroxidation. The degree of oxidation was affected by the number of frying. Other than the peroxides, there are other oxidized components that are formed during oil heating, such as oxidative dimers and

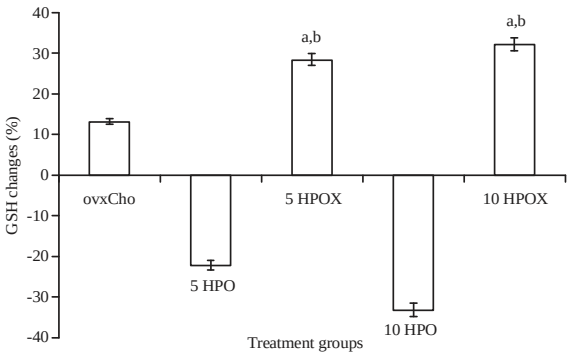

Fig. 7: Percentage of change in the reduced glutathione (GSH) level after 6 months of feeding with the cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

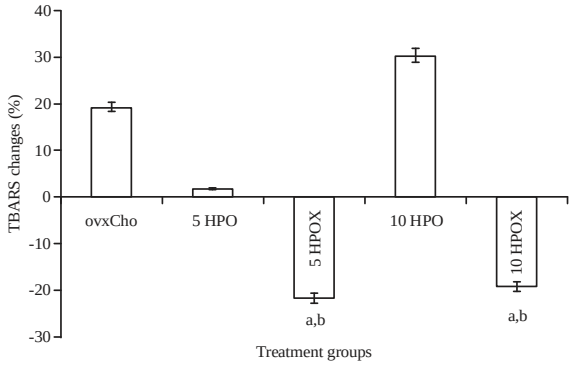

Fig. 8: Percentage of changes in the thiobarbituric acid reactive substances (TBARS) level after 6 months of feeding with the cholesterol diet (Chol-C), palm oil heated 5 times (5 HPO), ADD-X with palm oil heated 5 times (5 HPOX), palm oil heated 10 times (10 HPO), ADD-X with palm oil heated 10 times (10 HPOX). The bars represent Mean and error bars, SD, with n = 6 in each group, [a]<0.05: Chol-C vs 5 HPOX and 10 HPOX treated groups, respectively, [b]p<0.05: 5 HPO and 10 HPO vs 5 HPOX and 10 HPOX treated groups, respectively

oxidized triacylglycerols[25]. The peroxide value of the 10 times heated oil in this study was 12 mEq kg^{-1}. This value was exceeding 10 mEq kg^{-1} oil, which was the recommended the upper limit of peroxide value set by Rani *et al.*[26] the American Oil Chemists Society. This finding was in line with Awney *et al.*[27] who reported that the animals fed thermally oxidized soy oil had a peroxide value of 14 mEq kg^{-1} in various organs such as liver, kidney, testes and brain. In this study heated 5 HPO and 10 HPO increase mean, systolic and diastolic Blood Pressure (BP) of ovariectomized female rats. This finding was comparable to Jaarin *et al.*[28], Leong *et al.*[17] and Ng *et al.*[16], who reported that heated palm and soy oil increase BP in normal male rats.

Ovariectomized female rats were used in this study as it mimic post menopause modal in women. Ovariectomy induced an estrogen deficit state. The estrogen has been shown to protect against oxidative stress[29]. The increase in lipid peroxidation in the study was in part due to heated palm oil, which was further augmented by estrogen deficiency state. This finding was in agreement with Sanchez-Rodriguez *et al.*[30] who reported that menopause might be one of the major risk factors for oxidative stress. Supplementation of ADD-X to 5 HPO and 10 HPO significantly attenuated the blood pressure raising effect of heated palm oil.

This showed that heated 5 HPO and 10 HPO increased CAT, SOD and glutathione, which indicated oxidative stress, which was induced by heated oil and ovariectomy overwhelmed the body defense mechanism against oxidative stress. The ADD-X being rich in polyphenols were able to overcome the stress, oxidative condition leading to an increase in antioxidant enzymes defends mechanism. The protective effect of ADD-X in this study was due to its high contain of antioxidants such as polyphenols. The protective effect of antioxidant such as vitamin E on cardiovascular disease was already reported before[31]. Polyohenols have been reported to reduce blood pressure[32,33]. This theory was further supported by significant reduction in antioxidant enzyme concentrations such as CAT, SOD and glutathione reductase in ADD-X supplemented groups compared to heated palm oil groups. This change happened together with blood pressure changes. Apart from heated oil the changes in TBARS, GSH, SOD and CAT levels may be due to estrogen-deficient state in rats. This finding was comparable to Vaishali *et al.*[34] and Ogunro *et al.*[35] who reported that estrogen deficiency reduced those antioxidant enzymes.

In the enzymatic antioxidant defense system, SOD is one of the most important enzymes and scavenges O_2- anion (which is the first product of O_2 radicals) to form H_2O_2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions[36]. The O_2- anion is known to inactivate CAT[37].

Catalase has been regarded as a major determinant of hepatic and cardiac antioxidant status[38]. It is known to be involved in detoxification of H_2O_2 concentrations[39,40]. These enzyme activities were inactivated by ROS during diabetes and cardiovascular diseases[41]. The GSH concentration in blood was significantly reduced, which is also indicative of oxidative stress. Increasing of GSH concentration in cardiac tissue in obese rats give response to free radical scavenge and protect cells against oxidative damage[42].

CONCLUSION

Repeatedly heated palm oil caused oxidative stress and decrease antioxidant level in the blood and increases blood pressure. The treatment with ADD-X reduces the detrimental effect on blood pressure, which happened to a reduction in oxidative stress biomarkers and an increase in antioxidant enzymes. This finding may suggest that blood pressure lowering effect of ADD-X was mediated via its antioxidant property. Further pharmacological and biochemical investigations are under way to elucidate the mechanism of the blood pressure lowering effect of ADD-X.

ACKNOWLEDGMENT

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 study.

REFERENCES

1. Leong, X.F., C.Y. Ng, K. Jaarin and M.R. Mustafa, 2015. Effects of repeated heating of cooking oils on antioxidant content and endothelial function. Austin J. Pharmacol. Therapeut., Vol. 3, No. 2.
2. Leong, X.F., M.N.M. Najib, S. Das, M.R. Mustafa and K. Jaarin, 2009. Intake of repeatedly heated palm oil causes elevation in blood pressure with impaired vasorelaxation in rats. Tohoku J. Exp. Med., 219: 71-78.
3. Gunstone, F.D., 2002. Production and Trade of Vegetable Oils. In: Vegetable Oils in Food Technology: Composition, Properties and Uses, Gunstone, F.D. (Ed.). Blackwell Publishing, Oxford, pp: 1-17.
4. Ong, A.S.H. and S.H. Goh, 2002. Palm oil: A healthful and cost-effective dietary component. Food Nutr. Bull., 23: 11-22.
5. Danowska-Oziewicz, M. and M. Karpinska-Tymoszczyk, 2005. Quality changes in selected frying fats during heating in a model system. J. Food Lipids, 12: 159-168.
6. Hamsi, M.A., O. Faizah, S. Das, Y. Kamisah and Z.C. Thent *et al*., 2015. Effect of consumption of fresh and heated virgin coconut oil on the blood pressure and inflammatory biomarkers: An experimental study in Sprague Dawley rats. Alexendria J. Med., 51: 53-63.
7. Farag, R.S., M.S. Abdel-Latif, A.M.M. Basuny and B.S. Abd El Hakeem, 2010. Effect of non-fried and fried oils of varied fatty acid composition on rat organs. Agric. Biol. J. North Am., 1: 501-509.
8. Adam, S.K., S. Das and K. Jaarin, 2009. A detailed microscopic study of the changes in the aorta of experimental model of postmenopausal rats fed with repeatedly heated palm oil. Int. J. Exp. Pathol., 90: 321-327.
9. Eshak, M.G., I.S. Ghaly, W.K.B. Khalil, I.M. Farag and K.Z. Ghanem, 2010. Genetic alterations induced by toxic effect of thermally oxidized oil and protective role of tomatoes and carrots in mice. J. Am. Sci., 6: 175-188.
10. Khalil, W.K.B., H.A.M. Abd El-Kader, M.G. Eshak, I.M. Farag and K.Z. Ghanem, 2009. Biological studies on the protective role of artichoke and green pepper against potential toxic effect of thermally oxidized oil in mice. Arab J. Biotechnol., 12: 27-40.
11. Rossouw, J.E., 2002. Hormones, genetic factors and gender differences in cardiovascular disease. Cardiovasc. Res., 53: 550-557.
12. Lu, H., G.C. Melendez, S.P. Levick and J.S. Janicki, 2012. Prevention of adverse cardiac remodeling to volume overload in female rats is the result of an estrogen-altered mast cell phenotype. Am. J. Physiol.-Heart Circ. Physiol., 302: H811-H817.
13. Adam, S.K., I.N. Soelaiman, N.A. Umar, N. Mokhtar, N. Mohamed and K. Jaarin, 2008. Effects of repeatedly heated palm oil on serum lipid profile, lipid peroxidation and homocysteine levels in a post-menopausal rat model. Mcgill J. Med., 11: 145-151.
14. Azlina, A.A., H.S. Farihah, H.M.S. Qodriyah and M.F.N. Azlina, 2009. Effects of *Piper sarmentosum* water extract on 11-β hydroxysteroid dehydrogenase type 1 bioactivity in ovariectomy-induced obese rats. Int. J. Pharmacol., 5: 362-369.
15. Harrison, D.G., M.C. Gongora, T.J. Guzik and J. Widder, 2007. Oxidative stress and hypertension. J. Am. Soc. Hypertens., 1: 30-44.
16. Ng, C.Y., Y. Kamisah, O. Faizah, Z. Jubri, H.M.S. Qodriyah and K. Jaarin, 2012. Involvement of inflammation and adverse vascular remodelling in the blood pressure raising effect of repeatedly heated palm oil in rats. Int. J. Vasc. Med. 10.1155/2012/404025.
17. Leong, X.F., M.R. Mustafa, S. Das and K. Jaarin, 2010. Association of elevated blood pressure and impaired vasorelaxation in experimental sprague-dawley rats fed with heated vegetable oil. Lipid Health Dise., Vol. 9. 10.1186/1476-511X-9-66.

18. Mohamed, S. and F.M. Nor, 2013. Cooking oil composition with additive to reduce oil absorption. United States Patent US8425969 B2. http://www.google.com/patents/US8425969.
19. Owu, D.U., E.E. Osim and P.E. Ebong, 1998. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. Acta Tropica, 69: 65-73.
20. AOCS., 2003. Official Methods and Recommended Practices of the American Oil Chemists' Society. 4th Edn., AOCS Press, Champaign, Illinois, USA.
21. Aebi, H.C., 1974. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Academic Press, New York, USA., pp: 673-677.
22. Kakkar, P., B. Das and P.N. Viswanathan, 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys., 21: 130-132.
23. Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., 106: 207-212.
24. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
25. Subermaniam, K., Q.J.M. Saad, S. Das and F. Othman, 2014. Virgin Coconut Oil (VCO) decreases the level of malondialdehyde (MDA) in the cardiac tissue of experimental Sprague-Dawley rats fed with heated palm oil. J. Med. Bioeng., 3: 102-106.
26. Rani, A.K.S., S.Y. Reddy and R. Chetana, 2010. Quality changes in trans and trans free fats/oils and products during frying. Eur. Food Res. Technol., 230: 803-811.
27. Awney, H.A., 2011. The effects of *Bifidobacteria* on the lipid profile and oxidative stress biomarkers of male rats fed thermally oxidized soybean oil. Biomarkers, 16: 445-452.
28. Jaarin, K., M.R. Mustafa and X.F. Leong, 2011. The effects of heated vegetable oils on blood pressure in rats. Clinics, 66: 2125-2132.
29. Abbas, A.M. and A.Z. Elsamanoudy, 2011. Effects of 17β-estradiol and antioxidant administration on oxidative stress and insulin resistance in ovariectomized rats. Can. J. Physiol. Pharmacol., 89: 497-504.
30. Sanchez-Rodriguez, M.A., M. Zacarias-Flores, A. Arronte-Rosales, E. Correa-Munoz and V.M. Mendoza-Nunez, 2012. Menopause as risk factor for oxidative stress. Menopause, 19: 361-367.
31. Ismail, N.M., N.A. Ghafar, K. Jaarin, J.H. Khine and G.M. Top, 2000. Vitamin E and factors affecting atherosclerosis in rabbits fed a cholesterol-rich diet. Int. J. Food Sci. Nutr., 51: 79-94.
32. Li, S.H., P. Zhao, H.B. Tian, L.H. Chen and L.Q. Cui, 2015. Effect of grape polyphenols on blood pressure: A meta-analysis of randomized controlled trials. PLoS ONE, Vol. 10. 10.1371/journal.pone.0137665.
33. Diebolt, M., B. Bucher and R. Andriantsitohaina, 2001. Wine polyphenols decrease blood pressure, improve no vasodilatation and induce gene expression. Hypertension, 38: 159-165.
34. Vaishali, S., S. Singh, S. Neelima and S. Shaila, 2005. Status of antioxidant enzymes and trace metals in postmenopausal women. J. Obstet. Gynecol. India, 55: 64-66.
35. Ogunro, P.S., A.A. Bolarinde, O.O. Owa, A.A. Salawu and A.A. Oshodi, 2014. Antioxidant status and reproductive hormones in women during reproductive, perimenopausal and postmenopausal phase of life. Afr. J. Med. Med. Sci., 43: 49-57.
36. Bhattacharya, A., A. Chatterjee, S. Ghosal and S.K. Bhattacharya, 1999. Antioxidant activity of active tannoid principles of *Emblica officinalis* (Amla). Indian J. Exp. Biol., 37: 676-680.
37. Halliwell, B. and J.M.C. Gutteridge, 1984. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet, 323: 1396-1397.
38. Wohaieb, S.A. and D.V. Godin, 1987. Alterations in free radical tissue-defense mechanisms in Streptozocin-induced diabetes in rat: Effects of insulin treatment. Diabetes, 36: 1014-1018.
39. Yoshikawa, T., Y. Naho, A. Kishi, T. Tomii and T. Kaneko *et al*., 1993. Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut, 34: 732-737.
40. Manonmani, G., K. Anbarasi, K. Balakrishna, G. Veluchamy and C.S. Shyamala Devi, 2002. Effect of *Terminalia arjuna* on the antioxidant defense system in alloxan induced diabetes in rats. Biomedicine, 22: 52-61.
41. Ahmed, S., A. Rahman, A. Alam, M. Saleem, M. Athar and S. Sultana, 2000. Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride-induced oxidative stress. J. Ethnopharmacol., 69: 157-164.
42. Vincent, H.K., S.K. Powers, A.J. Dirks and P.J. Scarpace, 2001. Mechanism for obesity-induced increase in myocardial lipid peroxidation. Int. J. Obesity Relat. Metab. Disord., 25: 378-388.