

EFFECTS OF LONG TIME HEATED PALM OIL ON PHYSICO-CHEMICAL PROPERTIES AND PHARMACOLOGY OF RABBIT

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ABSTRACT

This study was carried out to evaluate the physico-chemical properties of heated palm oil and the effects of consumption of heated palm oil diet on body weight, blood profiles and histological changes in rabbit organs. It was found that the iodine value of heated palm oil was decreased whereas the acid value was increased. The body weight of all treated rabbits was increased remarkably in comparison to control one. Significant changes on WBC, RBC, platelet count, hemoglobin concentration and ESR were also observed. On the other hand, creatinine, SGPT, SGOT and uric acid of heated palm oil diet groups showed significant changes with respect to control group. The value of TC, HDL, LDL and TG of treated groups were found different compared to the untreated rabbits. Histopathological study of liver, kidney, heart, lung, brain and spleen of the heated palm oil treated rabbits showed remarkable abnormalities as compared to control group organs.

Keywords: Heated palm oil, Physico-chemical, Lipid profile, Biochemical, Hematological and Histopathology.

1. INTRODUCTION

Palm oil is one of the common edible oil and derived from the mesocarp of the fruits of the palm tree (*Elaeis guineensis*). Basically, there are two main products of the palm oil industry – palm oil and palm kernel oil. Ebong *et al.* (1999) described that fresh and oxidized palm oil usually used for cooking purposes. Palm oil contains a few highly saturated vegetable fats and is semi-solid at room temperature. The mentioned oil is plant based which contains cholesterol described by Behrman *et al.* (2005). Palm oil and palm kernel oil are 50% and 81% saturated respectively reported by Harold McGee (2004). Palm oil is equally composed of saturated and unsaturated fatty acids. It principally contains saturated palmitic acid which changes serum cholesterol reported by Keys *et al.* (1965). In Bangladesh fairly uses soybean oil, palm oil, mustard oil and so on. Those oils are using in cooking and frying food. Palm oil is most commonly used in hotel and restaurant for cooking and frying food because palm oil is cheaper than other vegetable oil in Bangladesh stated by Marco *et al.* (2007). During a cooking process, oil gets heated up to different temperatures. More heating oil produced smoking point. Oil starts to smoke when it is over-heated. If continuing to cook something in the same oil, the food product will taste poorly. Heated oil lost its characteristics and physical appearance when oil heated in more time reported by Rani *et al.* (2010). Choe and Min (2007) investigated that more heating oil causes oxidation, hydrolysis and polymerization reactions. These reactions changed in the physico-chemical, nutritional properties of the oil presented by Che Man and Jasvir (2000). Hydro peroxides and aldehydes are formed during the heated oil is heated at high temperatures and preparing food by heated oil are absorbed these toxic products and eventually into the gastrointestinal tract after ingestion reported by Grootveld *et al.* (1998). In addition, Leong *et al.* (2015) reported that long-term ingestion of foods prepared using more heated oil could severely compromise one's antioxidant defense network, leading to pathologies such as hypertension, diabetes and vascular inflammation. The tendency of repeated use of more heated oil leads to serious health abnormalities like as histological abnormalities presented by Farag *et al.* (2010). During deep fat frying, fats and oils are continuously or repeatedly heated at high temperatures for prolonged periods in the presence of air. During heat treatment, a progressive decrease in unsaturation was observed in all oils by measurement of iodine value reported by Gary *et al.* (1997). The acid value of the different oils subjected to microwave heating at different time intervals, increased generally by time of heating presented by Minar *et al.* (2003). On the other hand, fresh palm oil has no deleterious effects on blood pressure and cardiac tissue but prolonged consumption of repeatedly heated palm oil may result in an increase in blood pressure level with necrosis of cardiac tissue reported by Leong *et al.* (2008). Izaki *et al.* (1984) reported that consumption of oxidized oil caused liver dysfunction. Acceleration of fatty streak formation had been reported in rabbits fed oxidized lipid by Staprans *et al.* (1996). Consumption of thermally oxidized palm oil diets had deleterious effects on biochemical indices in rats investigated by Ayodeji *et al.* (2015). Mesembe *et al.* (2004) had earlier reported that thermally oxidized palm oil diet resulted in anemia because hemoglobin concentrations were decreased. Palm oil reduces the blood levels of total cholesterol, triglycerides, LDL-cholesterol, thrombotic eicosanoids (oxygenated metabolites of polyunsaturated omega-6 fatty acid and

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omega-3 fatty acid) implicated in several pathophysiological processes of the cardiovascular system presented by Edem (2002). In addition, heated palm oil caused an increase in serum LDL-cholesterol and caused transient changes in lipid profiles reported by Kamsiah *et al.* (2006). In Bangladesh, maximum restaurants and street food corners use more times heated edible oils. Therefore, this present study has been undertaken to investigate the physico-chemical properties of more time heated palm oil and to investigate the effects of consumption of long time heated palm oil on hematological, biochemical and lipid profiles of rabbits and to observe the organs tissue damaging nature.

2. MATERIALS AND METHODS

2.1 Materials

Palm oil was collected from local market. All chemical and reagents were of analytical grade and obtained from local suppliers. Twenty four healthy and mature rabbits (640–775g) were taken from local market, Khulna, Bangladesh.

2.1.1 Heating process

2 L of palm oil was placed in a saucepan and heated by electric heater. The heating process was conducted for 2 hr day⁻¹ and the oil was left to cool down then stored at room temperature. This process was repeated for 10 consecutive days.

2.2 Methods

2.2.1 Iodine value (IV) measurement

A known amount of palm oil sample was reacted with iodobromine (IBr) in glacial acetic acid and produced a di-halogenated single bond. After the completion of reaction, the unreacted iodobromine was reacted with potassium iodide which converted it to iodine. The liberated I₂ was then titrated with a standard solution of sodium thiosulfate. A blank test was carried out without the oil sample using exactly the same process. The iodine value was calculated from the equation,

$$IV = \frac{(V_2 - V_1) \times 127 \times N}{W} \times 100$$

Where, V_2 was the quantity of sodium thiosulphate used for blank, V_1 was the quantity of thiosulphate for sample, N was the normality of thiosulphate solution, W was the weight of the oil sample and 127 was the molecular weight of iodine.

2.2.2 Acid value (AV) measurement

The acid value was determined by taking 2 g of the palm oil samples and added to 25 ml of CHCl₃ in different conical flasks. Two drops of phenolphthalein was then added to the mixture. A similar titration was performed without the sample to determine the blank test and titration was carried out with 0.01 N potassium hydroxide until the color change occurred in the different conical flasks. The acid value was calculated from the equation,

$$AV = (V_2 - V_1) \times N \times \frac{56.1}{W}$$

Where, V_2 was the volume of titrant (ml) consumed by the oil sample, V_1 was the volume of titrant (ml) consumed by 1 ml of solution at the equivalent point, N was the normality of potassium hydroxide and 56.1 was the molecular weight of KOH, W is the weight of the oil sample in gram.

2.2.3 Experimental rabbit grouping and study design

Twenty four rabbits were divided equally into four groups and treated as follows: (i) control group (ii) mixed diet with 2 hr heated palm oil (P1) (iii) mixed diet with 10 hr heated palm oil (P2) and (iv) mixed diet with 20 hr heated palm oil (P3). Rabbits were kept in stainless steel cages at room temperature and were allowed to drink water *ad libitum* and finally were administered the test diet. The control group was fed fresh mixed diet and water only. On the other hand P1, P2 and P3 groups were fed mixed diet with heated oil respectively. Mixed diet and heated oil ratio was 85:15. The rabbits were maintained for a week before the treatment. The treatments were executed for 28 days. Body weight of all the rabbits was checked in every day using the weight measuring machine. After 28 days,

the rabbits were sacrificed under chloroform anesthesia, the blood and the organs heart, lung, liver, spleen, kidney, brain of all groups were collected. Biochemical and hematological profiles of rabbits were measured and histopathology of all the organs was studied.

2.2.4 Monitoring of the hematological profiles

The hematological profiles of the experimental rabbits were done to check the hematological abnormalities after administration of the heated palm oil. For this purpose, the following parameters were observed: total RBC count, total WBC count, Platelet count, Hemoglobin estimation, ESR (Erythrocytes Sedimentation Rate). Blood samples were analyzed using an automated cell counter.

2.2.5 Monitoring of the biochemical profiles

The following biochemical parameters were analyzed: Serum glutamate-oxalo-acetate transaminase (SGOT), Serum glutamate-pyruvate transaminase (SGPT), Serum alkaline phosphatase (SALP), Serum creatinine, Random plasma glucose (RBS), Lipid profile [triglyceride, total cholesterol (TC), High density lipoprotein cholesterol (HDL) and Low density lipoprotein cholesterol (LDL) and Uric acid.

2.2.5.1 Collection of serum

Blood samples were collected from the Jugular vein at fasting state. The blood was collected in plastic centrifuge tubes. These were then allowed to clot at 40°C for 4 hours. After clotting, the blood samples were centrifuged at 4000 rpm for 15 minutes using a WIFUNG centrifuge LABO-50M. The clear straw color serum was then collected in vials with Pasteur pipette and stored at -20°C.

2.2.6 Histopathological procedure

The treated rabbits were sacrificed, dissected and their liver, kidney, heart, lungs, brain and spleen were removed and sliced into pieces, each measuring a few millimeters of thickness. The sliced tissues were then immersed in 10% buffered formalin, processed and stained by haematoxylin and eosin stain for light microscopic examination.

3 RESULTS AND DISCUSSION

The iodine value of heated palm oil was decreased gradually whereas the acid value was increased in the present study (Table 1). The highest decrease of iodine value was observed in 20 hr heated oil. The body weights of each group of rabbits were measured before and after administration of the treatments (Table 2). Subsequently, all the rabbits were given mixed diet and heated oil. The body weight changes of all the rabbits were recorded in every day. The body weights of all groups were increased significantly. The highest body weight increase was observed in P3 group. The food intake per day was also found normal. It is remarkable to highlight that the body weight gain in the 20 hr heated oil treated group showed significant changes as compared with the other group.

Table 1: Iodine value and acid value of heated palm oil

Heating time	Iodine value n = 6 M±SD	Acid value n = 6 M±SD
Fresh Oil	50.70±0.05	2.65±0.06
2 hr	46.86±0.09	2.76±0.05
4 hr	43.69±0.12	2.92±0.03
6 hr	41.08±0.08	3.31±0.06
8 hr	40.36±0.08	3.54±0.04
10 hr	38.08±0.09	4.01±0.06
12 hr	35.01±0.09	4.60±0.06
14 hr	31.02±0.08	5.22±0.09
16 hr	28.96±0.07	6.06±0.07
18 hr	23.06±0.05	6.68±0.08
20 hr	18.02±0.05	7.01±0.03

n= Number of titrations, M= Mean value & SD= Standard deviation

The body weight increase may be due to the increase of fat tissue. Leong *et al.* (2008) investigated that five times heated palm oil treated rats did not gain body weight. But in this present study, the results showed that long time heated palm oil treated rabbits body weights were increased remarkably. So the present study agreed with the result obtained by Siti *et al.* (2008) and Hussein (2015) showed that the body weight of rats was significantly increased by feeding thermally oxidized palm oil. Thermally oxidized palm oil which generates free radicals, enhances the oxidative stress secondary to estrogen deficiency and high cholesterol diet reported by Hussein (2015). These parameters have been attributed to atherosclerosis presented by Maxwell and Lip (1997).

Table 2: Body weight of rabbit after heated palm oil treatment

Day of Observation	Body weight(g) C n = 6 $M_1 \pm SD_1$	Body weight (g) P1 n = 6 $M_2 \pm SD_2$	Body weight (g) P2 n = 6 $M_3 \pm SD_3$	Body weight (g) P3 n = 6 $M_4 \pm SD_4$
Fresh	731.43 $\pm$ 1.14	672.13 $\pm$ 2.05	693.45 $\pm$ 1.03	772.37 $\pm$ 1.94
1 st	731.74 $\pm$ 1.23	672.56 $\pm$ 2.17	694.12 $\pm$ 1.10	772.65 $\pm$ 1.89
7 th	733.49 $\pm$ 1.13	677.28 $\pm$ 1.95	701.02 $\pm$ 2.17	783.13 $\pm$ 2.32
14 th	735.42 $\pm$ 1.11	684.48 $\pm$ 2.62	707.85 $\pm$ 2.21	796.01 $\pm$ 1.05
21 th	738.13 $\pm$ 1.01	694.06 $\pm$ 2.67	716.87 $\pm$ 3.02	807.96 $\pm$ 2.38
28 th	742.30 $\pm$ 1.17	707.29 $\pm$ 2.29	726.91 $\pm$ 2.33	821.57 $\pm$ 3.29

n= Number of rabbit, M_1 , M_2 , M_3 , M_4 = Mean value of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively, SD_1 , SD_2 , SD_3 , SD_4 = Standard deviations of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively.

The present study, long term administration of heated palm oil treated rabbits have been observed to cause alterations in hematological profiles (Table 3) such as count white blood cell (WBC), count red blood cell (RBC), hemoglobin (Hb), erythrocyte sedimentation rate (ESR) and the platelet count (PC). The results showed that the total white blood cell count (WBC) of heated palm oil fed groups was increased. WBC count was increased in palm oil treated rabbits with respect to control. Total WBC of P1 group ($9.5 \times 10^3 \pm 0.16 \times 10^3$ blood cell/ μ l) was significantly higher than that of untreated rabbits. White blood cells (WBCs) also called leukocytes, are the cells of the immune system that are involved in protecting the body against infectious disease. The present study showed that the WBC of all heated oil treated groups was increased compared with control group. A high number of WBC is called leukocytosis. Leukocytosis may occur hepatic damage reported by Finlayson *et al.* (1999). This study agrees with Elemei *et al.* (2015) who reported that it may be an indication of inflammation, infection and stress to major organs or certain diseases. The red blood cell count (RBC) was increased in all heated palm oil fed groups with respect to control. The RBC of P3 group (6.58 ± 0.03 m/ μ l) was higher than that of untreated P1 and P2 groups. In previous study, Mesembe *et al.* (2004) and Hussein (2015) investigated that the RBC count was decreased in the rats fed thermoxidized palm oil compared with control group. This study showed that the RBC count of heated palm oil treated rabbits were increased with respect to control rabbits. This increase may be due to the suppressive effect of the hazardous constituents of thermally oxidized palm oil on the bone marrow reported by Mackie *et al.* (1999). The hemoglobin concentration of heated palm oil treated P1 and P2 groups were decreased compared to control rabbits. But the hemoglobin concentration of heated palm oil diet P3 group was increased. The hemoglobin concentration of P1 group (10.7 ± 0.21 g/dl) was lower than that of control (11.5 ± 0.08 g/dl). In addition, the hemoglobin concentration of P3 group (12.3 ± 0.21) was higher than that of untreated rabbits. The hemoglobin concentration was decreased in heated palm oil fed group except P3 group compared with control. In previous study, Mesembe *et al.* (2004) reported that this decrease in hemoglobin concentration may be a consequence of reduced uptake of iron by the damaged intestinal mucosa of rats resulting in a reduced bioavailability of iron in the system. The liver stores iron as ferritin and hemosiderin reported by Finlayson *et al.* (1999). The decrease in hemoglobin concentration may also be attributed to the decreased storage of iron in the liver as a result of damage to the liver presented by Elemei *et al.* (2015). Heated palm oil diets were significantly affected Erythrocyte Sedimentation Rate (ESR) in this study. ESR of all heated palm oil fed groups were decreased compared to untreated rabbits. ESR of P1 group (1.00 ± 0.0 g/dl) was lower than that of control group (10 ± 0.0 g/dl). The erythrocyte sedimentation rate (ESR) is the rate at which red blood cells sediment in a period of one hour. It is a common hematology test and is a non-specific measure of inflammation. The present study showed that the ESRs of heated palm oil treated groups were decreased with respect to control rabbits. It may be an indication of sickle cell anemia, leukemia. The platelet count (PC) was increased in P1, P2 heated palm oil fed groups compared with control. PC of P2 group ($593 \times 10^3 \pm 2.44 \times 10^3$ blood cell/ μ l) was higher than that of untreated and all heated oil fed groups. PC of P3 group was decreased compared

with control. PC of P3 group ($217 \times 10^3 \pm 0.81 \times 10^3$ blood cell/ μ l) was lower than that of control ($241 \times 10^3 \pm 0.81 \times 10^3$ blood cell/ μ l). Abnormalities in platelet number are an indication of a defect in primary hemostasis. An increase in platelet number above normal serves as a marker of vascular disease presented by Vidwan *et al.* (2010). This study agrees with Mohammad *et al.* (2010) who reported that the platelet count of high fat diet fed rabbits group were increased.

Biochemical profiles such as creatinine, SGPT, SGOT, ALP and uric acid of heated palm oil diets treated rabbits have been observed (Table 4). Creatinine was increased in heated palm oil treated groups except P2 group compared to untreated rabbits. Creatinine of P3 group (1.0 ± 0.14 mg/dl) was higher than that of control, P1 and P2 groups. The present study showed that serum creatinine in the heated palm oil fed rabbits group was significantly increased compared with control. Creatinine levels in plasma are usually measured to determine acute or chronic renal insufficiency reported by Luber (1988). They are usually raised in renal disease. So it may be suggestive of possible renal system damage agrees with the findings of Elemei *et al.* (2015). The serum glutamic pyruvic transaminase (SGPT) of heated palm oil treated groups were increased compared with control group. The SGPT of P3 group (81 ± 2.16 U/L) was higher than that of untreated rabbits (33 ± 0.82 U/L).

Table 3: Effect of heated palm oil diets on Hematological profiles

Parameters		Units	C n = 6 $M_5 \pm SD_5$	P1 n = 6 $M_6 \pm SD_6$	P2 n = 6 $M_7 \pm SD_7$	P3 n = 6 $M_8 \pm SD_8$
Total WBC		thousand/ μ l	2.8 ± 0.16	9.5 ± 0.16	6.1 ± 0.14	5.3 ± 0.16
Differential count of WBC in %	Neutrophils	%	50 ± 0.82	54 ± 0.82	37 ± 0.82	47 ± 1.14
	Lymphocytes	%	49 ± 0.82	45 ± 0.58	59 ± 1.41	50 ± 0.82
	Eosinophils	%	01 ± 00	01 ± 00	04 ± 0.82	02 ± 0.82
	Monocytes	%	00 ± 00	00 ± 00	00 ± 00	01 ± 00
Total RBC		m/ μ l	5.48 ± 0.01	5.74 ± 0.03	5.99 ± 0.09	6.58 ± 0.03
Hemoglobin		g/dl	11.5 ± 0.08	10.7 ± 0.21	11.1 ± 0.14	12.3 ± 0.21
ESR		g/dl	10 ± 00	1 ± 00	2 ± 00	2 ± 00
PC		thousand/ μ l	241 ± 0.81	412 ± 2.16	593 ± 2.44	217 ± 0.81

n= Number of rabbit, M_5 , M_6 , M_7 , M_8 = Mean value of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively, SD_5 , SD_6 , SD_7 , SD_8 = Standard deviations of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively. WBC= White Blood Cell, RBC= Red Blood Cell, ESR= Erythrocyte Sedimentation Rate, PC= Platelet Count.

Serum glutamic pyruvic transaminase (SGPT), an enzyme that is normally present in liver and heart cells. SGPT is released into blood when the liver or heart is damaged. The blood SGPT levels are thus elevated with liver damage or with an insult to the heart. The present study showed that the SGPT of all heated palm oil diet groups were significantly increased compared with control group. So, it indicates that the liver or heart of heated palm oil diet treated rabbit groups may be damaged. Rashid *et al.* (1999) who studied the effects of dietary cooked fats and oils on blood lipids reported that a relatively high SGPT level in palm oil fed group indicates the possible hepatic damage. The serum glutamic oxaloacetic transaminase (SGOT) was increased in all heated palm oil treated groups with respect to control. The SGOT of P3 group (147 ± 0.82 U/L) was higher than that of untreated and all heated oil diet groups. SGOT is a liver enzymes which made by liver cells. When liver cells are damaged, SGOT leaks out into the bloodstream and the level of SGOT in the blood becomes higher than normal. SGOT is found in parts of the body other than the liver including the heart, kidneys, muscles and brain. When cells in any of those parts of the body are damaged, SGOT can be elevated. In previous study, Nageswari *et al.* (1999) observed a maximum increase in SGOT in rats indicative of myocardial damage in the coconut oil fed group. In addition, this study showed that the SGOT of all heated palm oil fed groups were significantly increased compared with control group. So it indicates that the liver, heart, kidneys, muscles and brain of heated palm oil diet rabbit groups may be damaged.

Alkaline phosphatase (ALP) of heated palm oil fed of all group was decreased compared with control rabbits. ALP of P1 group (174 ± 1.63 U/L) was lower than that of control (260 ± 2.16 U/L). In previous study, Ayodeji *et al.* (2015) showed that ALP of heated palm oil diet group was increased. But in present study, the ALP of all heated palm oil fed groups was significantly decreased. Uric acid was increased in all heated palm oil fed groups compared with control. Uric acid of P2 group (1.9 ± 0.08 mg/dl) was significantly higher than that of control group (1.4 ± 0.00 mg/dl).

Table 4: Effect of heated palm oil diets on biochemical profiles

Parameters	Unit	C n = 6 $M_9 \pm SD_9$	P1 n = 6 $M_{10} \pm SD_{10}$	P2 n = 6 $M_{11} \pm SD_{11}$	P3 n = 6 $M_{12} \pm SD_{12}$
Creatinine	mg/dl	0.8 ± 0.00	0.9 ± 0.08	0.8 ± 0.08	1.0 ± 0.14
SGPT	U/L	33 ± 0.82	63 ± 1.41	53 ± 2.16	81 ± 2.16
SGOT	U/L	37 ± 1.41	45 ± 0.82	50 ± 1.41	147 ± 0.82
ALP	U/L	260 ± 2.16	174 ± 1.63	234 ± 1.41	257 ± 1.41
Uric acid	mg/dl	1.4 ± 0.00	1.8 ± 0.14	1.9 ± 0.08	1.6 ± 0.16

n= Number of rabbit, M_9 , M_{10} , M_{11} , M_{12} = Mean value of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively, SD_9 , SD_{10} , SD_{11} , SD_{12} = Standard deviations of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively. SGPT= Serum glutamic pyruvic transaminase, SGOT= Serum glutamic oxaloacetic transaminase, ALP= Alkaline phosphatase.

Biochemical profiles of serum lipid as total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglyceride (TG) in blood of heated palm oil diet rabbits group has been observed (Table 5). The total cholesterol (TC) of heated palm oil fed all groups were increased compared with control. The rabbits of group P3 showed the maximum level of total blood cholesterol (158 ± 2.16 mg/dl) which differ from all other groups. In previous study, Siti *et al.* (2008) who reported that the TC level of 5 hours heated palm oil fed group was increased but 1 hour heated palm oil fed group was not significantly changed compared with control group. But the present study, the TC of 2 hr, 10 hr and 20 hr heated palm oil diet groups were significantly increased. So, this study agreed with the study of Hur *et al.* (2005) who reported that heated oil reduced plasma cholesterol in rabbits. The blood High Density Lipoprotein (HDL) level after supplementation of heated palm oil in different groups of rabbits had significant differences from that of control group. The HDL level of rabbits of group P2 (57 ± 1.63 mg/dl) which differ significantly than control group (26 ± 0.82 mg/dl). The significant increase in HDL has been recorded in rabbits fed with heated palm oil group P1 (43 ± 1.4 mg/dl) and P3 (42 ± 0.82 mg/dl) that differ significantly from the value of control group. The present study supports the results of Chinu & Thankappan (2011) and Siti *et al.* (2008). The blood Low Density Lipoprotein (LDL) of heated palm oil fed P1 and P2 groups were decreased but P3 group was increased compared with control group. The rabbits of group P3 showed the maximum level of LDL (81 ± 1.41 mg/dl) which differ significantly from all other groups. This study showed that the LDL of all heated palm oil fed groups of rabbits except P3 were decreased compared with control group. So, this study partially agrees with the study of Kamsiah *et al.* (2006) who showed that the LDL of 5 time heated soybean and palm oil fed group were increased.

Table 5: Effect of heated palm oil diets on Lipid profiles

Parameters	Unit	C n = 6 $M_{13} \pm SD_{13}$	P1 n = 6 $M_{14} \pm SD_{14}$	P2 n = 6 $M_{15} \pm SD_{15}$	P3 n = 6 $M_{16} \pm SD_{16}$
TC	mg/dl	96 ± 0.00	128 ± 0.82	126 ± 0.82	158 ± 2.16
HDL	mg/dl	26 ± 0.82	43 ± 1.41	57 ± 1.63	42 ± 0.82
LDL	mg/dl	61 ± 2.45	53 ± 1.63	43 ± 0.82	81 ± 1.41
TG	mg/dl	44 ± 0.00	158 ± 2.16	129 ± 1.41	176 ± 0.82

n= Number of rabbit, M_{13} , M_{14} , M_{15} , M_{16} = Mean value of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively, SD_{13} , SD_{14} , SD_{15} , SD_{16} = Standard deviations of control (C) group, 2 hr heated palm oil (P1) group, 10 hr heated palm oil (P2) group and 20 hr heated palm oil (P3) group respectively. TC= Total Cholesterol, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein, TG= Triglyceride.

The effects of heated palm oil on triglyceride (TG) level of different groups of rabbits were increased compared with control group. TG of P3 group (176 ± 0.82 mg/dl) was significantly higher than that of control group. This study is in agreement with the results of Chinu & Thankappan (2011), Shastri *et al.* (2011) and Islam *et al.* (2015).

In the present study, the histopathology of liver, kidney, heart, lung, brain and spleen of rabbits were examined for the detection of pathological lesions if any (Figure 1-6). In histopathology, some specific lesions were found in the liver, kidney, heart, lung, brain and spleen of heated palm oil diet groups as compared with the control rabbits. In figure 1 histological structures of liver of control group showed that the liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from the central vein to periphery of the lobule. The hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and kupffer cells (a) (Image i). 2 hr heated palm oil diet group P1 showed fatty degenerative change and the portal area showing severe dilatation (b), vacuolation of central vein and hepatocytes (c) (Image ii). 10 hr heated palm oil diet group P2 showed abnormal shape of central vein (d), swollen cell with chronic inflammatory cell infiltration (e), tissue lost its attachment and vacuolation (f), (Image iii). 20 hr heated palm oil diet group P3 showed vacuolation and abnormal shape of central vein (g) and the liver cells were swollen (h), (Image iv).

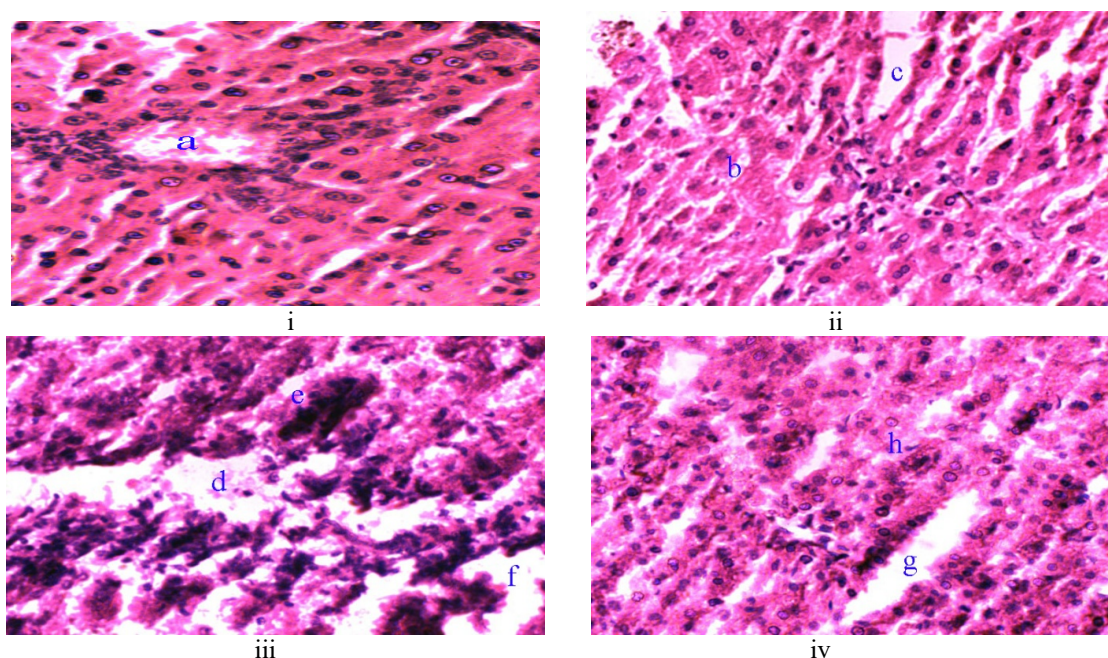


Figure 1: Histopathological section of liver of rabbits (Hematoxylin & Eosin x 200) (i) Control group liver, (ii) 2 hr heated palm oil diet group liver, (iii) 10 hr heated palm oil diet group liver and (iv) 20 hr heated palm oil diet group liver.

Figure 2 showing histological examination of the kidney of control group of rabbits which revealed the normal histological features. Control group showed normal histological structure of glomeruli (a) and renal tubules of kidneys in rabbits (b), (Image i). 2 hr heated palm oil diet group P1 showed cells in the medullary region vacuoles (c), focal inflammatory cells infiltration in between the tubules associated with dilatation in the blood vessels (d) and the glomerular tuft showed vacuolization in the lining endothelium (e), (Image ii). 10 hr heated palm oil diet group P2 showed abnormal shape of glomeruli (f), vacuolation (g), atrophy of a glomerulus with degeneration in the lining epithelial cells of renal tubules and edema of tissue (h) (Image iii). 20 hr heated palm oil diet group P3 showed blood capillaries in between the degenerated tubules which were congested (i), vacuolation (j), eroded wall of bowman's capsule (k), cell debris inside the glomeruli and edema of tissue (l), (Image iv).

Histological examination of the heart of rabbits are shown in figure 3. Control group showed normal arrangement of cardiac muscular layer (a), (Image i). 2 hr heated palm oil diet group P1 showed congested myocardial (b), few vacuolation in papillary muscle and splitting of longitudinal muscles (c), (Image ii). 10 hr heated palm oil diet group P2 showed hemorrhage (d), vacuolation (e), myocardial degeneration and necrosis (f), (Image iii). 20 hr heated palm oil diet group P3 showed hemorrhage (g), vacuolation (h), hypertrophy of cardiac muscle (i) and thickening of the ventricular septum and brown atrophy (j), (Image iv). Histological structures of lung of rabbits are shown in figure 4. Untreated rabbits showed the lung pulmonary tissues compact configuration with airway, interalveolar septa, regular alveolar sacs and capillaries (Image i). Bronchiolar (a) and alveolar (b) structures in the control group in

their normal structures. 2 hr heated palm oil diet group P1 showed lung tissue containing collagen fiber accumulation along with distinctive cell proliferation (c) and mononuclear cell invasion in the alveolar septa (d), (Image ii).

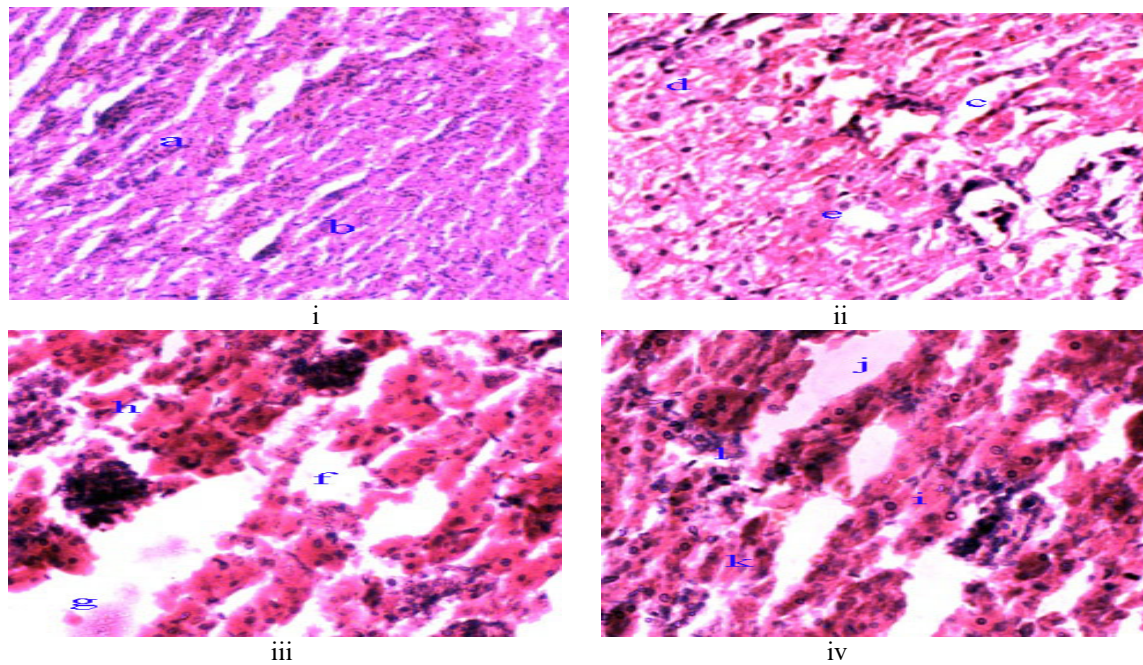


Figure 2: Histopathological section of kidney of rabbits (Hematoxylin & Eosin x 200) (i) Control group kidney, (ii) 2 hr heated palm oil diet group kidney, (iii) 10 hr heated palm oil diet group kidney and (iv) 20 hr heated palm oil diet group kidney.

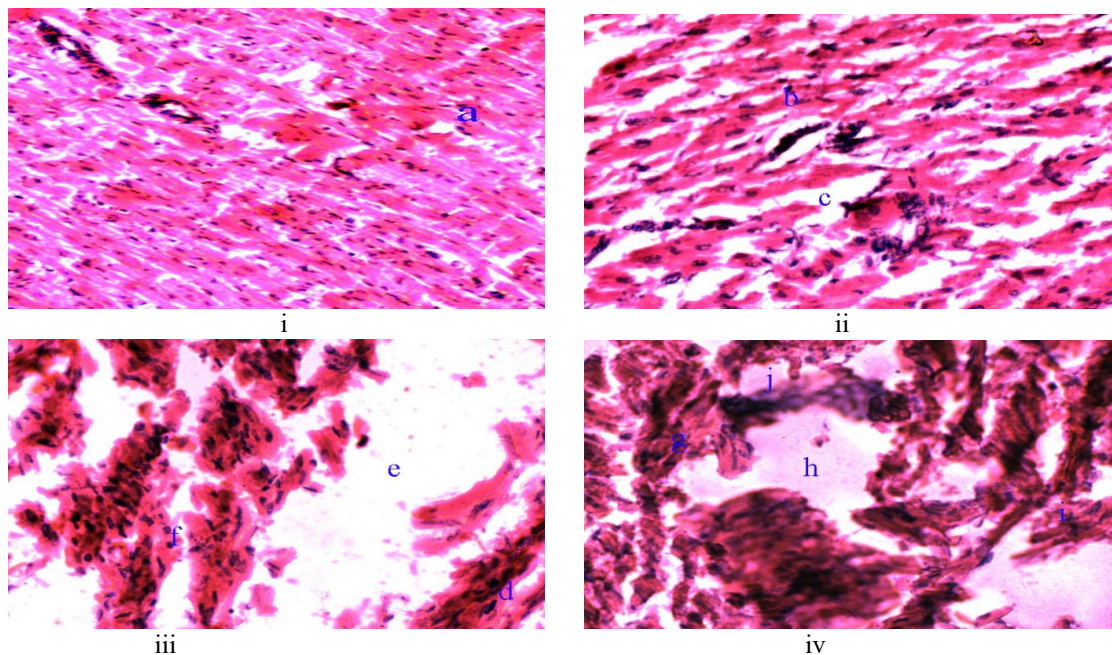


Figure 3: Histopathological section of heart of rabbits (Hematoxylin & Eosin x 200) (i) Control group heart, (ii) 2 hr heated palm oil diet group heart, (iii) 10 hr heated palm oil diet group heart and (iv) 20 hr heated palm oil diet group heart.

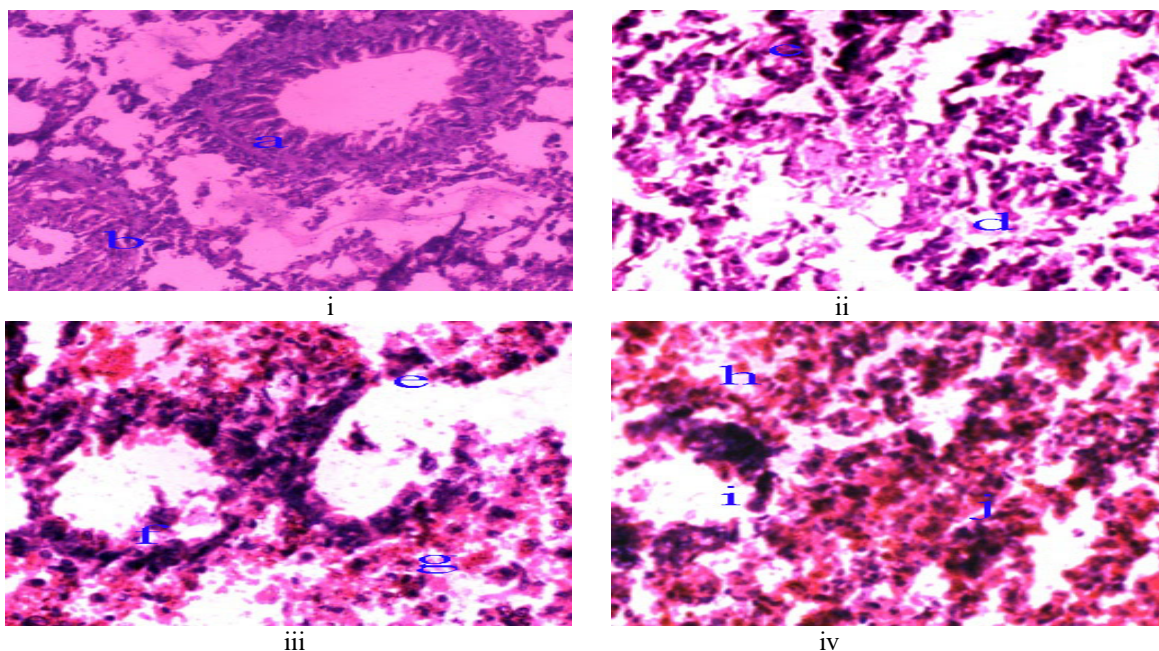


Figure 4: Histopathological section of Lung of rabbits (Hematoxylin & Eosin x 200) (i) Control group lung, (ii) 2 hr heated palm oil diet group lung, (iii) 10 hr heated palm oil diet group lung and (iv) 20 hr heated palm oil diet group lung.

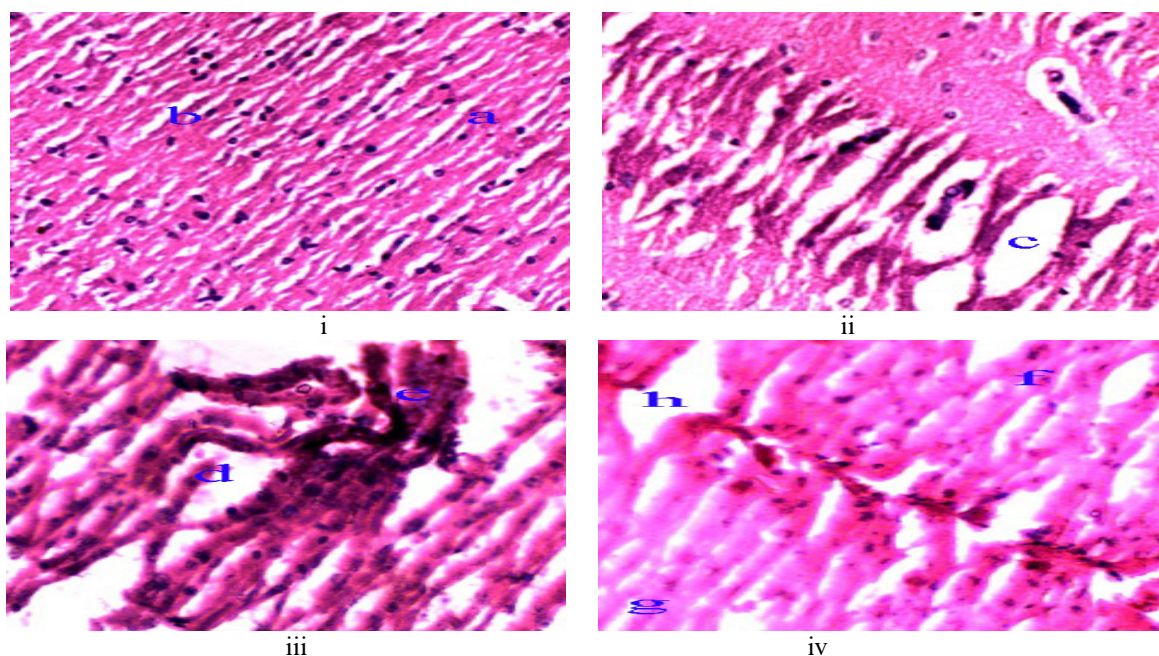


Figure 5: Histopathological section of Brain of rabbits (Hematoxylin & Eosin x 200) (i) Control group brain, (ii) 2 hr heated palm oil diet group brain, (iii) 10 hr heated palm oil diet group brain and (iv) 20 hr heated palm oil diet group brain.

10 hr heated palm oil diet group P2 showed a bronchus lined with pseudostratified epithelium and containing lymphocytes in their lamina propria and surrounding these structures the saccus alveolaris (e), alveoli with regular walls, interalveolar septa (f) and interalveolar connections connecting the alveoli to each other were observed in the lung parenchyma (g), (Image iii). 20 hr heated palm oil diet group P3 showed inhalation (h), revealed the lymphocyte infiltration into the interalveolar septa and also into the bronchiolar lamina propria (i) and the structural organization of the alveoli seemed to be disturbed and the interalveolar septa were thickened (j), (Image iv).

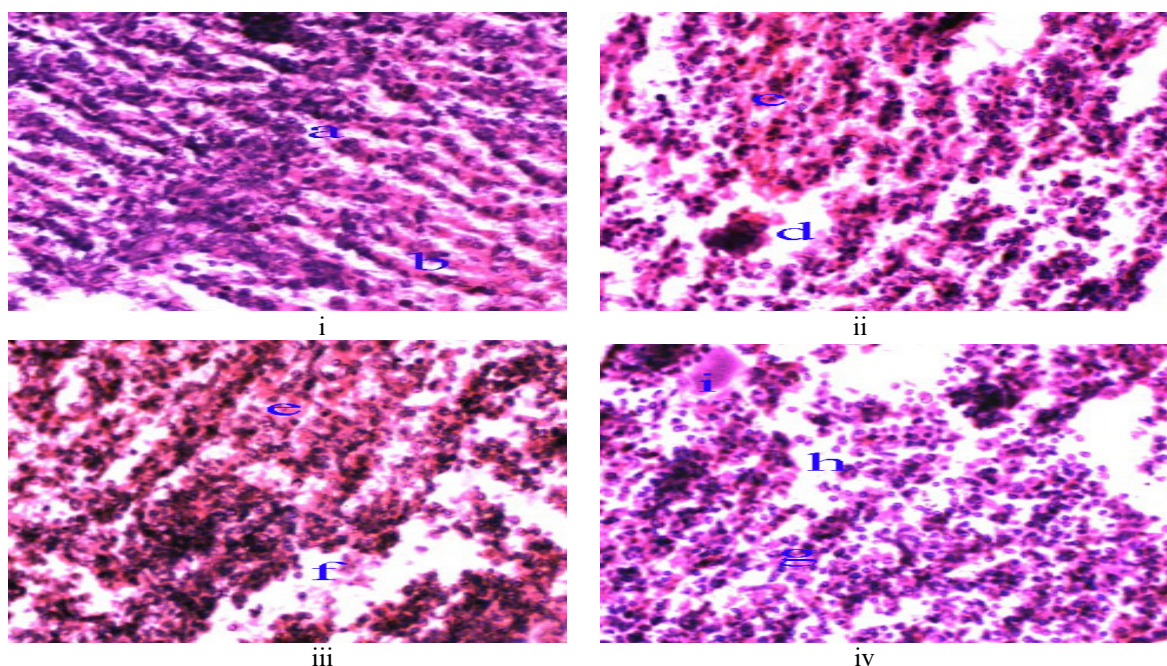


Figure 6: Histopathological section of spleen of rabbits (Hematoxylin & Eosin x 200) (i) Control group spleen, (ii) 2 hr heated palm oil diet group spleen, (iii) 10 hr heated palm oil diet group spleen and (iv) 20 hr heated palm oil diet group spleen.

Histological structures of brain of control and heated oil treated rabbits are presented in figure 5. Image i showed the histological structure of brain of control rabbits. Normal histological structure of the meninges (a) and cerebral cortex (b) were showed in brain of control rabbits. 2 hr heated palm oil diet group P1 showed the medulla oblongata vacuolation (c) in the matrix (Image ii). 10 hr heated palm oil diet group P2 showed the deep cerebrum had fat vacuoles in the matrix (d) as well as focal gliosis (e), (Image iii). 20 hr heated palm oil diet group P3 showed edema of tissue (f), necrosis (g) and vacuolation in the matrix with focal gliosis (h), (Image iv). In figure 6 image i showed the normal splenic structure of control rabbits. The histopathological examination of spleen of the control group showed normal structure which composed of normal white and red pulp (a), normal capsule and blood vessels (b). 2 hr heated palm oil diet group P1 showed atrophy in white pulp and edematous in red pulp (c), vacuolation in matrix (d), (Image ii). 10 hr heated palm oil diet group P2 showed atrophy of lymphoid tissue of white pulp (e), while the red pulp showed foamy vacuolated macrophages (f), (Image iii). In addition, 20 hr heated palm oil diet group P3 showed severe atrophy of lymphoid tissue of white pulp (g) while the red pulp showed foamy vacuolated macrophages (h), congestion of sinusoids (i), (Image iv).

4 CONCLUSIONS

Consumption of heated palm oil diets had deleterious effects on body weights, hematological parameters, biochemical profiles, lipid profiles and organs of rabbits. The results of this study indicate that heated palm oil may be hazardous to the consumer's health. Long time heated oil consumption may occur certain diseases such as infection, stress, inflammation, allergy, anemia, liver cirrhosis, hepatocellular disease, renal failure, pulmonary infarction, atherosclerosis (coronary artery disease) that means myocardial infarction of the consumers. Liver, heart, kidney, lung, brain, spleen and muscles of users may be damaged by chronic consumption of heated palm oil. So this study recommends that long time heated palm oil consumption might be harmful.

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