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ABSTRACT

Palm oil is cheaper than other edible oils, making it a promising source of frying oil in a major population; however some people use coconut oil. During deep frying, oils are continuously or repeatedly contact at high temperatures, are subject to a series of degradation reactions and formation of a variety of decomposition compounds. Hydrolysis due to the presence of moisture in the foods and thermal oxidation are major causes for frying oil decomposition. This decomposition product has negative effects on quality, flavor and color of frying oils and may influence nutritional quality and food safety. In an attempt to evaluate the influence of improperly used frying oil on lipid metabolism, 42 male Wistar rats were used in a 2x2 factorial arrangement. After 12 weeks on the experimental diets, no adverse effects were observed as judged by growth rate. Lipid profiles of the animals indicated that coconut oil had higher amounts of plasma cholesterol, low-density lipoprotein cholesterol and plasma triglyceride than did palm oil, but lower the high-density lipoprotein cholesterol. Feeding improper used frying oil caused damage to the liver, heart, endothelial aorta and kidney contrasted with fresh oil.

Keywords: *improper used frying oil, lipid metabolism, palm oil, coconut oil, lipid profiles, liver, heart, endothelial aorta and kidney*

INTRODUCTION

Frying oil is one of the most commonly used methods for the preparation of foods. The oil acts as a heat transfer medium and as an important ingredient of the fried food. However, repeated heating of edible oils, especially polyunsaturated fatty acid, markedly modifies various parameters in the oil which are considered good indices of degree of thermal oxidation (Fritsch, 1981). The thermal degradation results in accumulation of decomposition products which not only affect the quality of fried foods, but are also of much concern for human health (Al-Harbi and Al-Kahtani, 1993).

Thermally oxidized oils are known to cause growth retardation, increase in liver and kidney weights, damage to the liver, thymus and testes (Alexander *el al*, 1987 *in* Al-Harbi and Al-Kahtani, 1993), alteration of the production vascular eicosanoids (evaluation of the thromboxin formation) and decrease in vascular prostacyclin release (Giani *et al*, 1985 *in* Al-Harbi and Al-Kahtani, 1993). Further, heating of oil leads to reduction of vitamin E, the latter preventing formation of lipid hydro peroxide free radicals that are known to cause reduction of prostacyclin (and normally impair platelet aggregation), tumor formation, toxic liver injury, neuromuscular disorders, arthritis, iron overload, gastrointestinal disorders and damage to aortic endothelial cells (Yagi, 1987 *in* Al-Harbi and Al-Kahtani, 1993).

According to Finnish recommendations issued by the National Food Administration, oils can be used for frying if the Fritest gives a result less than 2, the acid value is less than 2.0 and the smoke point over 180ºC at the same time (Skrökki, 1995). If less than one point is assigned for both smell and color the quality is not good and the oil should be changed or in the other word these oil was improperly used for frying. Rukmini *et al* (2003) found that the fry-life of oil in carbohydrate foodstuff processing was 4 x 5 hours, since in protein foodstuff was 3 x 5 hours.

In a previous study (Rukmini *et al*, 2003), we also found that the difference foodstuff cause the changes saturated fatty acid of used frying oil, whereas the linolenic acid does not changes in experience. Several studies in animals and humans have established that saturated fatty acids increase plasma cholesterol concentrations whereas polyunsaturated fatty acids have hypocholesterolemic effect (Tony *et al*, 1991; Cottrell, 1991). Diets high in saturated fatty acids can promote thrombosis and hence atherogenesis (Sugano and Imaizumi, 1991). According to Cottrell (1991), an increase in saturated fatty acids in the diet in experiments generally leads to an increase in blood cholesterol content (LDL-cholesterol). Besides that, the saturated fatty acid, stearic acid (18:0), when ingested as a part of fat, does not tend to raise blood cholesterol concentration, whereas palmitic acid (16:0) does. The longer-chain-length fatty acids, with ≥ 18 carbon atoms in the chain, seem to have little effect, whereas those of medium length, with 10-16 carbon atoms, have a hypercholesterolemic effect.

These findings led us to study the combination effect between difference sources of oil (palm oil which has 50% saturated fatty acid and 50% unsaturated fatty acid and coconut oil which has more than 90% saturated fatty acid) and the condition of oil (fresh and improperly used for frying) simultaneously present in the diet on lipid metabolism in rats, including growth rate, blood lipid profiles and the histological organs.

MATERIALS AND METHODS

Frying oils

Commercial palm oil and coconut oil are the most widely used as fring oil in Indonesia, were obtained from local market. Each oil was place in 2-L capacity stainless-steel deep fryer with thermostatic control and heated to 180ºC. Catfishes were fried in 250-g batches at constant frying temperature. The batches were fried at half-hour intervals for 5 h/d to reach improperly used (the acid value was over than 2.0 and the smoke point was less than 180ºC). These oils were used for animals diet.

Animals and dietary treatments

Lipid metabolism studies were carried out in male albino rats of Wistar strain. Forty-two rats, 2 months of age and initially weighing 100 g were used as experimental animals. Following 2 weeks adaptation where animals were maintained on standard diet AIN-93 (Reeves *et al*, 1993), the rats were randomly allotted into 6 groups of 7 animals each. Each rat in each group was housed individually and fed experiment diets in isocalorie treatment as shown in Table 1. Food was given at 10-g/d, whereas water was given *ad libitum* for 12 weeks (3 months) and weekly body weights were recorded to monitor the growth rate. Blood samples for lipid profiles analysis were collected by cardiac puncture at the end of monthly.

Table 1. Composition of the experimental diets

Composition	Formulation diet in grams					
	O	A	B	C	D	E
Corn starch	620	327.50	327.50	327.50	327.50	327.50
Protifar formula milk	233	233	233	233	233	233

Sucrose	35.23	35.23	35.23	35.23	35.23	35.23
Soybean oil	37.67	167.67	-	-	-	-
Fresh palm oil	-	-	167.67	-	-	-
Improperly used palm oil	-	-	-	167.67	-	-
Fresh coconut oil	-	-	-	-	167.67	-
Improperly used coconut oil	-	-	-	-	-	167.67
Fiber (CMC)	50	50	50	50	50	50
Mineral mix	18.22	18.22	18.22	18.22	18.22	18.22
Vitamin mix	10	10	10	10	10	10
L-sistin	1.80	1.80	1.80	1.80	1.80	1.80
Kolin bitartrat	2.50	2.50	2.50	2.50	2.50	2.50
Tert butyl hydroquinon	0.008	0.008	0.008	0.008	0.008	0.008
Total calorie (kcal)	**3,800**	**3,800**	**3,800**	**3,800**	**3,800**	**3,800**

information :

O : formulation standard diet with 5% soybean oil
A : formulation control diet with 20% soybean oil
B : formulation diet with 20% fresh palm oil
C : formulation diet with 20% improperly used palm oil
D : formulation diet with 20% fresh coconut oil
E : formulation diet with 20% improperly used coconut oil

Plasma sample isolation

Whole blood samples were collected into 10% EDTA containing tube and allowed to stand at room temperature for at least 30 min, followed by 4500 rpm speed centrifugation for 20 min. After centrifugation, each plasma sample was withdrawn with a Pasteur pipette, place into an eppendorf tube and stored at cool room until the assays were conducted.

Lipid profiles analysis

Triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol concentration were evaluated in quantitative *in-vitro* determination using specific reagent carrier strip supplied by DiaSys.

Histological organs analysis

At the end of the experiment, the animals were anaesthetized with chloroform till dead and then the abdomen and thorax were opened. The liver, heart, aortic endothelial and kidney were then removed and soaked in 10% formaldehyde solution and then preparation to microscopic analysis with hematocylin eusin painting.

Statistical analysis

Data of the experiments were subjected to an analysis of variance (ANOVA) using factorial design. The difference was considered to be significant at the level of $p<0.05$ (Gill, 1981).

RESULTS AND DISCUSSION

The growth rate of rats

Feeding the animals with various sources of oil for 12 weeks (3 months) had no significant effect on body weight (Figure below). This was because all the groups of rats had given in isocalorie feeding (as shown in Table 1). It was suggest that the growth rate was under the influence of calories feeding, whereas the oil as the daily intake of fat in the rats dietary had no significant effect on the growth rate. The fresh or improperly used palm oil or coconut oil has same effect on the growth rate.

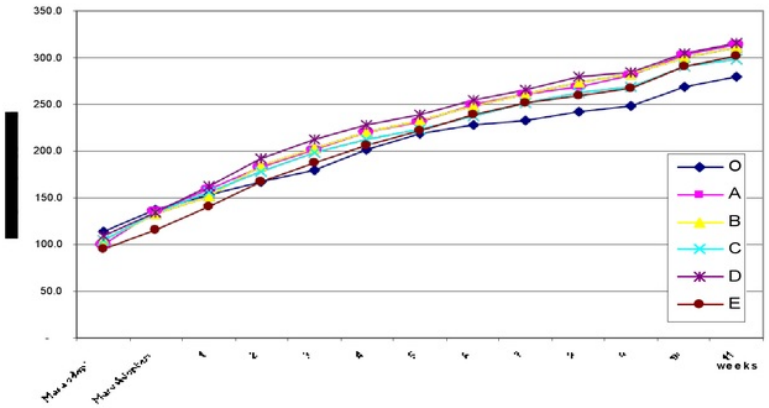

Fig. Body weight of the rats during feeding with a diet containing various oil sources

Lipid profiles

Lipid profiles include plasma triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol that monthly analysis are given below.

1. Triglyceride

Triglycerides are esters of glycerol with three fatty acids and are the most abundant naturally occurring lipids. They are transported in plasma bound to apolipoproteins forming very low density lipoproteins (VLDL) and chylomicrons. Measurement of triglyceride is used in screening of the lipid status to detect atherosclerotic risks and in monitoring of lipid lowering measures. Recent studies have shown that elevated triglyceride concentrations combined with increased low density lipoprotein (LDL) concentrations constitute an especially high risk for coronary heart disease (CHD). High triglyceride levels also occur in various diseases of liver, kidneys and pancreas. The mean plasma triglyceride concentrations in this experiment are given in Table 2.

Table 2. Effect of feeding diet on plasma triglyceride concentration

Dietary groups	Plasma triglyceride (mg/dL) in the-			
	earlier study	1st month	2nd month	3rd month
Standard (AIN'93)	106.10 ± 4.62	92.17 ± 7.07	89.66 ± 4.44	89.81 ± 2.02
Fresh soybean oil	107.64 ± 4.45	115.69 ± 4.00	97.21 ± 2.63	92.64 ± 4.71
Fresh palm oil	104.49 ± 4.06	126.71 ± 6.72	107.31 ± 6.67	84.47 ± 4.25
Improperly used palm oil	106.15 ± 5.35	126.05 ± 2.14	148.60 ± 6.29	145.83 ± 7.67
Fresh coconut oil	105.32 ± 4.52	120.58 ± 4.90	102.87 ± 8.96	99.29 ± 5.59
Improperly used coconut oil	103.33 ± 3.24	139.79 ± 6.65	154.68 ± 5.55	151.28 ± 5.39

The plasma triglyceride concentrations in the standard dietary group of rats received soybean oil were lower compared to than other dietary groups. The hypolipidemia activity of soybean is probably in part attributable to an enhanced fecal excretion of both neutral and acid steroids (Ogawa *et al*., 1992; Sugano and Koba, 1993). In addition, Heek and Zilversmit (1992) *in* Wuryastuti (2000) observed the close relationships between the activity of lipoprotein lipase (LPL) and the level of HDL-cholesterol and triglyceride. Thus, it is possible that soybean induces the activity of LPL that is subsequently causing drop in the level of plasma triglyceride. Epidemiological studies have observed that a combination of plasma triglycerides > 180 mg/dL and HDL-cholesterol < 40 mg/dL predict a high risk of CHD. Whereas borderline levels (> 200 mg/dL) should always be regarded in association with other risk factors for CHD.

Thus the analysis result showed that the plasma triglyceride at all the dietary groups were in desirable level. However, still need to be confirmed by kinetic studies of HDL-cholesterol.

Table 2 also present that the plasma triglyceride at the group of rats with the improperly used palm oil or coconut oil dietary were higher than the group of rats with fresh oil dietary. At the end of this experiment, it was also seen that the group of rats with coconut oil dietary had higher plasma triglyceride contrasted with the group of rats with palm oil dietary.

2. Total cholesterol

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins : high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for cholesterol uptake from the cells.

The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL-cholesterol contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with CHD and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-cholesterol indicates high risk. HDL-cholesterol has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor. The determination of the individual total cholesterol level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-cholesterol and LDL-cholesterol. The mean plasma cholesterol are presented in Table 3, whereas LDL-cholesterol in Table 4 and HDL-cholesterol in Table 5.

Table 3. Effect of feeding diet on plasma total cholesterol concentration

Dietary groups	Plasma total cholesterol (mg/dL) in the-			
	earlier study	1st month	2nd month	3rd month
Standard (AIN'93)	128.47 ± 5.53	127.37 ± 2.63	127.62 ± 6.44	120.00 ± 4.37
Fresh soybean oil	130.96 ± 4.52	147.26 ± 7.05	166.58 ± 3.33	129.08 ± 6.29
Fresh palm oil	128.53 ± 5.65	150.02 ± 5.91	111.82 ± 3.73	82.58 ± 3.48
Improperly used palm oil	120.89 ± 6.30	167.03 ± 1.16	191.84 ± 4.93	239.63 ± 4.24
Fresh coconut oil	133.04 ± 3.99	144.93 ± 7.70	172.53 ± 3.20	192.27 ± 5.19
Improperly used coconut oil	118.98 ± 7.33	168.67 ± 3.60	196.34 ± 3.32	252.88 ± 5.24

At the end of the experimental period, rats in the group received coconut oil, either fresh or improperly used, had significantly higher concentration of total cholesterol compared to those animals fed palm oil. According to Tony et al (1991), saturated fatty acids (especially lauric acid and myristic acid, the dominant fatty acid in coconut oil) increase plasma cholesterol concentrations whereas polyunsaturated fatty acids (PUFAs) have a hypocholesterolemic effect. The saturated fatty acids (12-16 carbons) have twice the cholesterol raising influence as PUFAs has in lowering it.

Improperly used frying oil has hypercholesterolemic effect contrasted with fresh oil. During the frying process, the unsaturated fatty acids decomposed to saturated fatty acids cause increased in saturated fatty acids content and a decreased in unsaturated fatty acids on the improperly used frying oil.

3. LDL-cholesterol

As the same as plasma total cholesterol concentrations, rats in the group received coconut oil, either fresh or improperly used, had significantly higher concentration of LDL-cholesterol compared to those animals fed palm oil. Although, the mechanisms by which dietary fatty acids regulate plasma LDL-cholesterol concentration have not been well characterized. Stucchi *et al* (1995) has suggested that saturated fatty acid may interfere a complex equilibrium between the rate of production and clearance by both receptor and non-receptor mediated pathways. In addition, studies done by Lin *et al* (1995) *in* Wuryastuti (2000) have further suggested that the saturated fatty acid induced rise in plasma LDL-cholesterol concentrations was the results of a lower-mediated LDL fractional catabolism compared to that in polyunsaturated fat-fed animals. The mean plasma LDL-cholesterol concentrations in this experiment are presented below.

Table 4. Effect of feeding diet on plasma LDL-cholesterol concentration

Dietary groups	Plasma LDL-cholesterol (mg/dL) in the-			
	earlier study	1st month	2nd month	3rd month
Standard (AIN'93)	104.76 ± 4.75	112.54 ± 2.66	122.72 ± 7.87	104.93 ± 3.94
Fresh soybean oil	104.88 ± 4.85	127.99 ± 7.25	154.37 ± 4.21	116.95 ± 6.23
Fresh palm oil	103.84 ± 5.19	129.79 ± 5.29	98.37 ± 4.04	71.48 ± 4.14
Improperly used palm oil	97.12 ± 7.13	159.53 ± 2.02	189.72 ± 3.71	230.55 ± 6.01
Fresh coconut oil	107.92 ± 4.90	133.97 ± 8.31	169.76 ± 3.20	182.05 ± 3.93
Improperly used coconut oil	91.91 ± 8.48	150.54 ± 3.43	192.44 ± 2.98	241.84 ± 3.37

Significant lower plasma LDL-cholesterol concentrations were observed in the group of rats received palm oil, especially fresh palm oil, with high polyunsaturated fatty acid diets. This result suggested that dietary with the fresh palm oil as opposed to coconut oil and improperly used oil, produced lower LDL-cholesterol concentrations in rats.

4. HDL-cholesterol

HDL have been described as lipoproteins which facilitate the clearance of triglyceride-rich lipoproteins. According to Grande *in* Varela *et al* (1988), there is negative correlation between cholesterol and the incidence of coronary disease is due primarily to the HDL-transported fraction. In contrast, the LDL-transported fraction is positively correlated with coronary disease incidence. The LDL carry most of the plasma cholesterol in man (some 60%) are therefore believed to have an atherogenetic effect, whereas the HDL are believed to have a protective or anti-atherogenic effect. The mean plasma HDL-cholesterol concentrations in this experiment are presented below.

Table 5. Effect of feeding diet on plasma HDL-cholesterol concentration

Dietary groups	Plasma HDL-cholesterol (mg/dL) in the-			
	earlier study	1st month	2nd month	3rd month
Standard (AIN'93)	67.77 ± 2.85	64.96 ± 2.89	59.95 ± 2.96	57.70 ± 2.82
Fresh soybean oil	69.31 ± 1.97	59.91 ± 3.62	58.04 ± 2.66	60.43 ± 1.74
Fresh palm oil	64.21 ± 2.10	48.05 ± 2.03	42.68 ± 1.82	38.23 ± 1.56
Improperly used palm oil	65.01 ± 3.36	47.37 ± 1.75	40.58 ± 2.82	36.26 ± 4.11
Fresh coconut oil	67.75 ± 3.82	52.29 ± 2.70	44.37 ± 1.68	43.84 ± 0.47
Improperly used coconut oil	62.14 ± 4.69	43.59 ± 2.06	33.66 ± 5.84	35.35 ± 7.33

Table 5 showed that consumption in high lipid for long time caused decreasing in HDL-cholesterol. Rats in the group with improperly used oils, either palm oil or coconut oil, had significantly lower concentrations of HDL-cholesterol compared to those animals fed fresh

oils. Oils with higher polyunsaturated fatty acids resulted in higher plasma HDL-cholesterol concentrations, in comparison with saturated fatty acids.

Histological organs

Several studies have shown a statistical association between the circulating concentration of certain lipoproteins, particularly LDL and HDL, and the risk of an individual suffering a heart attack. High blood concentrations of LDL have been shown to constitute one such risk factor. High circulating HDL concentrations, on the other hand appear to be beneficial in some studies but not others and according to some reports, low concentrations of essential fatty acids also appear to constitute risk (Cottrell, 1991).

One of the earliest events in atherogenesis is intracellular accumulation of lipids, particularly cholesterol esters, in the aortic intima. Lipids presumable come from uptake plasma lipoproteins, particularly LDL. The lipids are accumulated in the foam cells, which are predominantly macrophages. According to Sugano and Imaizumi (1991), diets high in saturated fatty acids can promote thrombosis and hence atherogenesis.

In this experiment, the pointed out that after heating, the more highly unsaturated oils are responsible for greater liver damage than oils of lesser unsaturation. Bucko *et al in* Varela *et al* (1988) have reconfirmed this : feeding guinea pigs olive oil, sunflower oil, butter and lard heated at 170°C and aerated for 1 h resulted in the appearance of severe fatty livers with granulomatous areas and hyperplasia of Kupffer cells. According to Cottrell (1991), fed a high-fat diet can develop arterial disease because the dietary fat might influence the development of the arterial lesions (atherosclerosis) thought to be a necessary precursor of a heart attact. The influence of dietary oils to the histological organs in this experiment presented below.

1. Liver cells

In the lipid profiles result we see that in rats fed improperly used frying oils are a rise in triglyceride, total cholesterol and LDL-cholesterol concentration. This is followed by a series of changes on the surface and within the wall of artery that are visible under the electron microscope like figure below.

In Fig.1 we see certain types of white blood cells (mainly monocytes) adhere to the arterial wall and then migrate under the (endothelial) lining of the wall. These cells subsequently group together and begin accumulate lipid (Fig.2). These clusters of "foamy" macrophages (as the monocytes have become) constitute the basic structural component of the earliest stage of the lesion visible under the lower magnifications of the light microscope.

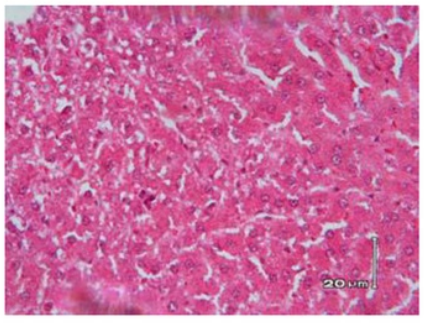

Fig. 1. Liver cells of rats on fresh oils dietary

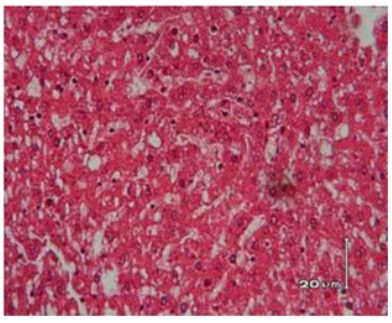

Fig. 2. Liver cells of rats on improperly used oils dietary

According to Cottrell (1991), more advanced lesions are formed when smooth muscle cells from the muscular arterial walls migrate to the lesion site and there accumulate lipid and proliferate. A connective tissue matrix is formed around and over this focus of cells, and lipid droplets, cholesterol crystals, areas of dead cells, and even calcification can be seen in very advanced lesions.

In compare to Fig.1 we can see many lipid droplets and injury wall cells in Fig.2. These figures suggested that improperly used frying oil cause damage the cells. This result was also seeing in heart and aortic endothelial cells like present below.

2. Heart cells

It is generally supposed that an acute interruption of the blood supply to the heart muscle arises when the artery supplying it becomes partly or wholly blocked by a clot (thrombus). If the artery has previously been narrowed by the presence of arteriosclerotic lesions (described as plagues), then complete occlusion is more likely occur. According to Cottrell (1991), it is not clear what precipitates the formation of the thrombus, but one possible explanation is that one of the arteriosclerotic plaques becomes ruptured and the release of its contents into the bloodstream precipitates clotting.

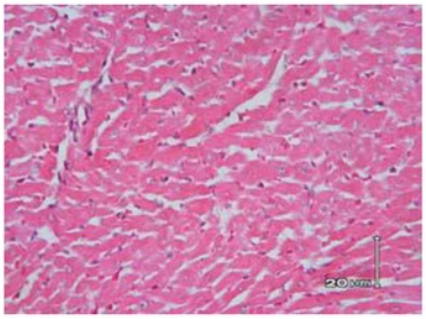

Fig. 3. Heart cells of rats on fresh oils dietary

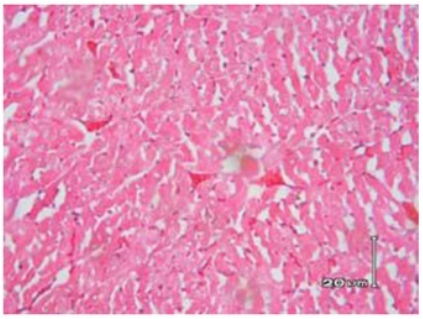

Fig. 4. Heart cells of rats on improperly used oils dietary

Improperly used frying oil cause accumulation of lipid droplets in vacuoles namely foam cells (Fig. 4). Those are developing and lead to atherosclerotic disease. It believe that lipid oxidation products (that present in used frying oils) may play a prominent role in promoting the disease.

3. Aortic endothelial cells

The groups of rats that have improperly used frying oils dietary who are prone to hyperlipidemia and subsequent aortic intimal thickening as shown in Fig.6. Oxidized oils (like in improperly used frying oils) has been shown to accelerate several steps in atherosclerosis including endothelial damage (Fig.6 contrasted with Fig.5), monocyte or macrophage recruitment and increased uptake of LDL by foam cells.

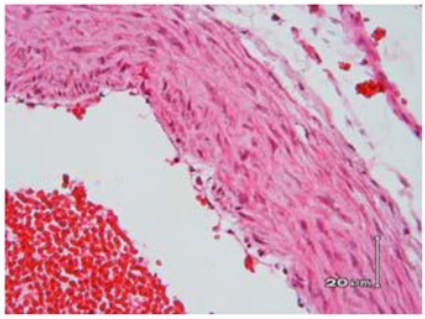

Fig. 5. Aortic endothelial cells of rats on fresh oils dietary

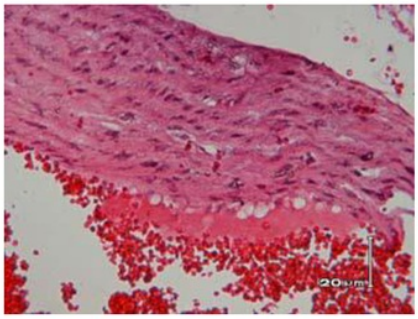

Fig. 6. Aortic endothelial cells of rats on improperly used oils dietary

4. Kidney cells

The kidney is generally described as increased in volume. The histologic modifications found are represented by cellular degeneration, tubular necrosis and granular clumps that block the tubular lumen (Fig.8 contrasted with Fig.7). Such lesions seemed more severe with improperly used frying oils, either palm oil or coconut oil, than with fresh oils, with which the damage observed was limited to an activation of the tubula epithelial cell nuclei.

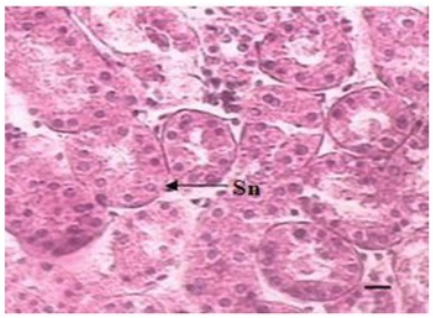

Fig. 7. Kidney cells of rats on fresh oils dietary

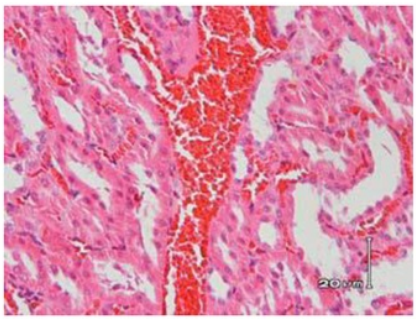

Fig. 8. Kidney cells of rats on improperly used oils dietary

CONCLUSIONS

There is no significant effect between difference sources and condition of oil on the growth rate of rats. Lipid profiles of the animals indicated that coconut oil had higher amounts of plasma total cholesterol, LDL- cholesterol and plasma triglyceride than did palm oil, but lower the HDL-cholesterol. Besides that, improperly used frying oils had hypercholesterolemic effect. Feeding improperly used frying oil cause damage to the cells of liver, heart, aortic endothelial and kidney contrasted with fresh oil.

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