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# Selective cholesterol deposition in the kidney tissue of rats fed palm kernel oil diet

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**Summary**

Plasma and tissue lipids were determined in twenty-four rats fed on locally prepared '*Ogi*' diet containing palm kernel oil (PKO), red palm oil (RPO) and mixture of both oils. Fasting blood sample was obtained from each animal by cardiac puncture under light ether anesthesia after feeding on different diets for twelve weeks. There were significant variations in the mean liver, kidney, spleen ($p < 0.001$, $p < 0, 03$, $p < 0.002$) tissue weights in the different dietary groups compared with the corresponding control values. The plasma total cholesterol, triglyceride and lipoprotein cholesterol concentrations in the dietary group showed no significant changes when compared with the corresponding control values. The liver, spleen and heart total cholesterol concentrations were not significantly different from the corresponding values in the control group, but within group analysis showed significantly elevated total cholesterol in the kidney tissue of rats consuming PKO diet ($p < 0.001$).The total cholesterol level in rats consuming PKO diet was significantly higher than the corresponding concentration in those consuming the diet containing a mixture of PKO + RPO [$p < 0.02$] and control ($p < 0.02$) diets. There was also a significant increased in the kidney tissue cholesterol of rats fed RPO diet when compared with the corresponding control value ($p < 0.05$). The histological findings revealed no abnormality except in rats fed on PKO and RPO diets where nephrocalcinosis was found.

**Keywords:** *Cholesterol, triglyceride, lipoproteins, vegetable oils and glomerulosclerosis.*

**Résumé**

Le plasma et les lipides dans les tissues ètaient determinès chez 24 rats nouris localement du regime d'Ogi » contenant d'huile des noix de palme (KRO) , L' huile rouge (RPO) et le mèlange de deux. L'echantillon de sang à jeune etait obtenu par puncture cardiaque avec une anesthèsie locale d'ether après nutrition des diffèrent regimens pour douze semaines. Ils avaient des variations significative du poids des tissues moyen du foi, pancrèas $P < 0.001$, $P < 0.$ , 0.3, $P < 0.0002$) dans chaque groupe de règimen comparè à leur contrôle. La concentration totale du cholestèrol dans le plasma, de triglycerine, de lipoproteine cholesterol dans le groupe de regime n'etait pas significatif comparès aux valeurs chez leurs controles respective La concentration du cholestèrol dans le foi , au pancreas , au cœur n 'etait pas significativemment diffèrent des valeurs chez leur controles mais l'analyse entre les groupes montrait que la concentration du cholestèrol dans les reins etait significativemment elevèe chez les rats consomant l'huile des noix de palme($P < 0.001$) > Le niveau total du cholesterol chez les rats consommants du PKO ètait elevè que ceux consommant le mèlange ou leur contrôle. L'augmentation de la concentration du cholesterol chez les rats consommant du RPO règime comparès aux valeurs obtenues chez leur controles etait significative ($P < 0.05$). Les rèsultats histologiques ne rèvelaient aucun anormalie a l'exception des rats nouris des règimes de PKO et PRO où la nèphrocalcinosie ètait observèe.

## Introduction

Sudden death due to coronary heart disease (CHD) is rapidly becoming a major health problem in developing countries of the world including Nigeria [1]. Dietary practices and life style may be important in this observation, since habitual diets and life style are some of the known modifiable "risk factors" associated with the development of premature CHD in different populations of the world. Previous studies have shown that the percentage total calorie provided by dietary saturated fatty acids is an important factor in the incidence rate of fatal and non-fatal myocardial infarction [2]. High calorie, high fat and the type of fatty acids in the diet are known to influence plasma lipid concentrations [3]. Elevated plasma cholesterol and triglyceride concentrations are associated with increased risk of developing premature atherosclerosis in man [3].

Red palm oil (RPO) is the most widely used cooking oil in West and Central Africa. RPO contains saturated and monounsaturated fatty acids [4]. Subsequently, it was suggested that RPO should be avoided along with certain animal fats as part of a "prudent diet" for control of heart

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diseases [4]. However, an earlier study by Alini et al. [5] among Malaysian male adolescents showed that the consumption of red palm oil did not cause any increase in the concentration of plasma cholesterol in the subjects.

Also consumption of palm kernel oil (PKO) in Nigeria is on the increase due to its low cost compared to the other vegetable oils. PKO is rich in saturated fatty acid such as myristic acid and this has been reported to be atherogenic in nature [6]. Studies on the effect of consumption of PKO in plasma lipids are still scanty. The present study was therefore designed to investigate the effects of the consumption of diets containing either RPO or PKO or a mixture containing fixed proportions of these oils on plasma and tissue lipids in albino rats.

## Materials and methods

### *Animals*

Twenty-four weanling male albino rats aged four weeks and weighing between 10 g and 15 g obtained from the Clinical Animal House, College of Medicine, University of Ibadan were studied. The animals were maintained in a well-ventilated room and were fed on locally prepared '*Ogi*' diet for 12 weeks. The diets were prepared from a mixture of maize starch *Ogi*, casein, vitamins, mineral salts, and the different vegetable oils according to the method of Agbedana and Taylor [7]. The control group was fed on commercial pellets (Ladokun Feeds Limited, Ibadan, Nigeria) for the same period of time. The rats were fed *ad libitum* and water was made freely available throughout the period of the experiment.

### *Experimental design*

The rats were divided into four different dietary groups. Each group was fed *ad libitum* with the specified diet as shown in table1. The daily food intake was recorded for each group, while the individual body weights were recorded every week throughout the period of twelve weeks. The animals were sacrificed after fasting overnight (14-16 hrs). Blood samples were collected by cardiac puncture after a light ether anesthesia. Plasma was separated from cells by centrifugation using an MSE minor centrifuge. The plasma samples were stored at -20 °C until analysed for total cholesterol, high density lipoprotein cholesterol and triglyceride. Low density lipoprotein (LDL) cholesterol was calculated using the Friedwald formula [8]. The liver, heart, kidney and spleen were quickly removed, blotted on filter paper to remove the blood and were weighed. A specimen of each organ was preserved in 10% formalin for histological examinations and the remaining part was stored frozen at – 20 °C until analyzed for total cholesterol and triglyceride using standard methods.

### Preparation of *'Ogi'* diet

'*Ogi*' was prepared from maize starch using the traditional Yoruba method; the dried maize grains were soaked in fresh water and fermented for three days at room temperature. The soaked maize grains were milled and sieved to extract the starch content. The starch was sun dried, powdered using a domestic grinder and the resulting powder was used for the preparation of the different diets as shown in table 1. The various food components were weighed, mixed thoroughly and grinded to achieve diets containing 5% PKO, 5% RPO and 2.5% PKO + 2.5% RPO. A small quantity of fresh water was added to each mixture to allow moulding of each diet into pellets for sun drying

**Table 1:** Composition of diets (weights in grams).

| Ingredients | Pko | Rpo | Pko + Rpo |
|---|---|---|---|
| | Diet | Diet | Diet |
| Maize starch ("ogi") | 71.75 | 71.75 | 71.75 |
| Palm kernel oil | 5.00 | - | 2.50 |
| Red palm oil | - | 5.00 | 2.50 |
| Vitamin mix | 0.25 | 0.25 | 0.25 |
| Vitamin-free casein | 18.00 | 18.00 | 18.00 |
| Mineral salts | 5.00 | 5.00 | 5.00 |

*Agbedana and Taylor [7]*

*PKO* = *Palm kernel oil*

*RPO* = *Red palm oil.*

**Table 2:** Mineral salts mixture was prepared using the method of Roger and Harper [9].

| | Weight in grams |
|---|---|
| Calcium carbonates | 29.29 |
| Di-sodium hydrogen phosphate ($Na_2HP0_42H_20$) | 0.43 |
| Potassium hydrogen phosphate ($KH_2P0_4$) | 34.31 |
| Magnesium sulphate. .. ... ... ($MgS0_4 . 7H_20$) | 9.98 |
| Ferric citrate ($FeC_2H_50_7$) | 0.623 |
| Copper sulphate ($CUS0_4\ 5H_20$) | 0.156 |
| Manganese sulphate ($MnS0_4\ H_20$) | 0.121 |
| Zinc chloride ($ZnC1_2$) | 0.2 |
| Potassium iodide (KI) | 0.005 |
| Ammonium molybdate | 0.0025 |
| Sodium selenate ($Na_2S0_1.5H_20$) | 0.0015 |

### *Biochemical analysis*

The plasma total cholesterol concentration was estimated by the modified method of Lieberman-Bur chard reaction as described by Searcy and Berquist [10]. The plasma HDL fraction was isolated from other lipoproteins using the

heparin-manganese chloride precipitation method as described by Burstein and Samaille [11]. The plasma HDL cholesterol in the supernatant was estimated as in the method for total cholesterol estimation. The (LDL) cholesterol was calculated using the Friedwald formula [8]. Plasma creatinine was measured using Jaffe reaction [13] after precipitation of the protein with sodium tungstate. Commercial Randox serum was used as quality control in all analytical procedures.

*Tissue lipids*

A known weight of each tissue was homogenised manually in chloroform: methanol (2:1 v/v) mixture using a Porter-Elvehjem homogenizer. The homogenate was made up to 20 fold (w/v) the weight of tissue sample using the same solvent mixture. The clear supernatant was assayed for total cholesterol and triglyceride by the methods of Searcy and Berquist [10] and Gottfried and Rosenberg [12], respectively.

*Histological procedures*

At the time of sacrifice, the heart, liver, kidney and spleen from each rat were quickly excised, blotted on filter paper to remove blood, weighed and a part of each organ was fixed in 10% formalin for a period of at least 24 hours. These specimens were subsequently dehydrated in upgraded concentrations of alcohol, cleared in various solutions of xylene and then impregnated and embedded in molten paraffin wax. Histological section was prepared for each specimen. The sections were stained with haematoxylin and eosin and were examined by light microscopy to determine the presence of any changes in the morphology of the different tissues.

*Statistical analysis*

The results are expressed as mean ± standard deviation. Statistical comparisons were made using the paired t-test and one way analysis of variance (ANOVA). Differences were regarded as significant at (P = 0.05).

## Result

*Food intake and body weight changes*

The food intake and growth patterns are shown in figure 1.

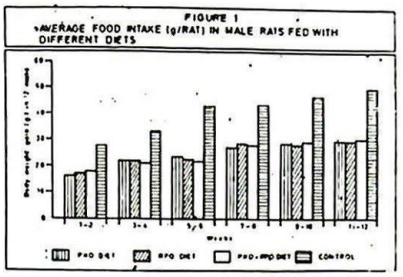


**Fig. 1:** Average food intake (g/rat) in male rats fed with different diets.

Throughout the period of feeding the control animals consumed approximately twice the amount of food consumed by the experimental groups. Food consumption by experimental rats stabilized in the second week of the study. In the fourth week, the food consumption in the PKO + RPO group was slightly decreased. The other groups however, showed a decreased food intake in the fifth week. Figure 2 shows the body weight changes in all the groups

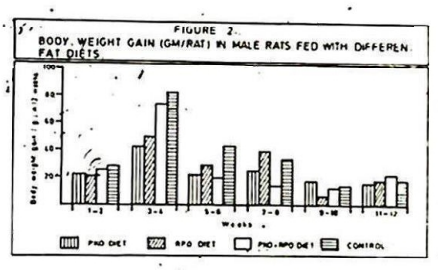


**Fig. 2:** Body weight gain (gm/rat) in male rats fed with different fat diets.

with consistent increases in the mean body weight for the different groups during 1-4 weeks of feeding. The PKO group showed identical growth pattern to the RPO group, the PKO + RPO and the control groups showed higher growth rates during the 3rd and 4th weeks of feeding (Fig. 2). The absolute body weight showed increases while growth rates decreased in all the groups from the 5th to 12th

**Table 3:** Mean tissue weights (X ± S.D) in all dietary groups

| Tissue | Control n = 6 | PKO n = 6 | RPO n = 6 | PKO + RPO n = 6 | p-value |
|---|---|---|---|---|---|
| Liver (g) | 7.50 ± 0.20 | 5.60 ± 0.20 | 5.30 ± 0.40 | 5.60 ± 0.20 | 0.001 |
| Heart (g) | 0.70 ± 0.10 | 0.70 ± 0.01 | 0.60 ± 0.04 | 0.60 ± 0.40 | ns |
| Kidney (g) | 1.40 ± 0.10 | 1.20 ± 1.0 | 1.20 ± 0.04 | 1.10 ± 0.04 | 0.03 |
| Spleen(g) | 0.90 ± 0.10 | 0.60 ± 0.10 | 0.50 ± 0.10 | 0.60 ± 0.10 | 0.002 |

*PKO = palm kernel oil, RPO= red palm oil, n=number, X=mean, S.D-standard deviation*

week with marked decreases in the 9th and 10th weeks in all groups.

**Tissue lipids**

Tables 4 and 5 show the mean tissue total cholesterol and triglyceride concentrations in the different dietary groups. The differences in the mean liver tissue total cholesterol concentrations in the different experimental groups were not statistically significant. Similarly, no statistically significant differences were observed in the mean total cholesterol concentrations in the heart and spleen tissues within the experimental groups and the control group. However, as shown on table 4, the mean total cholesterol concentration in the kidney showed significant intra-group variations within the different groups ($F = 4.99, p < 0.001$). The mean total cholesterol concentration in the kidney of the rats fed the PKO diet was significantly increased when compared with the mean values in rats fed on PKO + RPO

**Table 4:** Mean tissue cholesterol (X ± SD) in all groups.

| Tissue | PKO n=6 | RPO n=6 | PKO ± RPO n=6 | Control n=6 | F –value | P-value |
|---|---|---|---|---|---|---|
| Liver (mg/dl) | 885.2 ± 1119 | 890.73 ± 140.4 | 829.74 ± 156 | 867.2 ±127.9 | 0.2580 | ns |
| Heart (mg/dl) | 314.68 ± 88.7 | 269.30 ± 181.0 | 304.90 ± 148.9 | 261.02 ± 105 | 0.6461 | ns |
| Kidney (mg/dl) | 157.92 ± 57.1 | 142.63 ± 72.35 | 81.02 ± 19.15 | 113.75 ± 37.3 | 4.990 | 0.001 |
| Spleen (mg/dl) | 351.79 ± 2742 | 301.06 ± 171.96 | 332.52 ± 156.48 | 289.02 ± 165 | 0.1255 | ns |

*PKO= palm kernel oil*
*RPO = red palm oil*
*n = number*
*X= mean*
*PKO + RPO vs. PKO ($p < 0.01$)*
*PKO vs. Control [$p < 0.02$]*
*RPO VS control ($P < 0.05$)*
*SD=standard deviation.*

**Table 5:** Means tissue triglyceride (X ± SD) in all dietary group.

| Tissue | PKO n = 6 RPO | n = 6 PKO ± RPO | Control n = 6 | P value n = 6 | P-value | |
|---|---|---|---|---|---|---|
| Liver (mg/dl) | 206.17 ± 236.1 | 102.50 ± 26.4 | 101.61 ± 26.4 | 80.33 ± 7.4 | 0.1398 | ns |
| Heart (mg/dl) | 161.06 ± 88.8 | 247.50 ± 62.3 | 256.33 ± 74.1 | 199.83 ± 80.0 | 1.9161 | ns |
| Kidney (mg/dl) | 279.101±167.9 | 293.02 ± 167.9 | 217.95± 125.49 | 317.43 ± 112.52 | 0.9045 | ns |
| Spleen (mg/dl) | 251.43 ± 40.5 | 307.06 ± 261.8 | 209.63 ± 44.43 | 166.13 ± 81.04 | 0.9157 | ns |

*PKO = palm kernel oil*
*RPO = Red palm oil*
*n = number*
*X= mean*
*ns = not significant.*

*Tissue weights*

Table 3 shows the mean wet tissue weights in all the groups. The mean wet liver, heart, kidney and spleen weights were similar in all the experimental groups. Also as shown in table 3, the respective mean liver, kidney and spleen weights in the experimental groups were significantly reduced when compared with the corresponding values in the control group ($p < 0.001, p < 0.03. p < 0.002$). On the other hand no significant differences were observed in the respective heart weights in the experimental groups when compared with the corresponding levels in the control group.

**Table 6:** Plasma lipids and lipoproteins (Mean ± SD) in different dietary groups

| Dietary groups | T-C (mg/100ml) | HDL -C (mg/100ml) | LDL -C (mg/100ml) | T.G (mg/100ml) |
|---|---|---|---|---|
| PKO n = 6 | 183.16 ± 49.30 | 13.3 ± 4.13 | 146.83 ± 60.83 | 118.83 ± 19.54 |
| RPO n = 6 | 171.33 ± 59.38 | 17.67 ± 7.20 | 126.60 ± 60.83 | 139.00 ±5 0.35 |
| PKO+ RPO n = 6 | 179.16 ± 28.22 | 21.83 ± 5.38 | 126.88 ± 21.18 | 152.16 ± 37.01 |
| Control n = 6 | 152.66 ± 57.75 | 19.00 ±10.00 | 101.33 ± 58.88 | 137.17 ± 42.32 |

*PKO = palm kernel oil*
*RPO = red palm oil*
*n = number*
*T.C = total cholesterol*
*S.D = standard deviation*
*T.G = triglyceride*
*HDL -C = high density lipoprotein cholesterol*
*LDL -C = low density lipoprotein cholesterol*
*X = mean.*

and control diet ($p < 0.01, p < 0.02$), respectively. There was a significant decrease in the kidney total cholesterol concentration in the RPO group when compared with the corresponding control value ($p < 0.05$). There were no significant changes in the mean triglyceride concentrations in the liver, heart, kidney and spleen tissues in the experimental groups when compared with the corresponding values in the control.

*Plasma lipids and lipoproteins*

Table 6 shows the plasma total, HDL, LDL cholesterol and triglyceride concentrations in all the groups. There were slight increases in the plasma total and LDL cholesterol levels in all the experimental rats compared with the control group. These increases however, did not reach the level of statistical significance. Also the plasma high density lipoprotein and the triglyceride concentrations in all the experimental groups were similar to the corresponding control values.

Table 7 shows the correlation coefficients of plasma total cholesterol and tissue total cholesterol concentration

**Table 7:** Correlation coefficient for tissue and plasma cholesterol

| | | N | r-value | P-value |
|---|---|---|---|---|
| PKO | | 6 | | |
| | liver | | -0.150 | ns |
| | Heart | | -0.780 | < 0.05 |
| | Kidney | | 0.230 | ns |
| | Spleen | | 0.006 | ns |
| RPO | | 6 | | |
| | Liver | | 0.450 | ns |
| | Heart | | -0380 | ns |
| | Kidney | | 0.590 | ns |
| | Spleen | | 0.580 | ns |
| PKO + RPO | | 6 | | |
| | Liver | | 0.450 | ns |
| | Heart | | -0380 | ns |
| | Kidney | | 0.280 | ns |
| | Spleen | | 0.180 | ns |
| Control | | 6 | | |
| | Liver | | 0.910 | < 0.01 |
| | Heart | | 0.270 | ns |
| | Kidney | | -0.680 | ns |
| | Spleen | | -0.150 | ns |

*N = number* *ns = not significant*
*r = correlation coefficient* *P = level of significant*
*RPO = red palm oil* *PKO = palm kernel oil*

in all the different groups of rats. In the PKO group, the heart tissue cholesterol was negatively correlated with the plasma total cholesterol concentration (r = -0.78 P < 0.05). Also the positive correlation between liver tissue cholesterol and plasma cholesterol in the control group was statistically significant (r = + 0.910, $p < 0.010$).

The respective concentrations of plasma creatinine in the different experimental groups were not significantly different from the control value.

*Histological investigations*

Figures 3, 4, and 5 showed histological sections of the heart and kidney tissues in rats fed on PKO and control

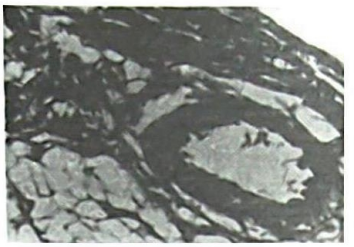

**Fig. 3:** A micrograph of a typical normal coronary artery in rat fed on PKO diet.

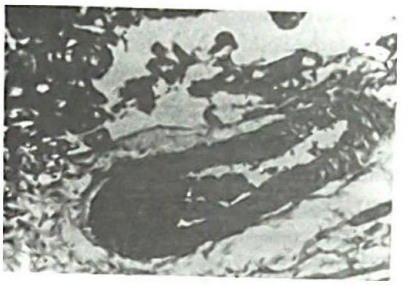

**Fig. 4:** A micr graph of a typical renal artery in rat fed control diet.

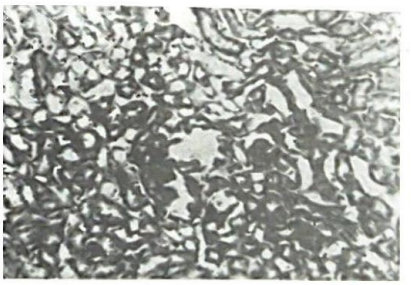

**Fig. 5:** A micrograph of a typical renal tubule with calcium deposit in rat fed PKO diet.

diets. The RPO and PKO + RPO experimental rats showed similar patterns. The tissue morphology was normal in all groups. The heart and kidney tissues of RPO, PKO + RPO groups also showed similar features. However as shown in figure 5, there were calcium deposits in the kidney tissue of rats fed on PKO diet. Similar features were found in a limited number of rats consuming RPO diet for twelve weeks.

## Discussion

The '*Ogi*' diet used for this study is similar to a local diet widely consumed by children in this community and this is different from most previous studies employing commercial rat pellets containing bleached and deodourized oils [14]. The control group consumed more food than the experimental groups because they were more familiar with the commercial diet. The experimental rats adjusted to the new diets with time and the total food intake in each dietary group was adequate as evident from the growth rates of the experimental rats. One striking observation in the present study is that the wet tissue weights of the liver, kidney and spleen were significantly reduced in rats consuming diets containing RPO, PKO or a mixture of both oils when compared with the corresponding values in the control group. The reason for this observation is unknown, since no available reports have suggested any specific relationship between organ weight and type of dietary fats consumed. Although all the rats gained weight throughout the period of feeding, the seeming decrease in the mean body weight gain after the 3rd week of feeding may suggest stabilization of body weight of the rats probably at full maturity

Plasma total cholesterol and HDL and LDL cholesterol levels were not significantly altered in rats consuming diets containing RPO, PKO or a mixture of both oils. Our results are at variance with those from an earlier study which found normal plasma cholesterol but a significantly increased LDL cholesterol concentration in rats fed on diets containing oxidised palm oil [14]. The difference may be attributed to a high concentration of saturated fatty acids in oxidised red palm oil when compared with natural red palm oil [14], probably suggesting that the form in which red palm oil is presented in the diet may play an important role in the modulation of plasma lipid levels in different populations where red palm oil is part of the habitual diet.

The mean plasma triglyceride levels were not significantly raised in the rats consuming diets containing these different oils. Similar findings were reported by some earlier workers in Malaysian young adults who were fed for five weeks on diets containing red palm oil [5].

The mean tissue total cholesterol in the heart, liver and spleen tissues were not increased in rats fed on the diets containing RPO or PKO + RPO. On the other hand, total cholesterol in kidney tissue from rats fed on the diet containing PKO only was significantly increased when compared with either the values in rats fed on RPO (110%) or the control (139%). This probably suggests a selective deposition of cholesterol in the kidney tissues of rats consuming protein '*Ogi*' diet containing PKO. The significance of an apparent organ-specific lipid deposition has been highlighted by some workers [15, 16]. Previous studies showed an abnormal lipid deposition in the heart tissue of puromycin amino nucleoside (PAN) induced nephrotic in rats [16]. The selective lipid deposition of lipid in the mesangial cells of the glomerulus has been linked with the incidence of glomerulosclerosis [15]. From the present study it could only be speculated that prolonged intake of PKO diet may be linked to development of glomerulosclerosis in albino rats.

The mechanisms for this increased tissue lipid deposition in the kidney of rats consuming PKO can only be speculated. The specific fatty acid composition in addition to the frequency and duration of consumption of these different vegetable oils could be important determinants of the altered tissue lipid metabolism. PKO is rich in myristic acid as compared to red palm oil and this fatty acid is known to be atherogenic in nature [15]. Therefore one possible explanation is that excess specific saturated fatty acid (myristic acid) in the PKO could lead to an increase in the uptake of cholesterol by kidney tissues in rats fed palm kernel oil diet. Such flux of cholesterol into the tissue pools may occur without being reflected in plasma cholesterol levels. The possibility exists that an accumulation of cholesterol in tissue such as the arterial wall could have a role in atherogenesis [17].

Although detailed histological studies revealed no evidence of athermanous changes in any of the dietary group rats, the evidence of nephrocalcinosis in a limited number of rats fed on PKO and RPO diets is striking.

## Conclusion

Consumption of red palm oil in the diet in a moderate amount did not cause any deleterious effects on plasma and tissue lipids in rats. It may be suggested that consumption of red palm oil may not be injurious to health; on the other hand excessive consumption of PKO could lead to the development of glomerulosclerosis.

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