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# Comparative Effect of Chronic Consumption of Some Edible Vegetable Oils on Lipid Profile and Some Haematological Parameters in Rats

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**ABSTRACT**

*Considering the fact that elevated serum concentration of cholesterol is a major risk factor for coronary artery disease, it became necessary to ascertain the effect of consumption of some edible oils on lipid profile and haematological parameters. Thirty six male albino wistar rats weighing 200 - 220 g were randomly divided into 6 groups (n = 6), thus; control group, red palm oil (RPO) fed group, palm kernel oil (PKO) fed group, coconut oil (CCO) fed group, soybean oil (SBO) fed group and sesame oil (SSO) fed group. 20 g of each edible oil was thoroughly mixed with 180 g of palletized grower feed, making a total of 200 g feed. The animals had access to food and water ad libitum. After 90 days of feeding, the animals were sacrificed and blood collected via cardiac puncture for lipid profile and haematological analysis. Results showed that total cholesterol (TC), triglyceride (TG), very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) were significantly ($P < 0.05$) reduced in the edible oil fed groups, compared with control. High density lipoprotein cholesterol (HDL-C) was significantly ($P < 0.05$) higher in the edible oil fed groups, compared with control. Red blood cell (RBC) count, haemoglobin (Hb) concentration and packed cell volume (PCV) were significantly ($P < 0.05$) higher in the edible oil fed groups, compared with control. White blood cell (WBC) count was significantly ($P < 0.05$) reduced in RPO and PKO groups, compared with control, and significantly ($P < 0.05$) increased in CCO and SSO groups, compared with control. We therefore conclude that the edible oils used in this study reduced the risk of CHD by reducing serum cholesterol concentrations, with CCO, SBO and SSO being more beneficial as serum TC concentrations were lowest in those groups than RPO and PKO. Also, the edible oils used for this study enhances erythropoiesis, but RPO, PKO and SBO are detrimental to leucopoiesis.*

**Keywords:** Coconut oil, haematology, lipid profile, palm kernel oil, red palm oil, sesame oil, soybean oil.

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## INTRODUCTION

Increased levels of cholesterol or triglycerides are most often consequences of genetic or inherited disorders of lipid metabolism. These lipids may also be increased by some common medical conditions such as diabetes mellitus (DM), hypothyroidism, kidney and liver disease [1,2,3]. Medications such as diuretics, prednisone, estrogens and testosterone, also adversely influence serum cholesterol levels [4]. In addition, diets high in fats, which is the basis of this study also affects serum cholesterol and TG concentrations [5,6].

Cholesterol is an essential part of every cell in the body. It is necessary for formation of new cells and for older cells to repair themselves after injury. Cholesterol is also used by the adrenal glands in the synthesis of some hormone, such as cortisol, by the testicles to form testosterone, and by the ovaries to form estrogen and progesterone [7].

Edible oils include red palm oil (RPO), palm kernel oil (PKO), coconut oil (CCO), soybean oil (SBO), sesame oil or beniseed oil (SSO), groundnut oil (GNO), etc. These oils are consumed not only for their supply of lipids in the diets but for their distinct aromas, colours, and palatability. These oils are rich in essential nutrients such as vitamins and anti-oxidant compounds.

Red palm oil, derived from the fruit of oil palm (*Elaesis guineensis*) has long been used as cooking oil. Red palm oil (RPO) is rich in mono unsaturated fatty acids, anti oxidant and vitamins, and is widely used as oil in diets in many part of the world. RPO has been reported to have beneficial effect in oxidative stress, arterial thrombosis and hypertension [8,9]. Reports of Zhang et al. [10], showed that RPO is a good source of carotenoids. They further stated that RPO increased plasma concentration of alpha carotene, beta-carotene lycopen and alpha-tocopherol.

Palm kernel oil (PKO) is derived from the seed of the fruit of *Elaesis guineensis*. PKO may not be a good edible oil since it has been reported to contain a very high percentage of trans and saturated fats, which increases blood cholesterol as well as low density lipoprotein cholesterol (LDL-C) levels [11].

Coconut oil (CCO) is derived from coconut (*Cocos nucifera*). CCO have been shown to inhibit lipo-protein oxidation, with hypolipidemic effects [12]. CCO has been proven to be beneficial in hair care, skin care, stress relief, maintaining cholesterol levels, weight loss, increased immunity, high blood pressure, diabetes, dental cares, and bone strength [13]. These health benefits have been attributed to the presence of lauric acid, capric acid and caprylic acid, and the possession of antimicrobial, antioxidant, antifungal and antibacterial properties [13].

Soybean oil is extracted from the seed of glycine max. Its seed contains about 40% protein and 20% oil. The oil is highly digestible, high in unsaturated fatty acids and also contains insignificant amount of cholesterol [14].

Sesame oil is obtained from sesame seed. The seed contains about 50% oil and 25% protein. It is widely used in baking, candy making and other food industries [15,16]. Oil from the seed is used in cooking and contains about 47% oleic and 39% linoleic acid. Sesame oil and food fried in sesame oil have a long shell-life because the oil contains an antioxidant. The oil is also used as confectionary fats in ice cream production [15,16].

Dietary oils remain the major source of lipid in diets. Considering the fact that elevated serum concentration of cholesterol is a major risk factor for coronary artery disease, it became necessary to ascertain the effect of consumption of these oils on biochemical and haematological parameters.

## MATERIALS AND METHODS

### 2.1 Plant Materials and Preparation of Edible Oils

Edible oils used for this study were red palm oil (RPO), palm kernel oil (PKO), coconut oil (CCO), soybean oil (SBO) and sesame oil (SSO). Red palm, palm kernel and soybean oils were purchased from Watt market in Calabar, Cross River State, Nigeria, while coconut and sesame oils were freshly prepared since they were not readily available in the market.

Coconut oil was prepared using the routine traditional method. Ripe coconuts of big sizes was purchased from Watt market in Calabar, Cross River State, Nigeria. The nuts were first broken manually and coconut mesocarp was removed, washed in water and grated using the local grater. The shredded coconut was then stirred with distilled water to give coconut milk. The coconut milk was allowed to stay for 12 hours, after which the curd was then scoped into a clean pot and gentle heat was applied (about 50 - 60$^{o}$C). As sufficient heat was applied, the curd caked at the bottom of the pot while the coconut oil came out as a light-yellowish oil. The oil was decanted into a glass bowl and allowed to cool before being put into a bottle with screw cap to prevent oxidation pending usage.

Beniseed was purchased from Obudu main market in Obudu Local Government Area of Cross River State, Nigeria. The seeds were rinsed to remove debris and sand, after which they were then sundried. The seeds were crushed using an electric blender and soaked in hot water of about 100$^{o}$C, stirred thoroughly to form a watery paste and allowed to cool. On cooling, the mixture was refrigerated at 4$^{o}$C for 24 hours, at the end of which it separated into 3 layers, thus, the oil floating on top, the water at the middle layer and the paste finally settling at the bottom of the glass container. The oil was poured into a screw bottle pending usage. All oils used for this study were refrigerated pending usage.

### 2.2 Animal Preparation and Protocol

Thirty six (36) male albino wistar rats weighing 200 - 220 g were used for this study. The animals were purchased from the Department of Physiology Animal House, College of Medical Sciences, University of Calabar, Nigeria.

Animals were randomly assigned 1 of 6 groups (n = 6), after which they were allowed to acclimatize for 7 days. All animals were allowed access to food and water *ad libitum* and 12 hours light/dark cycle. Group 1 served as control, group 2; red palm oil - treated group (RPO), group 3; palm kernel oil - treated group (PKO), group 4; coconut oil - treated group (CCO), group 5; soybean oil - treated group (SBO) and group 6; sesame oil - treated group (SSO).

### 2.3 Administration of Edible Oils

The edible oils were mixed with the palletized growers' feed in the ratio shown in table 1.

**Table 1. Diet Formulation**

| | Group I Control | Group II Red palm oil | Group III Palm kernel oil | Group IV Coconut oil | Group V Soybean oil | Group VI Sesame oil |
|---|---|---|---|---|---|---|
| No of Rat | 6 | 6 | 6 | 6 | 6 | 6 |
| Palletized grower feed | 200 | 180 | 180 | 180 | 180 | 180 |
| Red palm oil (RPO) | - | 20 | - | - | - | - |
| Palm kernel oil (PKO) | - | - | 20 | - | - | - |
| Coconut oil (CCO) | - | - | - | 20 | - | - |
| Soybean oil (SBO) | - | - | - | - | 20 | |
| Sesame oil (SSO) | - | - | - | - | - | 20 |
| Total feed (g) | 200 | 200 | 200 | 200 | 200 | 200 |

The diets were formulated daily to avoid fungi growth. During the formulation of the diet, the oils were added to the palletized growers feed and mixed thoroughly to allow for uniform distribution.

### 2.4 Sample Collection

At the end of 90 days of feeding, the animals were anaesthetized in chloroform vapour contained in a dessicator (3.5% soaked in cotton wool) and blood collected via cardiac puncture (blood was drawn from the heart) a modification of the method by Ohwada [17]. The samples were collected (using 5mls syringe attached to 21G needle) into plain capped bottles and EDTA - treated (ethylene diamine tetraacetate) bottles, for serum and whole blood samples respectively. The samples were immediately used for the estimation of the different variables.

### 2.5 Serum Lipid Profile Estimation

**Estimate of Total Cholesterol**

The concentration of cholesterol in blood serum was estimated using the enzymatic colorimetric test (CHOD –DAP) kit method of Sieldel et al. [18].

*Principle*

Cholesterol esterase catalyses the hydrolysis of cholesterol esters into free cholesterol and fatty acid. The free cholesterol is then oxidized to cholestene-3-one and hydrogen peroxide in the presence of cholesterol oxidase. Phenol and 4-amino-antipyrine then combine with the hydrogen perioxide in the presence of peroxidase to produce a red coloured quinonemine which is read colorimetrically at 540nm. The intensity of the colour produced is directly proportional to the total cholesterol concentration of the sample. Aliquots (0.1m) of the sample were used for these estimations.

**Estimate of Serum Triacylglycerol**

The triglyceride concentration in the test samples were estimated using the Chiron diagnostic Triglyceride GPO kit method of Negele et al. [19].

*Principle*

Lipase catalyses the hydrolysis of triacylglycerol to glycerol and fatty acids. The glycerol is then phosphorylated in a reaction catalysed by glycerol kinase to yield glycerol 3-phosphate, which is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide, then oxidized the chromogen comprising of n-ethyl-n-sulpholydroxypropyl-n-foludine. Sodium salt ion or (ESGIHPI) and 4-aminoantipyrine to form a purple coloured quinoneinine dye which is read colorimetrically at 540nm. All reagents and samples were brought to temperature (20 - 25$^{O}$C).

**Estimation of HDL-Cholesterol**

HDL-cholesterol was extracted from sample by precipitation using heparin sulphate and manganese chlorite. The supernatant obtained after gentrification was then used for HDL-cholesterol estimation according to the method of Siedel et al. [18], for total –cholesterol estimation previously described.

**Estimation of VLDL-C Concentration**
The VLDL-cholesterol concentration was obtained by dividing the serum triglyceride concentration by 5. This factor of 5 is based on the understanding that in fasting subjects with triglyceride concentration of 400mg/dl, the VLDL to total plasma triglyceride ratio is fixed relatively at 1:5.

$$\text{VLDL-cholesterol (mg/dl)} = \frac{\text{Triglyceride}}{5}$$

**Estimation of LDL-Cholesterol Concentration**
By the Friedewald's relationship, LDL-cholesterol is derived from the difference between the total serum cholesterol and sum of HDL-cholesterol and VLDL-cholesterol.

$$\text{LDL-cholesterol} = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C}).$$

**Atherogenic Index**
Atherogenic index was obtained using the formula below:

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

**2.6 Haematological Parameters Estimation**
Blood samples were analyzed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK), with standard calibration, according to the manufacturer's instructions for analysis of human blood and accurately programmed for the analysis of red blood cell (RBC) count, total white blood cell (WBC) count, hemoglobin (Hb) and packed cell volume (PCV).

**2.7 Statistical Analysis**
All data are expressed as the mean ± SEM. One - way ANOVA was used for analysis, followed by least square difference (LSD) post hoc, using SPSS software version 17.0. Significant difference was employed at $P < 0.05$.

## RESULTS

**Effects of Edible Vegetable Oils on Lipid Profile**
Table 2 shows the serum lipid profile of albino rats fed with experimental diets. The mean TC in the different experimental groups were 105.71 ± 1.21, 105.57 ± 0.97, 102.24 ± 1.83, 91.00 ± 1.23, 91.00 ± 1.23 and 95.00 ± 0.26 mg/dl for control, RPO, PKO, CCO, SBO and SSO respectively. Serum TC concentration was significantly ($P < 0.05$) lower in CCO, SBO and SSO treated group, compared with control and RPO group.

Serum total triglyceride concentration in the different experimental groups were 108 ± 1.14, 82.50 ± 2.43, 84.86 ± 5.88, 84.02 ± 2.96, 75.00 ± 0.28 and 97.00 ± 0.57 mg/dl for control, RPO, PKO, CCO, SBO and SSO respectively. Total triglyceride concentration was significantly ($P < 0.05$) reduced in all five (5) groups treated with edible oils, compared with control. It was also significantly ($P < 0.05$) lower in SBO group, compared with SSO group.

Serum concentrations of very low density lipoprotein (VLDL-C) in the different experimental groups were 22.03 ± 0.30, 16.20 ± 0.44, 17.40 ± 0.56, 16.80 ± 0.53, 15.00 ± 0.06 and 15.80 ± 0.14 mg/dl for control, RPO, PKO, CCO, SBO and SSO group respectively. Serum concentration of VLDL-C was significantly ($P < 0.05$) reduced in RPO, PKO, CCO, SBO and SSO group, compared with control.

Serum concentrations of high density lipoprotein (HDL-C) for control, RPO, PKO, CCO, SBO and SSO group was 18.43 ± 0.05, 30.80 ± 0.49, 37.05 ± 0.42, 39.96 ± 1.81, 38.50 ± 1.33 and 42.53 ± 0.56 mg/dl respectively. HDL-C was significantly ($P < 0.05$) increased in all the edible oils treated groups, compared with control.

The serum concentration of low density lipoprotein (LDL-C) for control, RPO, PKO, CCO, SBO and SSO group was 63.87 ± 1.22, 63.64 ± 0.28, 58.35 ± 0.99, 34.30 ± 0.73, 40.10 ± 0.4 and 36.03 ± 0.89 mg/dl. Serum concentration of LDL-C was significantly ($P < 0.05$) reduced in PKO, CCO, SBO and SSO groups, compared with control and RPO.

Atherogenic index (AI) for control, RPO, PKO, CCO, SBO and SSO group was 3.47 ± 0.04, 2.07 ± 0.01. 1.57 ± 0.01, 0.86 ± 0.05, 1.04 ± 0.01 and 0.85 ± 0.01 respectively. AI was significantly ($P<0.05$) lower in the edible oils fed groups, compared with control.

**Effects of Edible Vegetable Oils on Haematological Parameters**

Table 3 shows the haematological indices of albino rats fed with the different edible oils. The white blood cell (WBC) count recorded were 9.93 ± 0.09, 8.23 ± 0.18, 5.77 ± 0.25, 12.17 ± 0.42, 8.67 ± 0.37 and 10.50 ± 0.03 µ/L for control, RPO, PKO, CCO, SBO and SSO respectively. Total WBC count was significantly ($P < 0.05$) reduced in RPO and PKO treated groups, compared with control. It was also significantly ($P < 0.05$) increased in CCO and SSO groups, compared with control, RPO, PKO and SBO groups.

Red blood cell (RBC) count recorded were 7.13 ± 0.14, 7.92 ± 0.11, 8.03 ± 0.23, 8.40 ± 0.14, 9.00 ± 0.09 and 8.19 ± 0.12 µ/L for control, RPO, PKO, CCO, SBO and SSO respectively. RBC count was significantly ($P < 0.05$) increased in all edible oil fed groups, compared with control.

The haemoglobin concentrations recorded were 14.73 ± 0.06, 18.93 ± 0.12, 15.33 ± 0.08, 18.36 ± 0.14, 16.83 ± 0.14 and 17.13 ± 0.28 g/dL for control, RPO, PKO, CCO, SBO and SSO respectively. Haemoglobin concentration was significantly ($P < 0.05$) increased in RPO, CCO, SBO and SSO groups, compared with control.

The percentage packed cell volume (PCV) for control, RPO, PKO, CCO, SBO and SSO group was 36.69 ± 0.07, 45.20 ± 0.20, 50.13 ± 0.54, 42.90 ± 0.51, 54.67 ± 0.29 and 47.70 ± 1.41 % respectively. Percentage PCV was significantly ($P < 0.05$) increased in RPO, PKO, CCO, SBO and SSO groups, compared with control.

**Table 2. Serum lipid profile of the different experimental groups (mg/dl)**

| Parameters | Control | RPO | PKO | CCO | SBO | SSO |
|---|---|---|---|---|---|---|
| Total cholesterol | 105.71 ±1.21 | 105.57 ±0.97 | 102.24 ±1.83* | 91.00 ±1.23*,b | 94.40 ±0.49* | 95.00 ±0.26* |
| Total Triglyceride | 108.86 ±1.14 | 82.50 ±2.43* | 84.86 ±5.88* | 84.02 ±2.96* | 75.00 ±0.28*,a | 97.00 ±0.57* |
| Very low density lipoprotein | 22.03 ±0.30 | 16.20 ±0.44* | 17.40 ±0.56* | 16.80 ±0.53* | 15.00 ±0.06* | 15.80 ±0.14* |
| High density lipoprotein | 18.43 ±0.50 | 30.80 ±0.49* | 37.05 ±0.42* | 39.96 ±1.81* | 38.50 ±1.33* | 42.53 ±0.56* |
| Low density lipoprotein | 63.87 ±1.22 | 63.64 ±0.28 | 58.35 ±0.99* | 34.30 ±0.73* | 40.10 ±0.4* | 36.03 ±0.89* |
| Atherogenic Index (AI) | 3.47 ±0.04 | 2.07 ±0.01* | 1.57 ±0.01* | 0.86 ±0.05* | 1.04 ±0.01* | 0.85 ±0.01* |

**P < 0.05 vs control, a = P < 0.05 vs SSO, b = P < 0.05 vs RPO & PKO, values are mean ± SEM. n = 6.*

**Table 3. Haematological parameters of the different experimental groups**

| Parameters | Control | RPO | PKO | CCO | SBO | SSO |
|---|---|---|---|---|---|---|
| Total white blood cells (µ/L) | 9.93 ±0.09 | 8.23 ±0.18* | 5.77 ±0.25* | 12.17 ±0.42* | 8.67 ±0.37 | 10.50 ±0.03* |
| Red blood cells (µ/L) | 7.13 ±0.14 | 7.92 ±0.11* | 8.03 ±0.23* | 8.40 ±0.14* | 9.00 ±0.09* | 8.19 ±0.12* |
| Haemoglobin (g/dL) | 14.73 ±0.06 | 18.93 ±0.12* | 15.33 ±0.08* | 18.36 ±0.14* | 16.83 ±0.14* | 17.13 ±0.28* |
| Packed cell volume (%) | 36.69 ±0.07 | 45.20 ±0.20* | 50.13 ±0.54* | 42.90 ±0.51* | 54.67 ±0.29* | 47.70 ±1.41* |

**P < 0.05 vs control, values are mean ± SEM. n = 6.*

## DISCUSSION

The amount and type of fat contained in a diet has long been linked with the risk of Coronary Heart Disease (CHD), with saturated fats being adverse while polyunsaturated fats being protective factors.

Lipid profile assessment showed that serum TC was lowered in the edible oil fed groups, compared with control, with CCO fed group being lowest. Consequently, TG, VLDL-C and LDL-C were significantly reduced in the edible oils fed groups, compared with control. The lowest concentrations of VLDL-C and LDL-C were recorded in SBO and CCO respectively. HDL-C concentration in the edible oil fed groups was significantly higher than control, with SSO fed animals having higher values, compared to the other edible oil fed groups, an indication of a beneficiary effect of the oil. Consequently, atherogenic index (AI) showed that the edible oil fed groups have lower risk of developing coronary artery disease (CHD), compared with control.

Ide et al. [20] had earlier reported that edible oils are beneficial because they contain a variety of other chemical compounds which are known anti-oxidants that block oxidative damage, implicated in atherosclerosis and also reduce blood pressure, thus preventing hypertension. The protective effect of HDL-C is most widely attributed to its key role in mediating the reverse cholesterol transport from the peripheral tissues to the liver for re-utilization [21].

Saturated fatty acids usually increase the HDL-C concentration, which has been associated with increased Lecithin cholesterol acyl transferase [22]. It should be noted that oxidation of LDL-C is a risk factor for atherosclerosis and coronary heart disease. Since HDL-C enhances the inhibition of LDL-C oxidation [23,24,25], one can conclude that the edible oils are beneficial in preventing coronary heart disease.

Haematological investigations provide information on the general state of blood and the reticulendothelial system. In this study, some variations in hematological indices were observed following the feeding of rats on diets supplemented with different edible oils. RPO and PKO fed rats showed a lower WBC count, compared with control, CCO, SBO and SSO, while CCO fed group showed an increased WBC count, compared with control. Consistent with the findings of Abdul-Rahman et al. [26], WBC count was significantly increased in SSO fed group, compared with control. RBC count was increased in the edible oil fed groups, compared with control. Consequently, haemoglobin concentration and % PCV was increased in the different edible oils treated groups, compared with control. Haemoglobin concentration was highest in SBO fed group, consistent with the fact that RBC count was highest in the same group.

## CONCLUSION

We therefore conclude that the edible oils used in this study reduced the risk of CHD by reducing serum cholesterol concentrations, with CCO, SBO and SSO being more beneficial as serum cholesterol concentrations were lowest in these groups than RPO and PKO. Also, the edible oils used for this study enhances erythropoiesis, but RPO, PKO and SBO are detrimental to leucopoiesis.

## REFERENCES

[1] Navab M, Berliner J. A., Watson A. D., Navab M, Berliner J. A., Watson A. D. *Arterioscler Thromb Vasc Biol*. **1996**;16:831–42.
[2] Beckman J. A., Creager M. A., Libby P. *JAMA*. **2002**;287(19):2570–81.
[3] Gordon, L., Ragoobirsingh, R., Morrison, Y. A., Choo-Kang, E., McGrowder, D., Martorell, E. *J Lab Physicians*. **2010**;2(1):25–30.
[4] Mantel-Teeuwisse, A. K., Kloosterman, J. M., Maitland-van der Zee, A. H., Klungel, O. H., Porsius, A. J., de Boer, A. *Drug Saf*. **2001**;24(6):443-56.
[5] Romon, M., Nuttens, M. C., Thaeret, N. *Metabolism.* **1995**;44(9): 1139-45.
[6] Simon, J. A., Fong, J., Bernert Jr, J. T., Browner, W. S. *Stroke.* **1995**;26(5) 778-82.
[7] Jefcoate, C. R., McNamara, B. C., Artemenko, I., Yamazaki, T. *J Steroid Biochem Mol Biol.* **1992**;43(8):751-67.
[8] Edem, D. O. *Plant Food Nutrition*. **2002**;57:157-165.
[9] Bayorh, M. A., Abukhalaf, I. K., Ganafa A. A. *Asia Pacific Journal of Clinical Nutrition.* **2005**;14: 325-339.
[10] Zhang, J. C. R., Wang, A. N., Xue, L., Ge, K. V. *Biomedical and Environmental Science*, **2003**;16:348-354.
[11] Ascherio, A., Katan, M. B., Zock, P. L., Stampfer, M. J., Willet, W. C. *New England Journal of Medicine.* **1994**;340:1994-1998.
[12] Weiss, C., Maker, H. S., Lehrer, G. M. *Analytical Biochemistry*. **1980**;106:512-516.
[13] Hierholzer, J. C., Kabara, J. J. *Journal of Food Safety*. **2004**;4:1-12.
[14] Ivanov, D. S., Lević, J. D., Sredanović, S. A. *Journal of the Institute for Food Technology in Novi Sad*. **2010**;37 (2):65–70.
[15] Utsunomiya, T., Shimada, M., Rikimaru, T., Hasegawa, H., Yamashita, Y., Hamatsu, T., Yamasaki, M., Kaku, S., Yamada, K., Sugimachi, K. *Hepatogastroenterol.* **2003**;50:1609–1613.
[16] Wu, W. H., Kang, Y. P., Wang, N. H., Jou, H. J., Wang, T. A. *J Nutr*. **2006**;136:1270–1275.
[17] Ohwada, K. *Experimental Animals.***1986**; 35(3):353-5.
[18] Siedel, J., Hagele, E. O., Ziegenhorn, J., Wahlefeld, A. W. *Clin Chem.* **1983**;29:1075-1080.
[19] Negele, J. C., Dotosn, D. G., Liu, W., Putkey, J. A. *Journal of Biological Chemistry*. **1992**;276: 825-832.
[20] Ide, T., Ashakumary, L., Tahashi, Y., Kushiro, M., Fukude, N., Sugaro, M. *Biochemical Biophysical Acta*. **2001**;1534:1-13.
[21] Eckarstein, V., Nofer, J. R., Assmann, G. *Arterioscler, Thromb, Vasc Biol.* **2002**;21:13–27.
[22] Mensink, R. P., Katan, M. B. *Arterioscler Thromb*. **1992**;12:911 – 9.
[23] Diaz, M. N., Frei, B, Vita, J. A., Keaney, J. F. *N Engl J Med*. **1997**;337:408– 16.
[24] Nofer, J., Kehrel, B., Foker, M. *Atherosclerosis*. **2002**;161:1– 16
**[25]** Dillon, S. A., Burmi, R. S., Lowe, G. M., Billlington, D., Rahman, K. *Life Sci*. **2003**;21:1583–94.
[26] Abdul-Rahman, S. Y., Abdulmajeed, A. F., Alkatan, M. M. *Iraqi Journal of Veterinary* Sciences. **2009**:23(1):25-28.