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Study on Biochemical Indices of Liver Function Tests of Albino Rats Supplemented with Three Sources of Vegetable Oils

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ABSTRACT: Biochemical and histological analysis were conducted to determine the effects of palm oil, groundnut oil and coconut oil on the liver condition of albino rats. Thirty-two albino rats were divided into four groups of eight rats each and were fed with 90% rats chow supplemented with 10% of palm oil, coconut oil and groundnut oil for six weeks along with water *ad libitum*. Biochemical indices of liver function determined include serum total protein, albumin, conjugated and total bilirubin, aspartate aminotranferases (AST), alanine aminotransferases (ALT), and alkaline phosphatase (ALP) activities. Liver weight, body weight, feed intake and faecal output were also determined. Results show increase in liver weight, serum total protein, albumin, total bilirubin, ALT (in rats fed with palm and groundnut oil-based diet), AST (in rats fed with coconut oil-based diet only) as well as ALP compared with control. Histopathological examination show mild inflammation of the liver. These results therefore, indicate a compromise in liver of rats administered 10% oil - based diet.
Key words: Oil, Liver, Biochemical, Histopathological, Compromise.

INTRODUCTION

Fats and oils play important roles in the body, apart from imparting desirable taste and flavours to food, they are energy rich substances that serve as a major source of fuel for the body's metabolic processes. In addition, fats and oils are sources of essential fatty acids and carriers of fat soluble vitamins. Dietary fats and oils are mainly composed of triacylglycerols and cholesterol. Fats and oils which contain more unsaturated fatty acids are particularly susceptible to oxidation, intake of food containing oxidized lipid increased the concentration of secondary peroxidation products in the liver (Slater, 1972). However, fats and oils which contain high levels of saturated fatty acids increase blood cholesterol levels and hence the risk of cardiovascular disease (Heber *et al.,* 1992; Sundram *et al.,* 1995).

Coconut oil is a colourless to pale brownish yellow oil, rich in saturated fats containing medium chain fatty acids. It can be used in infant milk formulation to supply fats. Some studies have suggested that coconut oil is a chief source of myristic acid which has been attributed with the high cholesterolemic effect of coconut oil (Heber *et al.,* 1992; Ng *et al.,* 1992; Sundram *et al.,* 1995). Groundnut oil is non drying edible oil, the low level of linoleic acid present is said to be responsible for the excellent flavor stability of groundnut oil (Ayres, 1983). Groundnut oil contains about 50% monounsaturated, 30% polyunsaturated and 20% saturated fatty acids (Abaelu *et al.,* 1991).

Palm from the fruits of *Elaeis guineensis* is the cheapest and commonly used oil in Nigeria. It is used either in its fresh or thermally oxidized (bleached) form. Thermally oxidized palm oil is different from the fresh palm oil because of the presence of free radicals released as a result of heating (Owu *et al.,* 1998). The fresh palm oil contains about 50% saturated and 50% unsaturated fatty acids, it also contains vitamins A, E and carotenes which are antioxidants (Ghafourunisa, 1995).

Workers such as Ahmad *et al.*, (2007), studied the effects of beef fat, fish fat and soybean oil on the weights of the liver, kidney and heart of rats and reported significant increases in the liver and heart weight compared with the control. Monserrat *et al.,* (1995), reported a dose dependent protective effect of coconut oil on renal necrosis occurring in rats. In addition, Edem and Akpanabiatu (2006) reported a dose effect relationship between the amount of oil in the diet and activities of some enzymes such as lipase, alkaline phosphatase (ALP) and alanine transaminases (ALT). This work therefore, investigates the effects of 10% edible oils on some biochemical parameters of liver function.

MATERIALS AND METHODS

This work was conducted in the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin city, Nigeria.

Source of Materials/Animals

The rats (Weighing 190-210 g) used for this study were obtained from Ambrose Alli University Ekpoma, Edo State, Nigeria. The feed used (rats chow) were products of Edo feed and flour Mills, Limited, Ewu, Edo state. Palm oil was obtained from Palm oil Research Company (PRESCO), Edo State, and sudan IV was obtained from a chemical store, Lagos state, Nigeria.

Treatment of Animals

Thirty two albino rats were divided into 4 groups of eight rats each. They were acclimatized for 2 weeks on rats' chow only. Group 1 (control) was given 100% rats' mash, while the other groups were given 90% rats' chow supplemented with either palm oil (group 2), coconut oil (group 3) and groundnut oil (group 4). The animals were given these diets for six weeks along with water *ad libitum*. Weekly measurements of weight were recorded. Feed intake and faecal output were also recorded daily.

Collection of Blood and Liver Samples

The rats were subjected to an overnight fast after which they were anaesthesized and blood collected by cardiac puncture into sterile containers without anticoagulant. The liver was excised, blotted dry and weighed and placed in 10% formalin for histopathological analysis.

Analytical Techniques

Biochemical analysis were carried out to determine the serum concentrations of total protein, albumin, conjugated and total bilirubin, and the activities of liver enzymes such as AST, ALT and ALP using diagnostic kits (Quimica Clinica Applicada, S. A. Spain). Total protein was determined by the Biuret method (Peters, 1968), albumin by the bromocresol green method (Doumas *et al.,* 1971), bilirubin was estimated by the method described by Jendrassik and Grof (1938). Alanine and aspartate aminotransferases were determined based on the colourimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957), alkaline phosphatase by the phenolphthalein monophosphate method (Babson, 1965).

Histopathological examination were done using the method of Humason (1962). The liver tissues were fixed in 10% neutral formalin, dehydrated embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical Analysis

All data were expressed as mean± SEM. One way analysis of variance was used to test for difference among the all the groups. Duncan's multiple range test was used to test for significant differences among the means. A p – value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Weight gain did not differ significantly ($p>0.05$) between the test rats and the control. Liver size was significantly ($p<0.05$) increased in all the test rats compared with control (Table 1). Similarly, total protein, albumin, total bilirubin concentration and alkaline phosphatase activity were significantly increased in all the test rats compared with the control (Table 2). However, while conjugated bilirubin and ALT levels were significantly increased in rats fed coconut oil based diets, they were not significantly altered in rats fed coconut oil based diets. AST levels were significantly ($p<0.05$) increased in rats given coconut oil based diet while it remained unaltered in others.

Table 1: The Effect of Palm, Groundnut and Coconut Oil-Based Diets on Body Weight, Feed Intake, Faecal Output (g) and Liver Weight (g).

Diet	Weight Gain	Feed Intake	Faecal Output	Liver Weight (g)
Palm oil	27.9± 8.5	142.4± 4.2*	58.04± 6.5	5.7± 1.2*
Groundnut oil	26.5± 4.2	130.5±3.6	53.7±3.2	5.5 ± 0.8*
Coconut oil	27.5±4.4	123.1±2.4	45.9±2.8	6.3 ± 1.4*
Control	26.0±3.8	128.3±2.6	53.7±2.4	5.0 ± 0.8

Results are expressed as mean ± SEM (n=8). *Significant at $p<0.05$ compared with control using analysis of variance.

Table 2: The Effects of Palm, Coconut and Groundnut Oils on some Biochemical Parameters

Diet	Control	Palm oil	Coconut oil	Groundnut oil
Total Protein (g/L)	14.50±0.04	20.80± 0.32*	20.25±0.19*	28.20±0.35*
Albumin (g/L)	1.80±0.01	4.40±0.17*	6.10±0.01*	5.20± 0.2*
Conj. Bilirubin (µmol/L)	0.34±0.01	3.08±0.01*	0.86±0.01*	2.74±0.02*
Total Bilirubin (µmol/L)	7.18±0.01	16.42±0.05*	13.0±0.04*	10.94±0.02*
ALT (µ/L)	18.2±2.17	29.17±3.65*	15.0±1.00	22.3±4.5*
AST (µ/L)	22.0±0.76	27.0±0.49	31.8±3.59*	27.8±1.92
AST (µ/L)	15.22±2.2	24.16±0.09*	24.40±2.50*	24.66±1.80*

Results are expressed as mean ± SEM (n=8). * Significant at p<0.05 compared with control with control using analysis of variance.

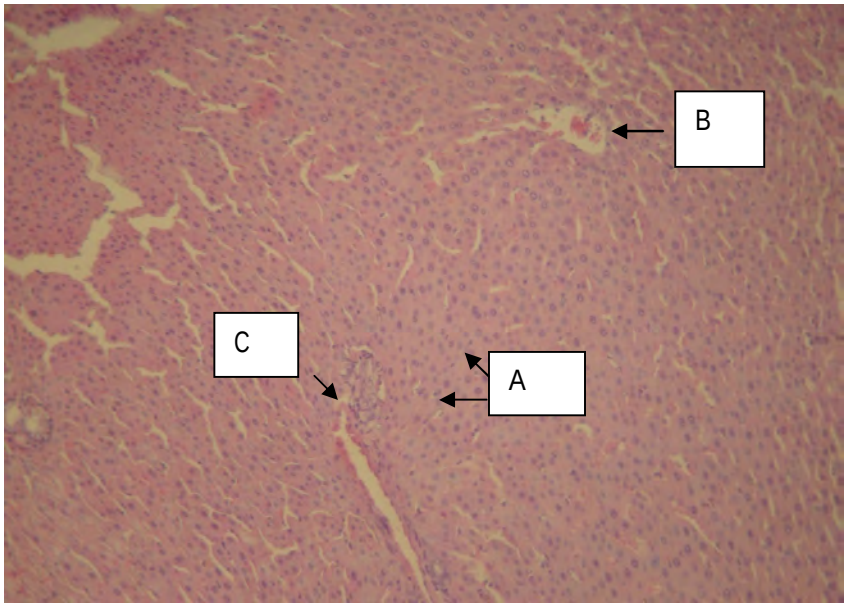

Plate 1: Microscopic representation of the liver of control rats. Normal liver with hepatocytes (A), central vein B, and portal triad (C) {X40 H & E}

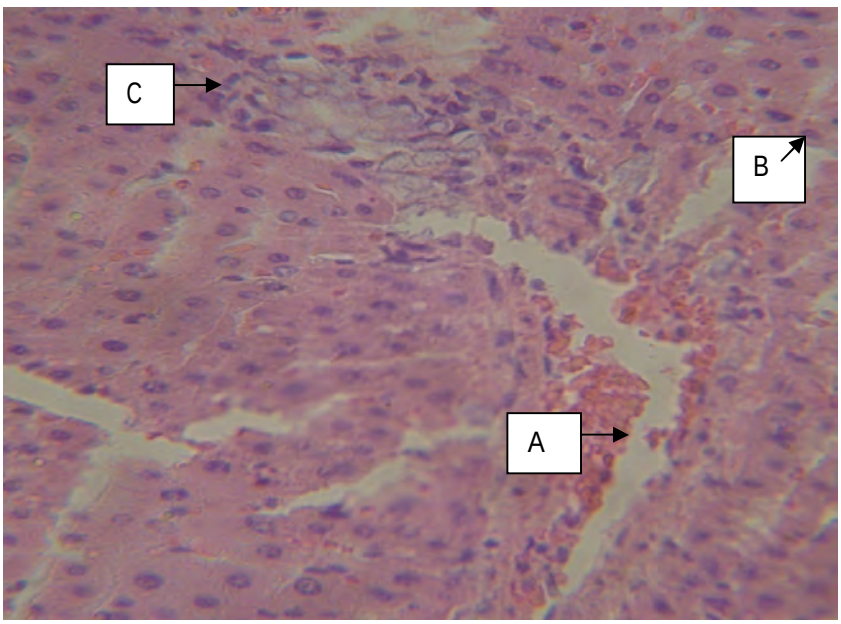

Plate 2: Microscopic representation of the liver of rats administered with 10% palm oil based diets (X40 H & E) showing mild portal (A) and sinusoidal (B) congestion and mild periportal infiltrate of chronic inflammatory cells (C)

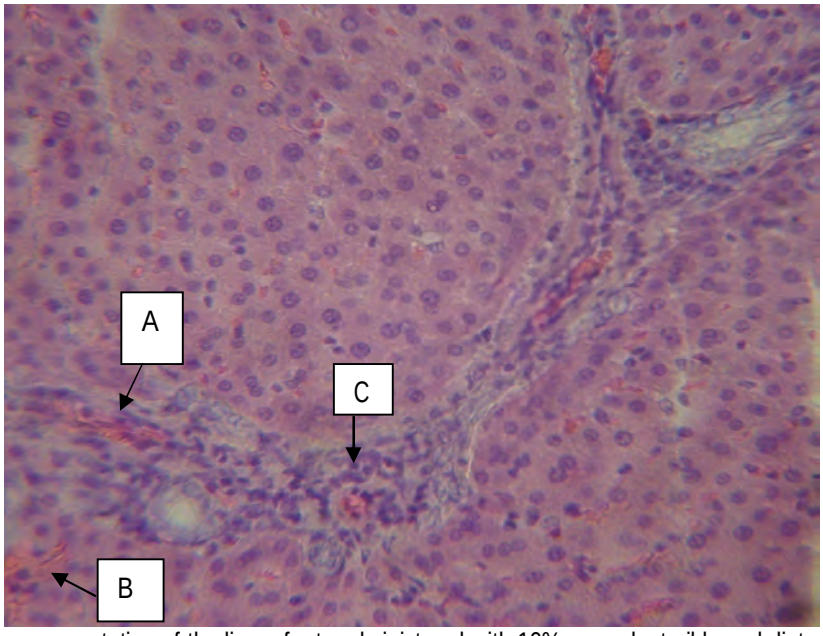

Plate 3: Microscopic representation of the liver of rats administered with 10% groundnut oil based diets showing mild portal (A) and sinusoidal (B) congestion and mild, periportal infiltrates of chronic inflammatory cells (C) (X40 H & E)

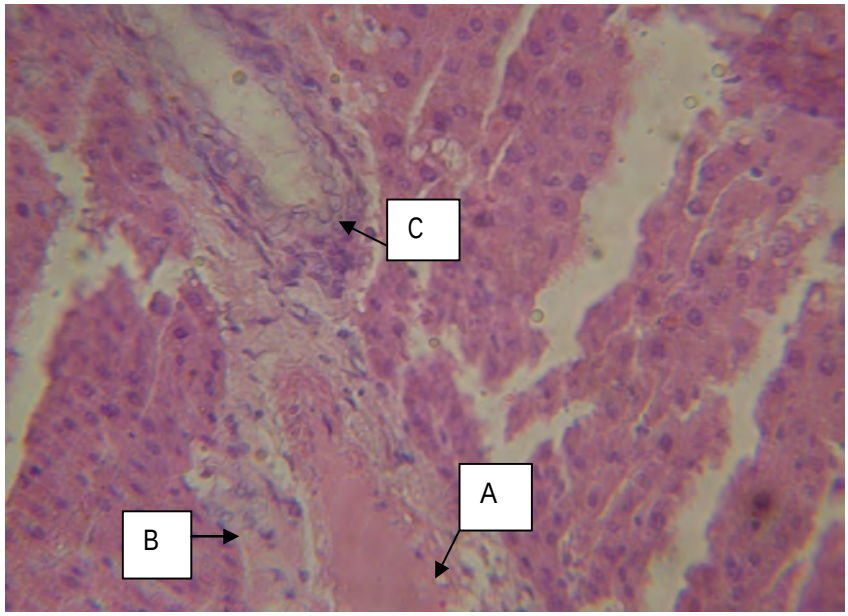

Plate 4: Microscopic representation of the liver of rats fed with 10% coconut oil based diet, showing mild portal congestion (A), vascular dilation (B) and mild periportal infiltrate of chronic inflammatory cells (C) (X40 H & E)

Plates 1-4 show the results of the histological examination of the livers of control and test rats. The liver of control rats (Plate. 1) showed normal hepatocytes with prominent central vein and portal triad. Histological evaluation of the liver of rats fed palm oil, groundnut oil and coconut oil - based diets showed congestion (increase in blood flow) in the portal region as well as the sinusoids. Dilation (widening) of the blood vessels of the portal zone of the liver was observed in those rats administered coconut oil diet.

There was no significant difference ($p>0.05$) in weight gain and the amount of faecal output between the control and the test rats. Feed intake was significantly increased in rats given palm oil based diets compared with control. However, there were significant ($p<0.05$) increases in organ weight compared with control. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an indication of the toxic effect of that chemical (Orisakwe *et al.,* 2003).

The result of body weight and increased organ weight is consistent with the report of Ahmad *et al.* (2007), however, Saha *et al,* (2005), observed that a significant difference exists on the effects of different edible oils on growth performance. Any change in the concentration of serum protein and albumin indicate a change in the normal liver functions (Ahmed *et al.,* 1992). The increased protein and albumin levels associated with the test rats indicate impairment in the normal function of the liver. With the exception of those rats fed coconut oil based diet, increases were observed in the levels of conjugated bilirubin.

Many diseases of the liver are accompanied by Jaundice, a yellowing of the eyes and skins caused by increased levels of bilirubin in the system. Bilirubin accumulate from the breakup of haemoglobin present in red blood cells. During normal function, the liver removes bilirubin from the blood and excretes it through bile. Increases in total bilirubin and conjugated bilirubin observed, compared with the control rats also indicates a compromise in the normal function of the liver in rats administered 10% oil- based diets.

The enzymatic activity of alanine (ALT) and aspartate (AST) aminotransterases and alkaline phosphatase were studied to evaluate liver malfunctions. Liver enzymes levels are usually raised in acute hepatoxicity, but tend to decrease with prolonged intoxication due to damage to the liver (Obi *et al.,* 2004). ALT levels increased only in those rats fed palm oil and groundnut oil- based diets compared with the control, while AST levels increased only in those rats administered coconut oil based diet.

Histological evaluation of the liver of rats fed palm oil, coconut oil and groundnut oil based diets revealed increased activities in the blood vessels of the liver as well as inflammation especially around the portal zone. The control rats however had normal liver, no inflammation. The results of this study reveal that 10% oil based diets might produce deleterious effects on the liver of rats. Further work needs to be done however to ascertain whether reducing the amount or quantity of edible oils below the 10% level would ameliorate this effect.

REFERENCES

Abaelu, A.M., Okochi, V.I., Oyesile, O.O., Akinyele, J.O. and Akinrimisi, W.E.O. (1991). Nigerian dietary oils and transport of amino acids in rats. Nigeria. *Journal of Physiological Science,* **7:** 32 -37.

Ahmad, N., Majumder, S., Miah, M.A. and Uddin, M.J. (2007). Effects of edible fats and oils on the body weight gain and on weights of some selected organs in rats removing the impact of unequal feed intake. *Bangladesh Journal Veterinary Medicine,* **5 (1&2):** 107-110.

Ahmed, M., Saeed, M.A. Alam, H. and Ashgar, Z. (1992). Biological Studies of indigenous medicinal plants II: Effects of Aplotaxis lappa Dcne. On various parameters of liver metabolism in rabbits. *Journal of Islamic Academy of Science,* **5***:51-56.*

Ayres, J.L. (1983). Peanut oil. *Journal of the American Oil Chemists Society,* **60(2):** 357-59

Babson, L.A. (1965). Estimation of Alkaline phosphatase activity. *Clinical Chemistry,* **11**:789.

Doumas, B.T., Watson, W.A. and Briggs, A.G. (1971). Albumin Estimation*. Clinica Chimica Acta*, **31**:87-96.

Edem, D.O. and Akpanabiatu, M.T. (2006). Effects of palm oil containing diets on enzyme activities of rats. *Pakistan Journal of Nutrition,* **5(4):** 301-305.

Ghafourunissa, K . (1995). Nutritional and Health implications of palm oil. *Journal of Medical Research,* **102**:233-234.

Heber, D., Ashley, J.M., Solares, M.C., Wang, H.J. and Alfin-Slater, R.B. (1992). The effects of palm oil enriched diet on plasma lipids and lipoproteins in healthy young men. *Nutritional Research,* **12:**553-560.

Humason, G.L. (1962). *Animal Tissue Techniques*. Freeman, W.H. and Co. publishers, London. Pp 57-61.

Jendrassik, L. and Grof, V. (1938). Estimation of direct and total Bilirubin. *Biochemische Zeitschrift,* **297**:81-89.

Monserrat, A.J., Romero, N., Lago, N. and Aristi, C. (1995). Protective effect of coconut oil on renal necrosis occurring in rats fed a methyl deficient diet. *Renal Failure*, **17(5):** 525-37.

Ng, T.K.W, Hayes, K.C., Dewitt, G.T., Jegatheson, M. Satgunasingham, N., Ong, A.S.H., and Tan, D.T.S. (1992). Palmitic and Oleic acids exert similar effects on lipid profiles in normocholesterolemic humans, *Journal of American College of Nutrition,* **11**:383-390.

Obi, E., Orisakwe, O.E., Asomugha, L.A and Udemezue, O.O (2004). The hepatotoxic effect of halofantrine in guinea pigs. *Indian Journal of Pharmaceutical Sciences,* **36(5):**303-305.

Orisakwe, O.E., Hussaini, D.C. and Afonne, O.J. (2003). Testicular effects of sub-chronic administration of Hibiscus sabdariffa calyx

aqueous extract in rats. *Reproductive Toxicology,* **18**:295-298.

Owu, D.U., Osim, E.E. and Ebong, P.E. (1998). Serum liver enzymes profile of wistar rats following chronic consumption of fresh or thermo-oxidized palm oil diets. *Acta. Tropical,* **69:** 65-73.

Peters, T. (1968). Determination of total proteins. *Clinical Chemistry,* **14**:1147-1159.

Reitman, S. and Frankel, S. (1957). Determination of glutamic-oxaloacetic transaminase. *America Journal of Clinical Pathology,* **28:**56-59.

Saha, S.K., Ahmad, N., Majunder, S., Hosain, M.Z and Mials, M.A. (2005). Effects of different edible oils on growth performance, different organ weight and serum transaminases in rats. *Bangladesh Journal of Veterinary Medicine,* **3(1):**79-81.

Slater, T.F. (1972). *Free Radical Mechanisms in Tissue injury*. Pion Ltd London. Pp 21.

Sundram, K., Hayes, K.C. and Siru, O.H. (1995). Both dietary 18:2 and 16:0 may be required to improve the serum LDL/HDL cholesterol ratio in normocholesterolemic men. *Journal of Nutritional Biochemistry,* **4:**179-187.