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Histological Changes And Demonstration Of Glycogen Distribution In The Gonads Of Male Wistar Rats Following Long-Term Ingestion Of Mixed Palm Oil Diets

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ABSTRACT

Histological changes and of glycogen distribution in the gonads of male Wistar rats following long-term ingestion of mixed palm oil diets were investigated. Twenty-four young Wistar rats initially weighing between 60 and 80g were divided into 3 groups (A, B and C) of 8 rats each. Group A rats were fed with thermoxidized palm oil diet, obtained by mixing 15g of thermoxidized palm oil with 85g of rat chow (15% w/w). group B rats were fed the fresh palm oil diet, obtained by mixing 15g of fresh palm oil with 85g of rat chow (15% w/w). group C served as the control, and were given normal rat chow only. The animals were allowed feed and water ad libitum for 14 weeks. The weight of the testes of the group A animals (1.96 ± 0.06) was significantly ($P<0.01$) lower than those of groups B and C [(2.75 ± 0.08) and (2.98 ± 0.06)] respectively. The difference between groups B and C was not significant. There were some discolouration in the testes of the group A animals compared to the other two groups. Histological observation showed mild degeneration of some of the germinal epithelia and disruption of some of the interstitial cells compared to the control. The glycogen deposition was decreased in the group A and B animals compared to the control, with the effect being more pronounced in group A. these results suggest that chronic consumption of thermoxidized palm oil diets may have adverse effect on the testis of Wistar rats.

Keywords: Testis; Wistar rats; Palm oil diets may have adverse effect on the testis of Wistar rats.

Palm oil is a redviscucous liquid, obtained from the fleshy mesocarp of the fruit, produced by the oil palm tree, *Elaesis quineensis*. The two forms of the oil commonly employed preparation of dishes are the fresh form and the thermally oxidized form. The fresh form, which is reddish in colour, is obtained from fruit mesocarp by squeezing and then boiling at low temperatures. The thermally oxidized form is darker in colour and is obtained when the fresh oil is subjected to several rounds of heating at veryhigh temperatures.

Palm oil, like any other oil becomes oxidized when heated at a very high temperature. Thermodixation has a deteriorative effect on dietary oil (Perkins and Van Akkerren, 1965; Peers and Swoboda, 1982; Isong 1988), because there is a concomitant evolution of toxic by-products during the process (Artman, 1969;Dhopeshwarker, 1980). Uncontrolled oxidation results in the formation of peroxides and other products, which are known to be veryreactive, cytotoxic and destructive compounds when ingested into the organism (Plea, 1975; Lake and Water-worth, 1980, Frankel, 1980, Ziombski, 1982).

Several studies have demonstrated the destructive effects of thermoxidized palm oil diets on mammalian systems (Abaelu et al., 1999, Osim et al., 1992, Osim et al., 1994, Osim et al., 194, Isong, 1988, Mesembe, 2002).

Thermally oxidized fat has been reported to adversely and severally affected reproductive capacities. These include functional changes in the testes of rats (Krivenkova and Treschuk, 1978), twin syndrome in female rats, discolouration in the ovary and testes in rats (Giassudin 1975), delay in gestation, reduced fetility, and embryo-fetal toxicity (Isong et al, 1997).

The foregoing serving as a background, this study therefore examines the possible effect of the two forms of diet on the testicular morphology, histology, as well as the distribution of glycogen within the testicular tissue.

MATERIALS AND METHODS

Twenty-four young Wistar rats initially weighing 60-80g and aged between 6-8 weeks were divided into 3 groups (A,B and C) of 8 rats each. The rats in the first two groups form the test groups, while group C rats served as the control. Fresh palm oil of about 20 litres was divided into two equal potions. One part was theremally oxidized as described by Isong (1988), Osim et al., (1992) and Mesembe (2002). The other part was given to the animals in the fresh form, since there are the two common forms of palm oil used for cooking. The following method was used to obtained the oxidized palm oil. Fresh palm oil was heated at 150°C in a stainless steel pot intermittently for five different sessions. Each heating session lasted twenty minutes. At the end of each heating session, the oil was allowed to cool for

five hours. Since the level of palm oil in most West African dishes is about 15% (Umoh, 1972), 15g of the cooled thermally oxidized oil were mixed with 85g of rat feed, and given as the second test diet. The diets were stored in black containers to prevent further oxidation of the oil components. The control group was placed on laboratory rat chow. The chow and water were allowed *ad libitum* fo r14 weeks. The rats were weighed before the commencement of the feeding experiment, and thereafter, the animals were weighed weekly. The rats were kept free from drought at room temperature throughout the feeding period. At the end of the administration of the diets, the animals were sacrificed with chloroform and their testes were excised and placed on cold normal saline. The testes were subsequently blotted dry on filter papers, weighed and preserved in Bouins fluid. The tissues were processed using the Haematoxylin/Eosin (H & E) technique for histological examination and Periodic Acid-schiff (PAS) reaction for glycogen demonstration. Photomicrographs were taken.

Statistical Analysis

Analysis of variance (ANOVA) was used to compare the means; thereafter the student t-test was used to compare the control and experimental values to find the level of significance. All the results were expressed as mean $\pm$ standard error of mean.

RESULTS

The mean body weight gain of the thermoxidized palm oil group was significantly ($p < 0.01$) lower than that for the fresh palm oil and control groups. The weights of the testes in the thermoxidized group (1.96 ± 0.08), and (2.98 ± 0.06), respectively. There was no significant difference between the fresh and control groups. There was a small reduction in the testes sizes in the thermoxidized and fresh palm oil groups compared to the control, with some colourations in the testes. These were more in the thermoxidized tubules cut at different sections, separated by the interstitial cells of Leydig. Within each seminiferous tubules, are cells of spermatogenic series (germinal epithelia) lying on basement membranes, while scattered within are Sertoli cells. The thermoxidized group (fig. 3) showed shrinkage of the germinal epithelia, the seminiferous tubules are almost occluded and separated from one another, indicating the wearing-out of the interstitial cells. The fresh palm oil group (fig. 2) showed some mild degenerated interstitial cells.

The control (fig. 4), shows scattered glycogen, especially within the seminiferous tubules. The fresh and thermoxidized palm oil groups (fig. 6 and fig. 5, respectively) show scanty glycogen deposition compared to the control.

Table 1: Mean body weights and organ weights of rats fed on control diet, fresh and thermoxidized palm oil diets.

GROUP (n =8)	Diet type	Weight gain (g)	Testes weight (g)
A	Thermoxidized Palm oil	$146.25 \pm 1.62^{**}$	$1.96 \pm 0.06^{**}$
B	Fresh palm oil	184.37 ± 1.13^{Ns}	$2.75^{**} \pm 0.08^{Ns}$
C	Control	196.25 ± 1.44	2.98 ± 0.06

Results are presented as mean $\pm$ SEM
** = P<0.01 in comparison with the control group.

DISCUSSION

Chronic consumption of thermally oxidized palm oil diet resulted in little but significant gain in weight of the rats. This may be due to low food and water intake recorded in this study (Osim et al., 1994). The low weight gain may also be a consequene of some tissue wasting (Osim et al., 1994; Mesembe, 2002).

The testis weight was significantlylower in the thermoxidized group compared to those for the fresh palm oil and control groups. This could be as a result of inadequate nutrient supply due to intestinal mucosal damage (Osim et al., 1994). It may also be indicative of some tissue wasting (Osim et al., 1994).

The shrinkage and partial occlusion of the seminferous tubules, as well as, loss of interstitial cells indicate that tissue necrosis occurred. Implicated here are toxic by-products (Artman, 1969, Dhopeshwarker, 1980); peroxides and other reactive and cytotoxic components (Plea, 1975; Lake and water-Worth, 1980; Frankel, 1980; Ziomski, 1982), released from the thermoxidized oil. This may affect the formation of sperm cells and also the production of testosterone which is essential for germ cell development. The development and maintenance of male secondary sexual characteristics are hindered, thus leading to reduced sexual capacities. The reduced distribution of glycogen in the thermoxidized palm oil group indicates that less energy will be available for normal cell function. The results obtained are consistent with reports that oxidized oil is toxic to the reproductive organs (Krivenkova and Treschuk, 1978; Giassundin, 1975; Isong et al., 1997).

In conclusion, one may safely speculate that, chronic consumption of thermoxidized palm oil diets may lead to infertility in males. Therefore, the consumption of this variety of treated palm oil should be discouraged.

REFERENCES

Abaelu A.M, Okochi U.I, Oyesile O.O, Akinyele KJ. O. and akinrimisi E.O (1991). Nigerian Dietary Oil and Transport of amino acids in rats intestine Nig. J. Physiology Sci. 7:32-37.

Artman N.R. (1969). The chemial and biological properties of heated and oxidized fats Adv. Lip. Res. 7:245-330.

Dhopeshwarker G.A (1980). Naturally occurring food toxicants: toxic lipids. Prog. Lip Res. 19: 107-118.

Frankel E.N. (1980). Lipid oxidation, Prog. Lipid Res. 19: 1-22.

Isong E.U. (1988). Biochemical and nutritional studies on rats fed thermally oxidized palm oil. Ph.D Thesis, Department of Biochemistry, University of Calabar.

Igiri A.O., Ibegbu A.O and Osim E.E. (1994). The Morphological and histological changes in the small intestine induced by chronic consumption of palm oil diets in rats. Tropical Journal for Applied Sciences. Vol. 3.

Krivenkova E.M and Treschuk I.J (1978). Morphological and functional investigations or the testis of albino wistar rats after long-term inclusion in their ration of thermallyoxidized fats. Veprosy Pitaniya 6: 59-64.

Lake B. and Waterworrth M. (1980). Food and Nutrition. Mills and Boon. London. Pp . 180-201.

Mesembe O.E. (2002). The effects of fresh and thermoxidized palm oil diets on some haematological indices in the rat: MSc. Thesis, University of Calabar, Nigeria.

Murray R.K., Granner D.K, Mayes P.A and Rodwell V.N. (2000). Harper's Biochemistry 23rd ed. New Jersey, USA. Prentice Hall International Inc. pp. 199 and 594.

Osim E.E, Owu D.U. Isong E., and Umoh I.B. (1992). Influence of chronic consumption of thermoxidized oil on platelet aggression in the rats. Discov. Innov. 4:83-87.

Osim E.E., Isong E.U. and Umoh I.B. (1994). Influence of chronic consumption of thermoxidized and fresh palm poil diets on basal metabolic rate, body weight and morphology of tissues in rats. Discov. Innov. 6: 389-396.

Peers K.E. and Swoboda P.A.T (1982) Deterioration of sunflower seed oil under stimulated frying conditions and during small scale frying of potato chips. J. Sc. Food Agric. 33(4): 389 -395.

Plea R.M. (1975). Protein synthesis and absorption in rats fed on oxidized oil. Practitioner 224; 306 421.

Perkins E. G and Van Akkeren I.A (1965). Heated Fats, IV: Chemical changes in fats subjected to deep frying processes. J.AM Oil Chem. Soc. 42: 161-166.

Umoh I.B. (1972). Change in nutritional value of some Nigerian diets after cooking by certain traditional methods Ph.D Thesis, University of Ibadan, Nigeria.

Ziombski H. (1982). Physical, Chemical and Nutritional changes in heated fats. Changes in nutritive value. Raezinki Panstwowego Zadkladu Higeny, 33(3); 162 -170.

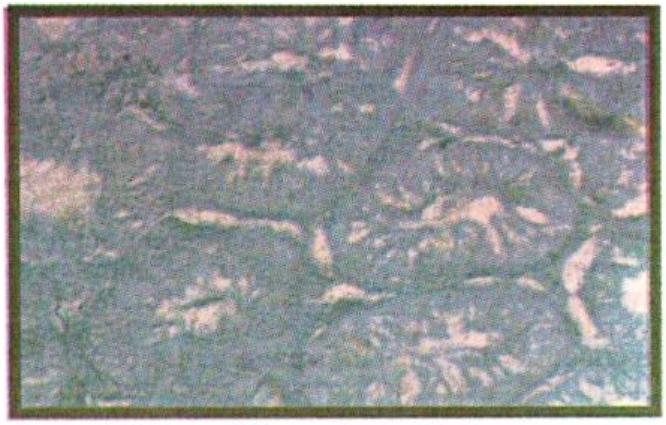

Fig 3: Shrinkage of the germinal epithelia

Fig 4: Control: Scattered glycogen

Fig 1: Seminiferous tubules (Control)

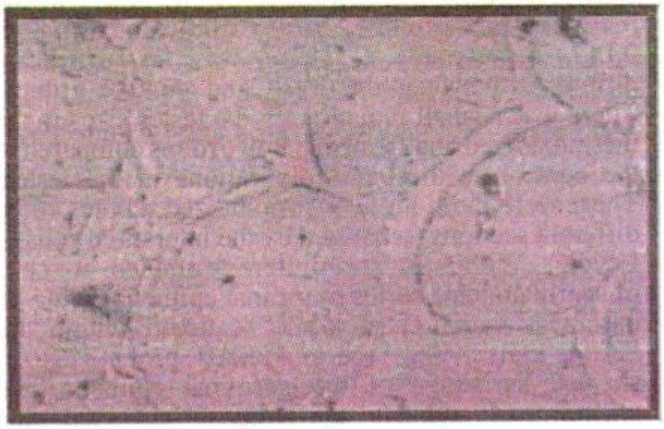

Fig 5: Fresh palm oil treated groups

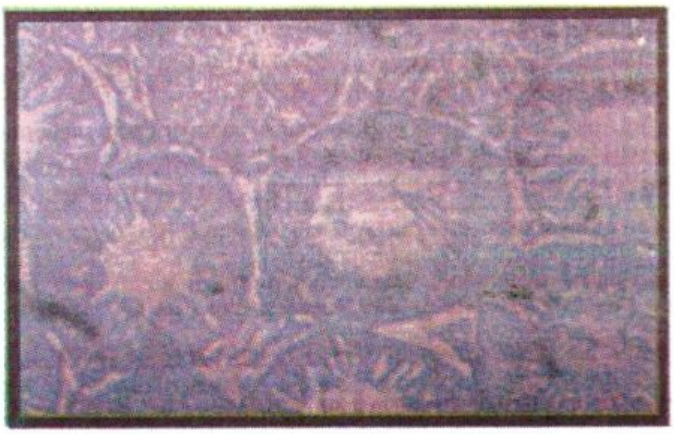

Fig 2: Treated group showing degeneration of intersitial cell

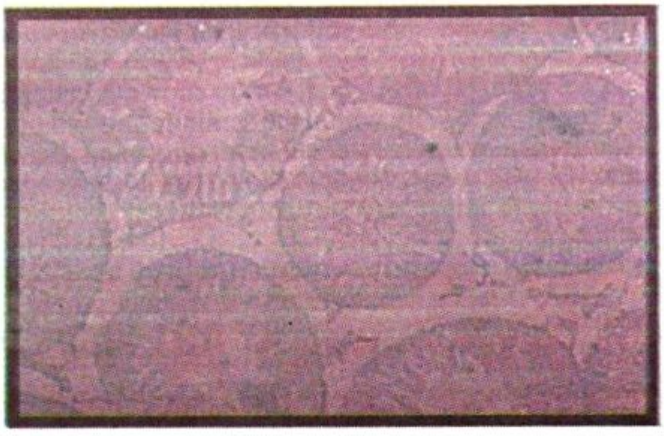

Fig 6: Palm oil treated groups