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Effects of reused high-fat diet on male rat livers: A histological assessment using H&E stain

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Abstract

Fat associated liver conditions may be related to humans consuming foods heated at high temperatures in degraded and reused unstable fats. Consumption of these deep fried foods and the highly oxidized lipids is an emerging public health issue in human populations where the quality of frying fat is neither monitored nor regulated. This study was carried out to histologically assess the effects of reused high fat diets on male rat livers. Eighteen (18) male Sprague Dawley rats were randomly assigned three groups of six rats each and fed a neat 20% high fat diet, a reused 20% high fat diet and a normal rat pellet diet for two months. The animals were sacrificed and their processed livers examined under light microscope. In the study groups' slides; hepatocellular ballooning, microvesicular steatosis, lobular inflammation, portal inflammation and fibrosis were determined. Gross examination of livers indicated macro nodular cirrhosis. Finally, it was observed that 20% reused high fat diet had caused liver steatosis, in the study animals. The Haematoxylin and eosin light microscopy assessment would contribute to clarify some aspects of liver pathogenesis and public health implications of reused fats.

Keywords: *Reused fat, Haematoxylin-eosin, Liver steatosis, Light microscopy, Non alcoholic fat liver disease*

1. Introduction

Frankel *et al*. (1984) observed that when saturated fats are excessively heated under high temperatures for a prolonged time, they undergo decomposition. Chow and Gupta (1994) were able to demonstrate that cooking fats start breaking down producing free fatty acids, diacylglycerols, and monoaclyglycerols that break down further to produce hydro peroxides and finally transformed into polymers that are of high molecular weight. Ingestion of these decomposition products or unstable oxidized lipids causes several alterations in the lipid and fatty acid metabolism that might be of great physiological relevance as suggested by Crnjar *et al*. (1981). Oxidized fats in deep fried foods have been demonstrated to play a highly significant role in the pathogenesis

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of various diseases. Eder (1999). A report of November, 2018 by Euromonitor International titled "edible oils in Kenya" predicts an increased consumption especially in densely populated areas in the country.

The biological and toxicological properties of oxidized fats in study animals include diarrhea, increased liver and kidney mass, induction of cytochrome P-450 activities in the liver and colon, increased cell proliferation in the gastrointestinal tract and induction of oxidative stress as observed by Adams *et al.* (2005), however, they further concluded that the process of histological damage and courses of nonalcoholic fatty liver are unclear. A previous study by Brunt *et al.* (2009) observed that patients who presented with NAFLD had marked lobular inflammation, hepatocellular ballooning, hepatocellular necrosis, lobular inflammation, pericellular fibrosis and portal fibrosis.

This study proposes use of 20% repeatedly used high fat diet model on adult male rats as a possible accelerator and risk factor in pathophysiology of NAFLD, liver cancer and cirrhosis.

Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) Histological grading system was used to assess the stained liver tissues.

2. Materials and methods

2.1. Experimental animals

Eighteen (18) weaned male Sprague-Dawley rats were acquired from the School of Biological Sciences and housed in the animal house of the Department of Medical Physiology, University of Nairobi. At a density of 6 animals per cage, the animals were acclimatized for seven days while being fed on normal rat pellets, (Unga Farm Care (E.A) Ltd, Nairobi. Apendices1.0) and allowed to freely access water by nipple bottles.

2.2. Preparation of reused cooking Fat

Ten kilograms of Frymate™ brand of cooking fat (Pwani Oil Products Ltd) was used. The brand was purchased at a local retail store and handed over to a road side fish vendor in Kayole estate, Nairobi, Kenya. The cooking fat was repeatedly used to deep fry fish by the road side for a period of two weeks. The repeatedly used deep frying fat was then collected and filtered to remove food particles and mixed with the previous collection and stored in a freezer.

Plate 1: Sample of neat cooking fat, (Frymate™ brand)

Plate 2: A road side deep frying fish kitchen establishment

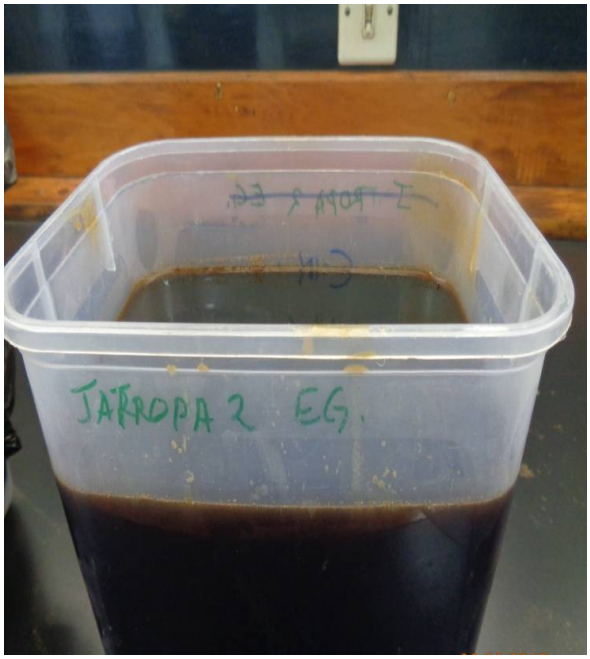

Plate 3: Sample of used cooking far

2.3. Preparation of the experimental diets

The 20% neat high fat and reused high fat diet was prepared by adding 200 g of either neat or reused cooking fat to 800 g of normal rodent pellets. The mixture was then gently heated over low heat for 15 min, allowed to cool and stored in air tight polythene bags for future use.

2.4. Experimental design

After seven days of acclimatization, they were fed appropriate pellet diet *ad libitum*.

Group I: High Fat Diet (HFD),

Group II: Reused High Fat Diet (RHFD),

Group III: Normal rat pellet's diets (ND) from Unga Farm Care (E.A) Ltd, Nairobi. (Control group).

After eight weeks, all animals were sacrificed after an overnight fast by chloroform sedation. Immediately the livers were fixed in 10% neutral buffered formalin overnight and embedded in paraffin wax employing an automatic tissue processor, (Leica TP 1020, Germany). The tissue blocks were sliced with a microtome machine, mounted on microscope slides and stained according to the standard hematoxylin-eosin procedure, (Malatesta, 2016) for examination of general tissue composition and structure.

3. Results

There were no deaths of animals in this study; all groups were used for histological assessment. Histological assessment of the stained slides was done according to non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) Histological grading system and the grades scored for each liver section.

Histological assessment of liver samples from the normal diet group (fig. I); the hepatocytes showed epithelial cell with one or more centrally located round nucleus. There were no morphological changes: normal lobules, lack of pigmentation, absence of congestion, steatosis nor necrosis indicating no hepatic injury.

Histological examination of the HFD study stained liver samples (Figure 3) indicated micro-vesicular steatosis.

Gross observation of the RHFD study liver sample (Figure 4) showed macro-nodular liver cirrhosis in two of the six experimental animals. On histological examination (Figure 2) the stained liver cells in this group exhibited micro-vesicular steatosis in H & E indicating an abnormal accumulation of lipids. It was further observed that the RHFD liver sections showed mild portal inflammation, an indication of cell death and mild hepatocellular ballooning; a key indicator in NASH, Caldwell (2010).

Histological results

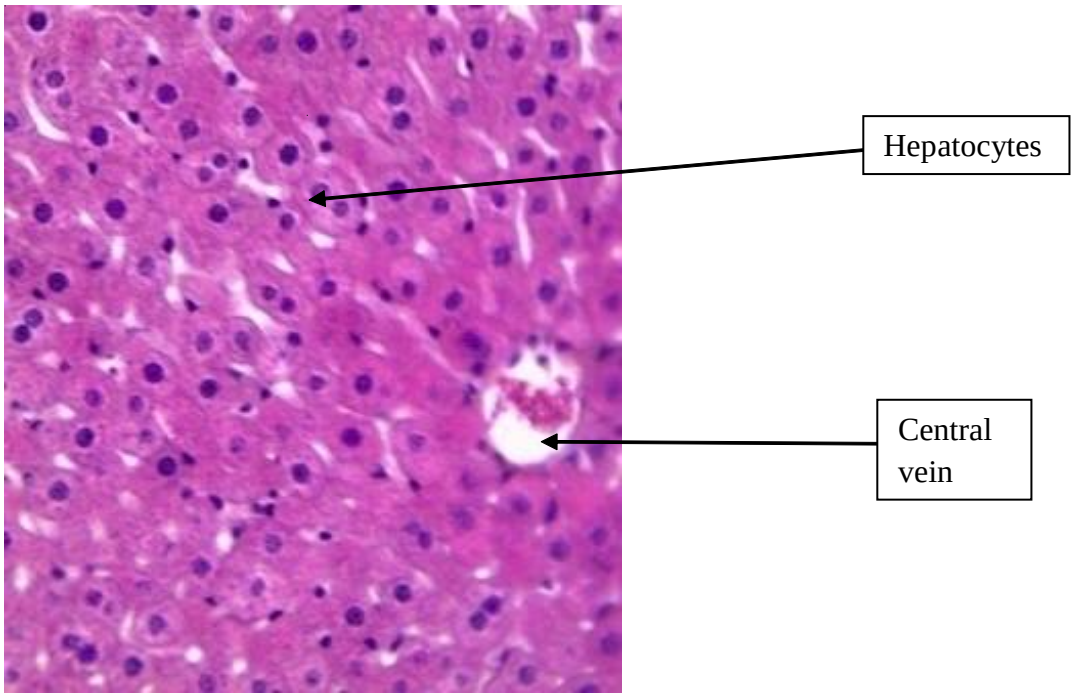

Figure 1: Liver section of normal diet group indicating normal hepatocytes (H) with central nucleus and a Central vein (V). X500 μm

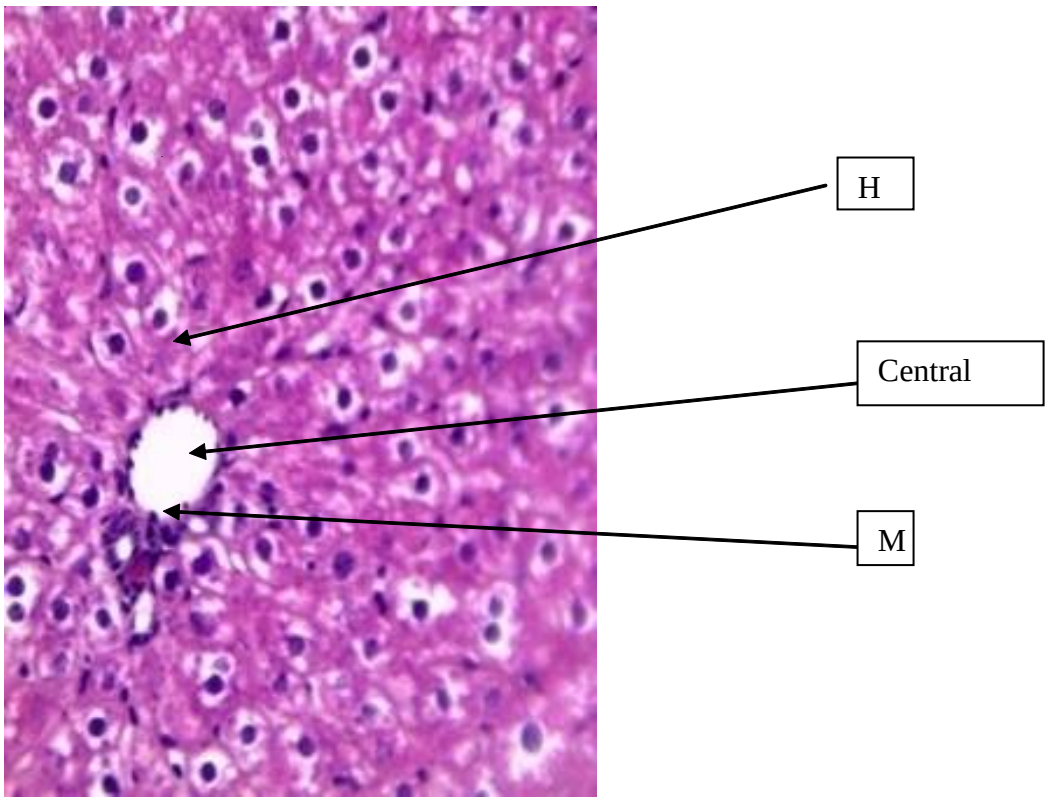

Figure 2: Liver section from RHFD group; portal cell inflamation (M) and mixed steatosis and mild hepatocellular ballooning (HP), X500 μm

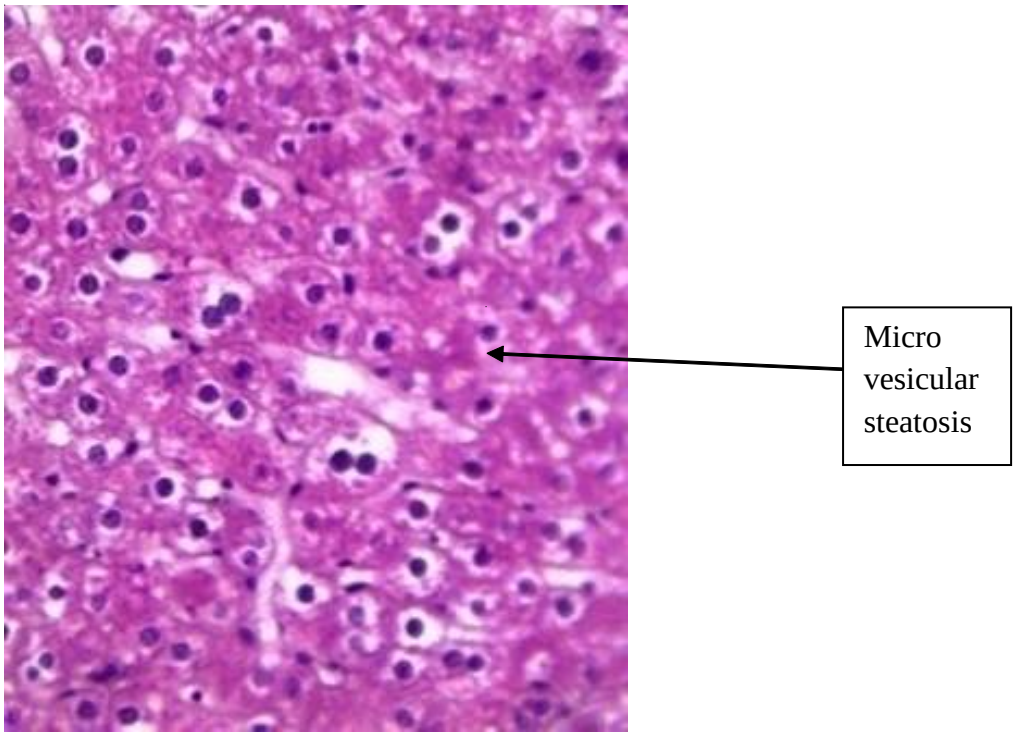

Figure 3: Liver section from HFD group; micro vesicular steatosis (MV). X500 μm

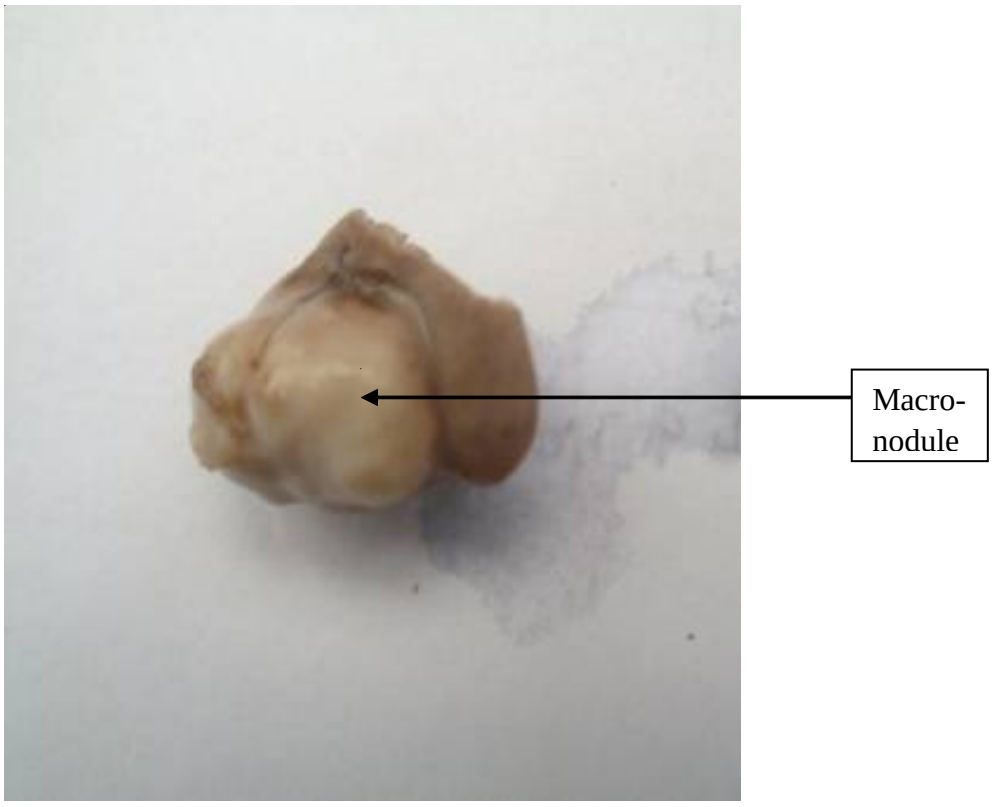

Figure 4: A fixed liver from RHFD group, macro-nodular cirrhosis

Table 1: Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) histological grading system of the stained liver slides

Histological feature	HFD	RHFD	ND
Macro-vesicular steatosis	_	_	_
Micro-vesicular steatosis	2	2	_
Hepatocellular ballooning	_	1	_
Lobular inflammation	_	_	_
Portal inflammation	_	1	_

4. Discussion

In this study, the effects of the reused high fats in the rat liver are attributed to the concentration of secondary lipid peroxidation products and total polar compounds.

Oxidized fats are known to induce oxidative stress that can be mitigated by intake vitamin E as demonstrated by Yoshida and Kajimoto (1989). It's therefore probable that the bioavailability and metabolism of vitamin E among other nutrients is also adversely affected by the reused fats; Vitamin E is a powerful antioxidant that can mitigate against oxidative stress as demonstrated by Eder *et al*. (2003).

The role of oxidative stress in the pathophysiology of non alcoholic fat liver disease has been demonstrated and reviewed severally; Masarone *et al.* (2018) in their review suggest that progressive increase in oxidative stress may likely be the primary initiator in non alcoholic fat liver disease by inducing mitochondrial dysfunction and endoplasmic reticulum stress (ER). ER is majorly involved in folding and assembly of proteins; saturated fatty acids have been theorized to cause liver injury by among other means, disruption of ER homeostasis.

Nonalcoholic fatty liver disease (NAFLD) results in histologically complex specific and nonspecific injury patterns as observed by Kleiner and Brunt (2012). In this study hepatocellular ballooning, mononuclear cell infiltration, mild macro and micro vesicular fat globules and fibrosis were observed in the liver sections of animals fed repeatedly used high fat diet. These changes are progressive to liver failure and are known to occur as a result of oxidative damage in the hepatocytes proteins as indicated by Altunkaynak (2005). He observes that inflammation is indicative of cell death (necrosis). Feeding reused high fat may have reduced lipogenic enzymes (fatty acid synthase and glucose -6- phosphate dehydrogenase) activity by suppression of gene expression of these enzymes as suggested by Klaus *et al*. (2003). In his study he postulates that oxidized fats contain substances that suppress gene expression of lipogenic enzymes. The animals were also on a continuous high fat feeding that may have caused an influx of fatty acids.

5. Conclusion

The fatty changes in the livers under study reflected an impairment of normal synthesis and elimination of lipids from the affected hepatocytes. Therefore, in this study, of which 20% repeatedly used high fat diet was used, we suggest that reused high fat diet is the main cause of accelerated progression of steatosis to non alcoholic fat liver disease and finally liver cirrhosis. Further implications of these changes are failure of the liver to synthesis and secrete major plasma proteins, which will consequently affect other body systems.

A comprehensive assessment by other histochemical staining methods; staining carbohydrates by periodic Acid-Schiff, demonstration of fatty material by Oil red O dye and defining connective tissue elements (collagen) by Masson's Trichrome Technique could have greatly benefitted this study.

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Appendix

The nutritional composition of rat pellets used as control (GP1) the present study

Nutrition information of rat pellets

Parameters	%
Moisture	Max 12
Crude protein	Min 17.7
Fat	Min 2.2
Ash	Min 5.1
Crude Fibre	Max 6.0
Calcium	0.85
Phosphorous	0.79

Source: Unga Farm Care (E.A), limited.

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