RESEARCH ARTICLE

Amal M. Hashem
Elsaid N. salama

THE AMELIORATIVE EFFECT OF OLIVE OIL AGAINST THE HISTOPATHOLOGICAL LESION OF THE LIVER OF MICE FED ON REPEATEDLY HEATED FRIED OIL

ABSTRACT:
The effects of long term feeding (12 weeks) of deep fried oils on the histology and ultrastructure of adult male albino mice liver are studied. The animals are divided into three groups (15 mice each). The first group is fed on a standard diet and served as a control. The second group is fed on a diet containing 25% of heated fried oil. The third group is fed on a diet contains a mixture of olive and fried oil in equal ratio. Histopathological examination showed many alterations in the liver tissue. The observed hepatic lesions are mild to severe distortion of the normal architecture of the liver, prominence widening of the central veins, fatty degeneration, peri-vascular and focal necrosis associated with granulomas. At the ultrastructure level, vesiculatation of rough endoplasmic reticulum, electron-dense mitochondria and convoluation of the nuclear envelope with dense peripheral clumping of heterochromatin particles as well abundant distribution of cytoplasmic lipid globules with almost distortion of cytoplasmic organelles were observed. Olive oil is found to induce partial recovery of these hepatic abnormalities.

KEY WORDS:
Fried oil, Histopathology, Liver, mice

INTRODUCTION:
The world wide uses fat frying foods is considered to be the most common unit operation in food preparation. During frying, the oil is continuously and repeatedly degraded at high temperature in the presence of air and moisture. Accordingly, oxidation, isomerization, polymerization, hydrolysis and cyclization reactions occur. The decomposition products of these reactions were found to produce off-flavor in the oil (Farag El-Anany, 2007; Romero et al., 2007). Gabriel et al. (1977) reported lipidosis, necrosis, and calcified lesions in the heart, liver, and kidney of rat fed thermally oxidized fats. Thermally oxidized oils produced by repeated frying, contain a complex mixture of toxic products such as oxidized monomers, dimers, polymers, as well as free radicals, hydroperoxides, aldehydes, and unsaturated hydrocarbons (Grootveld et al., 1998). These products had been reported to be mutagenic and carcinogenic to experimental animals (Shamberger et al., 1979; El-Bohi et al., 2011), increased the risk of hypertension (Serjouie et al., 2010; Leong et al., 2009). Reusing frying oil was found to exert harmful effects on human health (Moreira et al., 1999; Nicolle et al., 2003; Romero et al., 2006; Leong et al., 2010) and may cause histological abnormalities in liver and kidney (Leong et al., 2008; Farag et al., 2010).

The present study aimed to investigate the histopathological and ultrastructure effects of long term feeding on deep fried oil on the liver of albino mice, as well as the possible protective effect of olive oil.

MATERIAL AND METHODS:
Experimental Animals:
This study was carried out on forty five mature apparently healthy male albino mice, Mus musculus with an average weight of 20 ± 0.5 g; obtained from the Faculty of Veterinary Medicine, Zagazig University Egypt. The mice are kept in cages at animal house room temperature of 27 ± 2°C with a 12 hr
light/dark cycle. After two weeks of acclimation, the mice are randomly divided into three groups (15 mice each). The first group is fed on a standard diet and served as a control. The second group is fed a diet contain 25% repeatedly used fried oil. Mice of the third group are fed on a mixture of fried and non-cooked olive oil in equal proportions (25% each). The experimentation period lasted 12-weeks. The animals had free access to water and food throughout the experimental period.

Oil samples:
The deep-fried oil samples were obtained from a popular restaurant, in Zagazig City, Sharkia Province, that have a capacity of preparing food for hundreds peoples everyday. The fresh olive oil was purchased from the local supermarkets.

For the light microscopy, the animals are sacrificed at the end of experiment. The liver is excised and fixed in Bouin’s solution, routinely processed for histological investigation. Five µm sections are stained with hematoxylin & eosin stain and are examined under bright field light microscopy.

For TEM, the animals were perfused with freshly prepared 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.6) containing 2.5% polyvinyl pyrrolidone (Sigma), pH 7.3. The liver was immediately removed, transferred to fresh fixative for 20 min at 4°C and cut into 60–80-µm slices, using an Oxford vibratome. After rinsing in 0.1 M cacodylate buffer (pH 7.6), the slices were post-fixed for 1 h with 1% reduced osmium tetroxide (Karnovsky, 1971). The material is then rinsed in cacodylate and 0.05 M maleate buffer (pH 5.2) then stained with 1% uranyl acetate in maleate buffer overnight. The specimens are dehydrated in a graded series of ethanol and embedded in Luft’s medium (Luft, 1961). Ultrathin sections (80–90 nm) are stained with 1% alkaline lead citrate (Reynolds, 1963) for 4 min and examined under a Philips 301 electron microscope in The Regional Centre of Mycology, Al-Azhar University, Cairo Egypt.

RESULTS:
Light microscopic observations:
Light microscopic examination of paraffin and semi thin sections of liver of the control group revealed normal histological picture of hepatic tissues. The hepatocytes are arranged in an anastomosing cords separated from each other by blood sinusoids that arranged in a solar architectural manner around the central veins. The hepatocytes appeared polyhedral in shape with large rounded vesicular nuclei containing prominent nucleoli. Their cytoplasm showed bright eosinophilic appearance (Fig. 1).

Microscopic observation of almost all livers of rats fed on fried oil only (group II) showed a noticeable dilation of central vein and manifested focal hepatic necrosis associated with nuclear pyknosis (Figs 2 & 3). Granulomatous lesions are distributed throughout the hepatic tissues associated with periportal infiltration of inflammatory cells mostly composed of lymphocytes. The parenchyma cells of mice fed on the deep-fried oil exhibited widespread of hydropic and lipid degeneration.

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Fig. 1. Photomicrograph of a portal area of control group liver section showing binucleated hepatocyte (h: solid arrow) arranged in cords around the central vein (cv). H&E × 400.

Fig. 2. Photomicrograph of a liver section of group II showing enlargement of central vein (cv) and perivascular necrosis (→) with hepatocytes having pyknotic nuclei (P) and vacuolation (V). H&E × 400.

Fig. 3. Photomicrograph of a liver section of group II showing focal granulomass (arrow) with lymphocytic infelitation and loss of liver architecture, dilatation of sinusoids (s). H&E × 400.
Feeding mice on diet containing olive oil mixed with fried oil showed improvement of the hepatic lesions. Most of the hepatic lesions are encountered by few granulomatous lesions and small necrotic foci (group III) (Figs 4 & 5). Periportal leukocytic infiltrations were also detected adjacent to the dilated central vein. There was a marked reduction of disorganized hepatic cords and vacuolar degeneration of hepatocytes.

**TEM observations:**

Control hepatocytes possessee a spherical nucleus with a vasicular nucleolus, as well as cell organelles including numerous mitochondria contained medium density homogeneous matrices and many fine cristae. Endoplasmic reticulum confined to numerous small stacks is detected scattered throughout the cytoplasm (Fig. 6).

Hepatocytes of mice fed with deep fried oil (group II) showed areas of cytoplasmic dissolution, partial clumping of nuclear chromatin, and convoluted nuclear envelope. Extensive lipid droplets of different size are detected widespread throughout of cytoplasm (Fig. 7). Cytoplasmic organelles exhibited massive alterations including vesiculation of rough endoplasmic reticulum with no obvious detachment of their content of ribosomes and swollen mitochondria with apparent missing cristae (Figs 8 & 9). Lipid globules, progressive depletion of almost all cell organelles and clumping of heterochromatin and partial degenerated nuclear envelope are detected (Fig. 10).
Feeding mice on diet containing olive oil in combination with fried oil showed improvement of the ultrastructural changes represented by decreasing fat deposition (Fig. 11). Mitochondria retain its normal appearance but still showed electron dense while, nuclear envelope is still partially deteriorated (Figs 12 & 13). The rough endoplasmic reticulum was still disorganized with long profiles woven between mitochondria and in some cases encircling it forming a bizarre pattern.
Kupffer cells of group II; fed on fried oil showed nuclear membrane shrinkage, few phagocytic vacuoles and less condensed organelles. The kupffer cell appeared with irregular amoeboid structure shaving condensed peripheral heterochromatins. Their cytoplasm enclosed by many phagocytic vacuoles and mitochondria (Fig.14). However, it become more intact in experimental group fed on olive oil in combination with fried oil (Fig. 15).

![Fig. 14. An electron micrograph of hepatocyte of group II showing Kupffer cell with its special shaped nucleus (N), chromatin (ch), mitochondria (m), large fat globule F and nuclear membrane shrinkage (→).](image1)

![Fig. 15. An electron micrograph of hepatocyte of group III showing activated kupffer cell with many cytoplasmic pseudopodia-like projections (arrow head), phagocytic vacuoles V, mitochondria (m), fat globule F, nucleus (N) with chromatins Ch. (→) indicates phagocytosis.](image2)

**DISCUSSION:**

The present study is conducted to evaluate some of the toxicological hazards of repeatedly used fried oil on the liver of male mice and the antidotal effect of olive oil.

From the present findings, feeding on repeatedly deep fried oil is found to induce hepatotoxicity confirmed by widespread of hepatic necrosis, manifested by focal granulomatous lesions. Necrotic foci associated with hepatocellular ballooning degeneration and widespread of leukocytic infiltration were seen.

Similar vacuolar degeneration of the hepatocytes around central veins may due to fatty accumulation (Gabriel et al., 1977). Giacometti et al. (2012) stated that thermally oxidized oils produced by repeated frying, contained a complex mixture of toxic products which contributed to increase the risk of change in the phospholipid fatty acid composition in mice liver.

The observed mild to severe necrosis of the liver cells of mice fed on deeply fried oil were also detected. The central veins are found to be widened. Peri-vascular, as well as focal necrosis of the liver is reported as one of the consequences of feeding deep fried oil. These findings agree with the work of Jimoh and Odutuga (2004), Sanders (1983) and Gar et al. (2007). Similar necrosis and lymphocytic infiltration was reported also by Klastaskin and Oconn (1993) who observed that the majorities of lesions occurred at sites of transport of the toxic fat degradation products across blood vascular membranes of hepatocytes, inhibited normal metabolic pathways with resulting fatty accumulation and cellular necrosis.

On the contrary, Narasimhamurthy and Raina (2000) described the absence of any significant change in histology of rats fed thermally oxidized fat. Several authors have demonstrated that different types of saturated and unsaturated fatty acids induced substantially different effects following human feeding. Nutritional studies have indicated that the saturated fatty acids are not induced hazards. Repeatedly heated oil may deteriorate the activity of blood pressure, regulating enzymes and increase lipid peroxidation (Leong et al., 2012). The effectiveness of virgin olive oil in protecting biological membranes by increasing their resistance to free radicals liberated by fried oil as mentioned by Gibis (2010).

Furthermore, Quils et al. (2006) stated that mono-unsaturated fatty acids (such as those of olive oil) have been associated with greater longevity and a high degree of protection against age-related cognitive decline in humans. Bogani (2007) concluded that the protective properties of the Mediterranean diet are in part due to the consumption of antioxidant-rich olive oil. Moreover, Napolitano et al. (2008) stated that olive oil has a high concentration of polar antioxidants which are able to reduce acrylamide formation during frying. Scaccini et al. (1992) found that rats fed diets of olive oil had a decreased concentration of reactive lipoproteins, as end products of lipid peroxidation. These may explain the positive effects of olive oil in our work.

Kupffer cells constitute about 15% of the liver cells which exhibited different functions such as phagocytosis, detoxication and mediation of the innate immune response in
the liver (Bouwens et al., 1986). Activation of Kupffer cells led to the development of localized inflammation (Besaratinia and Pfeifer, 2004). Feeding on boiling oils contributed to the formation of genotoxic and cytotoxic oxidative compounds such as acrylamide and non-metabolizable fatty acid which supresses Kupffer cell activation (Dung et al., 2006; Ramadan et al., 2012). This may explain why kupffer cells from fried oil fed specimen appeared inactive. The changes in hepatocytes coexisted with marked stimulation and enhanced phagocytic activity of kupffer cells. Nevertheless, evidences on the beneficial prosperities of highly bioactive compounds of olive oil which had been proved the ameliorated effects of the hepatotoxicity of deeply heated fried oil poisoning in mice.

Further investigations are required to indicate the molecular rule of olive oil in minimizing the adverse toxic effects of fried oil.

REFERENCES:


