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RESEARCH ARTICLE

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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 deeply 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, vesicuolation of rough endoplasmic reticulum, electron-dense mitochondria and convolution of the nuclear envelope with dense peripheral clumping of heterochromatin particles as well abundant distribution of cytoplasmic lipid globules with almost distorstion of cytoplasmic organelles were observed. Olive oil is found to induce partial recovery of these hepatic abnormalities.

KEY WORDS:

Fried oil, Histopathology, Liver, mice

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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 kideny (Leong *et al.,* 2008; Farag *et al.*, 2010).

The present study aimed to investegate 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 ± 2ºC 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 every day. 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 Boun`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 are 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^{o} C and cut into 60–80-lm 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).

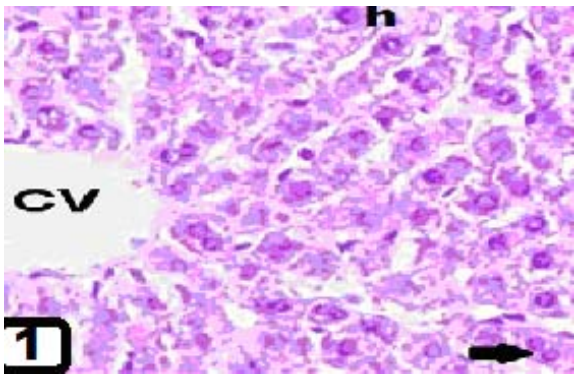

Fig. 1. Photomicrograph of a portal area of control group liver section showing binucleated hepayocyte (h: solid arrow) arranged in cords arround the central vein (cv). H&E × 400.

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.

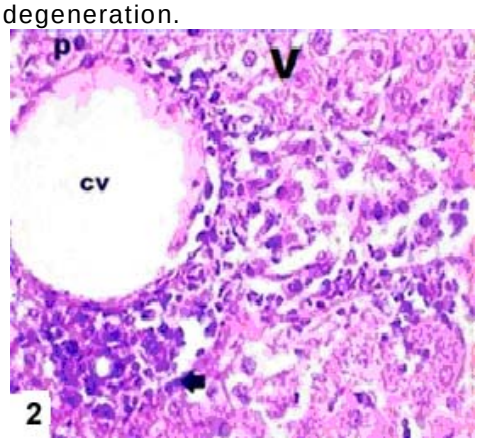

Fig. 2. Photomicrograph of a liver section of group II showing enlargement of central vein(cv) and perivascular neecrosis (→) with hepatocytes having pykntic nuclei (P) and vacuolation (V). H&E × 400.

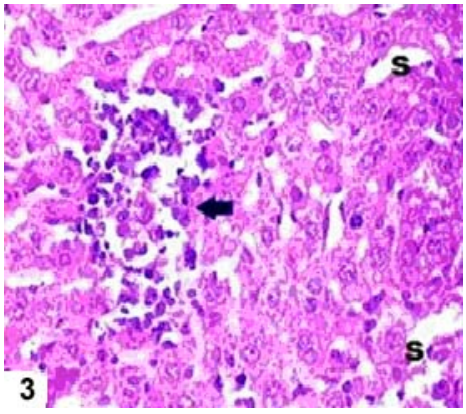

Fig. 3. Photomicrograph of a liver section of group II showing focal granulomass(arrow) with lympocytic infelteration and loss of liver architecture,diletation of sinusoids (s). H&E × 400.

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.

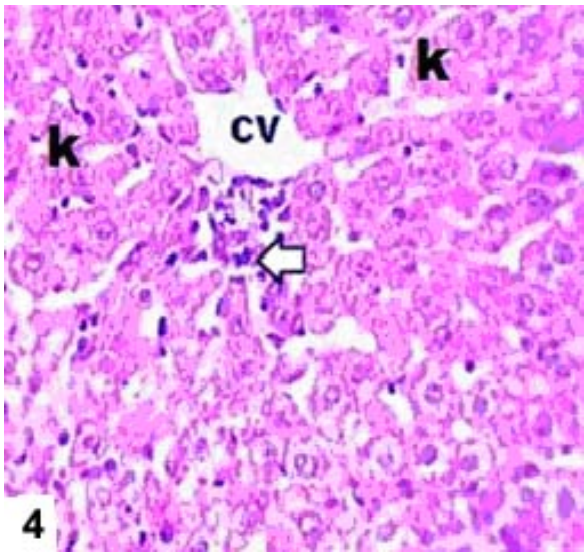

Fig. 4. Photomicrograph of a liver section of group III showing less dilated central vein (cv), small granulomass (→) and kupffer cells (k). H&E × 400.

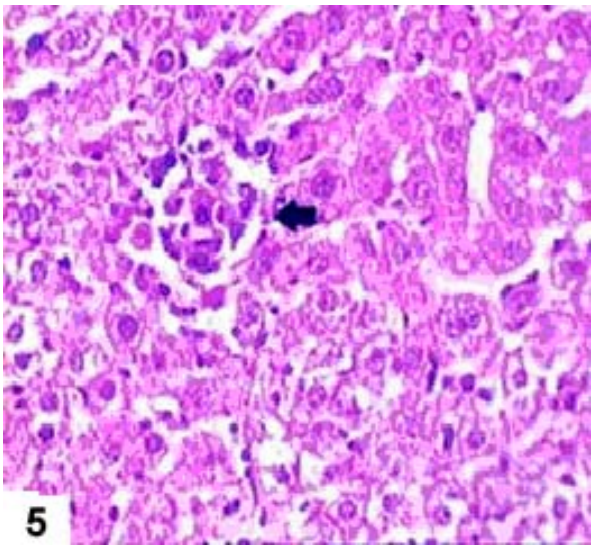

Fig. 5. Photomicrograph of a liver section of group III showing small sized granulomass (→) and less dilated sinusoids. H&E × 400.

TEM observations:

Control hepatocytes possesse 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).

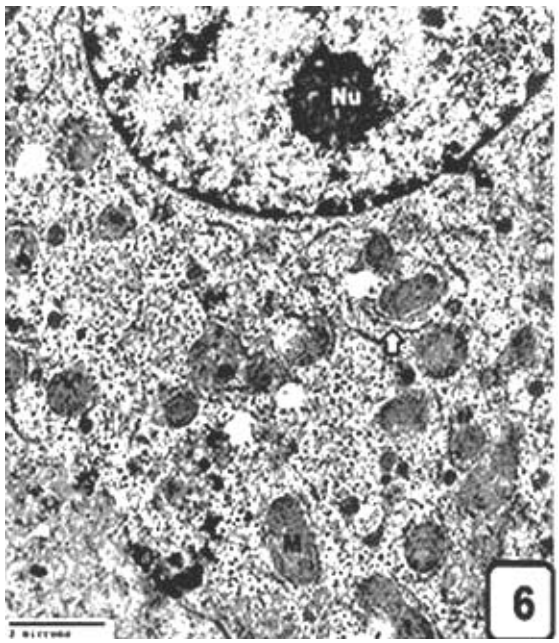

Fig. 6. A electron micrograph of a control hepatocytes showing spherical nucleus (N), nucleolus (Nu), spherical mitochondria (M) and rough endoplasmic reticulum (→).

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 vesicuolation of rough endoplasmic reticulum with no obvious detachement 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).

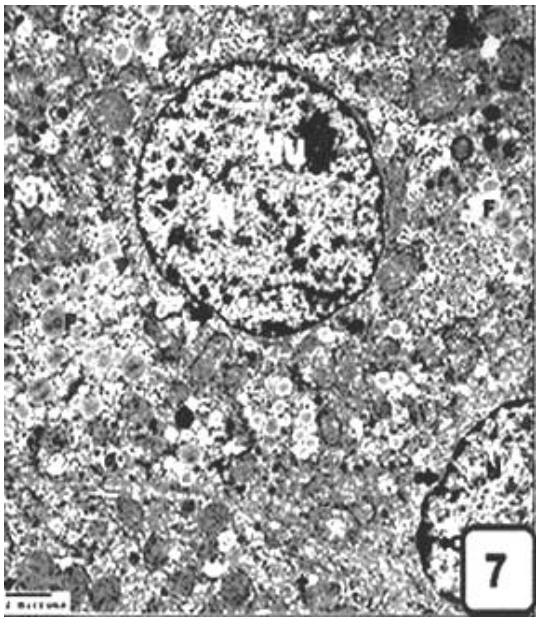

Fig. 7. An electron micrograph of hepatocyte of group II showing excessive fat droplets (F) in the cytoplasm, atrophied nuclei (→), and nucleolus.

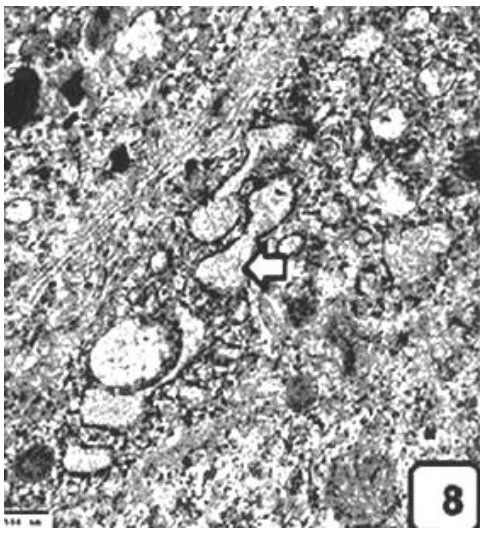

Fig. 8. An electron micrograph of hepatocyte of group II showing extensive abnormalities and hypertrophy in the endoplasmic reticulum (→).

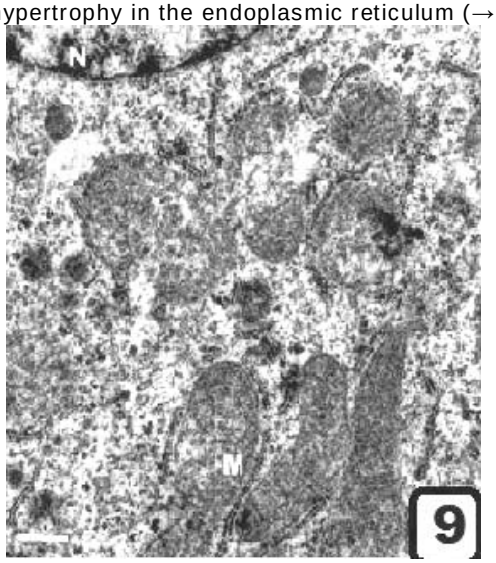

Fig. 9. An electron micrograph of hepatocyte of group II showing abnormal mitochondrial shape (M), swelling and loss of cristae, and blood sinusoid (BS).

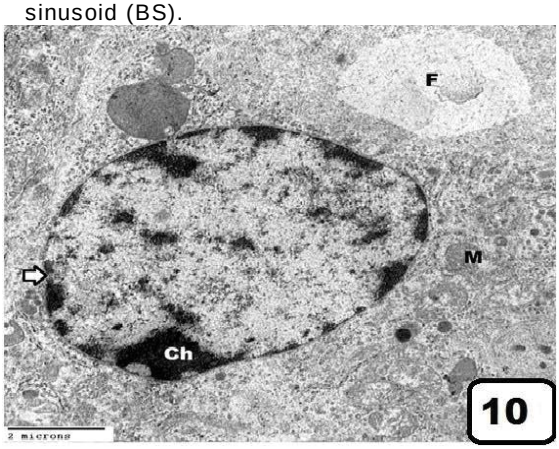

Fig. 10. An electron micrograph of hepatocyte of group II showing advanced stages of lipid vacuolation (F), depletion of almost all cell organelles, peripheral clumping of chromatin bodies (ch) and lysis of nuclear membrane (→)

Feeding mice on diet containing olive oil in combination with fried oil showed improvement of the ultrastructural changes represented by decreasing if 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.

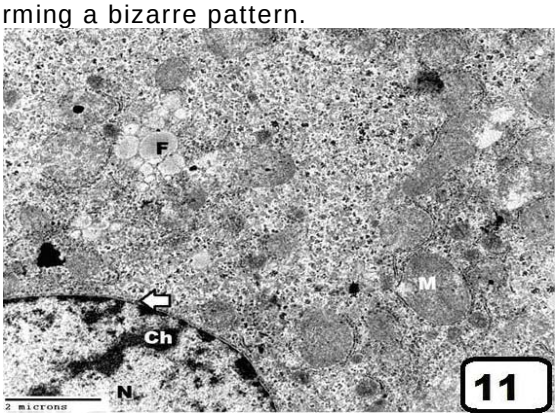

Fig. 11. An electron micrograph of hepatocyte of group III showing reorgnization of mitochondria (M), few different sized fat droplets (F), dispersed chromatin (ch), nuclear membrane (→).

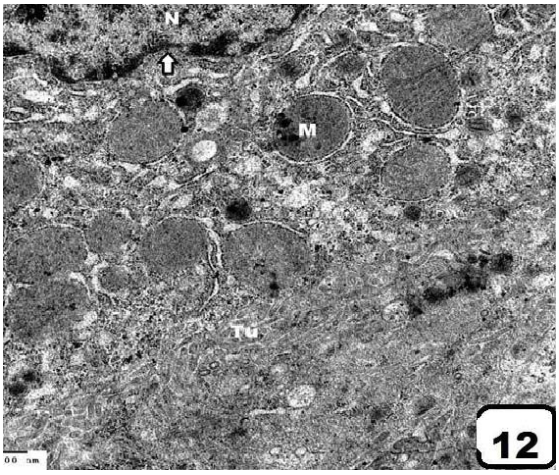

Fig. 12. An electron micrograph of hepatocyte of group III showing agregatin of mitochondria M, nuclear membrane shrinkage (→) tubular endoplasmic reticulum (Tu), nucleus (N).

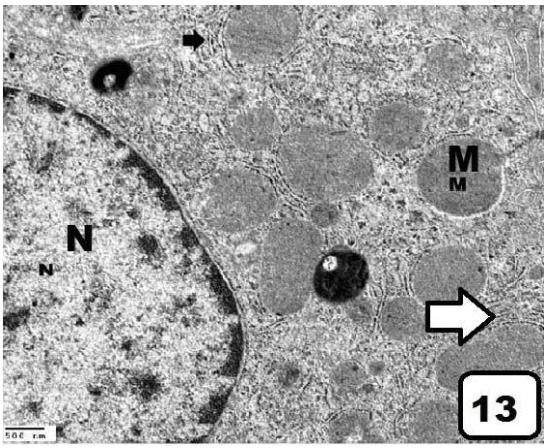

Fig. 13 An electron micrograph of hepatocyte of group III showing organelles retain its normal appearance, nucleus (N), mitochondriaM, endoplasmic reticulum (→).

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).

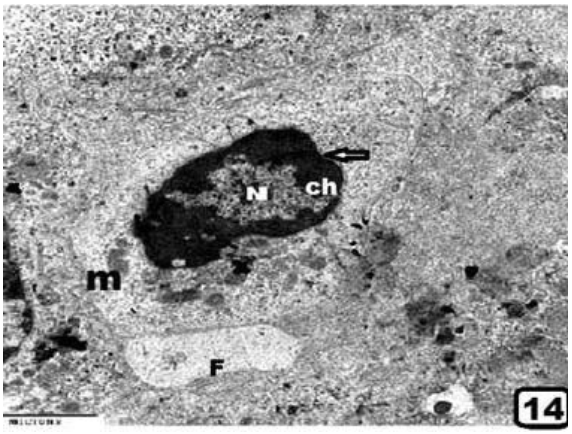

Fig. 14. An electron micrograph of heparocyte of group II showing Kupffer cell with its special shaped nucleus (N) ,chromatin (ch), mitochondria (m), large fat globule F and nuclear membrane shrinkage (→).

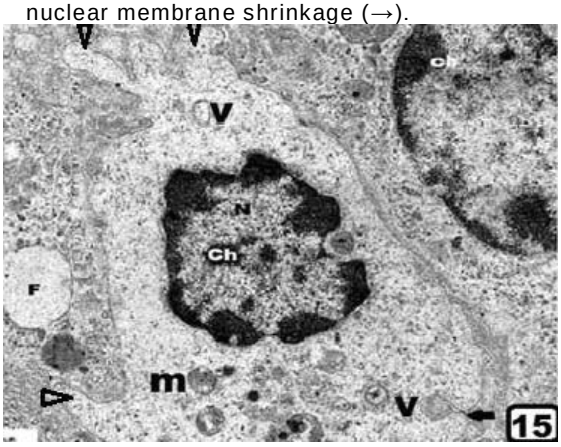

Fig. 15. An electron micrograph of heparocyte of group III showing activated kupffer cell with many cytoplasmic pseudopodia-like projections (arrow head), phagocytic vacuoles V,mitochondria (m), fat globule F, nucleus (N) with chromatins Ch. (→) indicates phagocytosis.

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 productswhich 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 Garib *et al.* (2007). Similar necrosis and lymphocytic infiltration was reported also by Klastskin 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:

Bawazir AE. 2011. Chronic effect of olive oil on some neurotransmitter contents in different brain regions and physiological, histological structure of liver and kidney of male albino rats. World J. Neurosci., 1: 31-37

Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, Lee CH, Smith AB, and Breslin PA. 2005. Phytochemistry: ibuprofen like activity in extra-virgin olive oil. Nature, 437: 45-46.

Besaratinia A, Pfeifer P. 2004. Genotoxicity of acrylamide and glycidamide. J. Natl. Cancer Inst., 96(13): 1023-1029

Bogani P, Galli C, Villa M, Visioli F. 2007. Postprandial anti-inflammatory and antioxidant effect of extra virgin olive oil. Nutrition, 190(1): 181-185.

Bounwens L, Knoop DL, Wisse E. 1986. Local proliferation and extrahepatic recruitment of liver macrophages (Kupffer cells) in partial-body irradiated rats. J. Leukoc. Biol., 39(6): 687-697.

Dung CH, Wu SC, Yen GC. 2006. Genotoxicity and oxidative stress of the mutagenic compounds formed in fumes of heated soybean oil, sunflower oil and lard. Toxicol. In Vitro, 20(4): 439-447.

El-Bohi KM, Moustafa GG, El-Sharkawi NI, Sadik LM. 2011. Genotoxic effects of acrylamide in adult male albino rats liver. J. Am. Sci., 7(1): 1097-1108.

Farag SR, Abdel-Latif MS, Basuny MM, Abd El Hakeem BS. 2010. Effect of non-fried and fried oils of varied fatty acid composition on rat organs. Agric. Biol. J. N. Am., 1(4): 501-509.

Farag SR, El-Anany AM. 2007. Influence of fried and fried-treated oils with different filter aids on rat health. J. Sci. Food Agric., 87(3): 485-495.

Gabriel HG, Alexander JC, Valli VE. 1977. Biochemical and histological effects of feeding thermally oxidized rapeseed oil and lard to rats. Can. J. Comp. Med., 41(1): 98-106.

Garib M; Zeybek U, Erdamar S, Cevik A, Funda E. 2007. The hepatotoxic effects of deep-fried sunflower oil on rat livers. Adv. Mol. Med., 3(1): 35-40.

Giacometti1 J, Tomljanovic AB, Milin C, Cuk M, Stasic BR. 2012. Olive and corn oil enriched diets changed the phospholipid fatty acid composition in mice liver after one-third hepatectomy. Food Nutr. Sci., 3(2): 240-248

Gibis M. 2010. Inhibitory effect of lipophilic antioxidants in frying oil on formation of heterocyclic amines in fried patties. Meat Science, 85(4): 735-742.

Grootveld M, Atherton MD, Sheerin AN, Hawkes J, Blake DR, Richens TE, Silwood CJ, Lynch E, Claxson AW. 1998. In vivo absorption, metabolism, and urinary excretion of alpha, beta-unsaturated aldehydes in experimental animals. Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturated-rich culinary oils. J. Clin. Invest., 101(6): 1210-1218.

Jimoh FO, Odutuga AA. 2004. Histological changes of selected rat tissues following the ingestion of thermally oxidized groundnut oil. Biokemistri, 16(1):1-10.

Karnovsky MJ. 1971. Use of ferrocyanide-reduced osmium tetroxidein electron microscopy. J. Cell Biol., 51: Abst. 284.

Klastskin G, Oconn H. 1993. Abnormalities of the hepatic parenchyma In Histopathology of liver. Oxford Uni. Press, New York, 1: 40-55.

Leong XF, Aishah A, Nor Aini U, Das S, Jaarin K. 2008. Heated palm oil causes rise inblood pressure and cardiac changes in heart muscle in experimental rats. Arch. Med. Res., 39(6): 567-572.

Leong XF, Mustafa MR, Das S, Jaarin K. 2010. Association of elevated blood pressure and impaired vaso-relaxation in experimental Sprague-Dawley rats fed with heated vegetable oil. Lipids Health Dis., 23(9): 66.

Leong XF, Najib MN, Das S, Mustafa MR, Jaarin, K. 2009. Intake of repeatedly heated palmoil causes elevation in blood pressure with impaired vasorelaxation. Tohoku J. Exp. Med., 219(1): 71-78.

Leong XF, Salimon J, Mustafa MR, Jaarin K. 2012. Effect of repeatedly heated palm oil on blood pressure regulating enzymes activity and lipidperoxidation in rats. Malasian J. Med. Sci., 19(1): 20-29

Luft JH. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., 9(2): 409–414.

Moreira RG, Castell-perez EM, Barufet M. 1999. Deep-fat frying: Funda and Application, Maryland: Aspen Publishers, Inc. pp. 350.

Napolitano A, Morales F, Sacchi R, Fogliano V. 2008. Relationship between virgin olive oil phenolic compounds and acrylamide formation in fried crisps. J. Agric. Food Chem. 56(5): 2034–2040.

Narasimhamurthy K, Raina P.L. 2000. Long-term feeding effects of thermally oxidized oils on the absorption, storage and excretion of fat in rats. Eur. Food Res. Technol., 210(6): 402–406.

Nicolle C,Cardinault N, Aprikian O, Busserolles J, Grolier P, Rock E, Demigne C, Mazur A, Scalbert A, Amouroux P, Remesy C. 2003. Effect of carrot intake on cholesterol metabolismand on antioxidant status in cholesterol-fed rat. Eur. J. Nutr., 24(5): 254-261.

Quiles JL, Barja G, Battino M, Mataix J, Solfrizz V. 2006. Role of Olive Oil and Monounsaturated fatty acids in mitochondrial oxidative stress and aging. Nutr. Rev., 64(s4): 531–539

Ramadan AM, Ali N, Sayed HM, Abd El-Monem DD. 2012. Potato chips and roasted bread induced chromosomal aberrations and micronuclei formation in albino rats. J. Am. Sci., 8(1): 474-480.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208–212

Romero A, Bastida S, Sànchez-Muniz FJ. 2007. Cyclic fatty acids in sunflower oils during frying of frozen foods with oil replenishment. Eu. J. Lipid Sci. Technol., 109(2): 165-173.

RomeroA, Bastida S, Sanchez-Muniz FJ. 2006. Cyclic fatty acid monomer formation in domestic frying of frozen foods in sunflower oil and high oleic acid sunflower oil without oil replenishment. Food Chem.Toxicol. 44(10): 1674-1681.

Sanders TAB. 1983. Nutritional significance of rancidity. In: “Rancidity in foods. (Allen JC, Hamilton RJ. Eds)”. Applied Sci. Publ. Ltd. pp: 59-66.

Scaccini C. Nardini m, daquno M, Gentili V, Oilfelice M, Tomassit G. 1992. Effect of dietry oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. J. lipid res., 33(5): 627-633.

Serjouie A, Tan CP, Mirhosseini H, Man YBC. 2010. Effect of vegetable based oil blends on physicochemical properties of oils during deep-fat frying. Am. J. Food Tech., 5(5): 310-323.

Shamberger RJ, Corlett CL, Beaman KD, Kasten BL. 1979. Antioxidants reduce the mutagenic effect of malon-aldehyde and propiolactione. Mutat. Res., 66(4): 349-355.

الدور الوقائي لزيت الزيتون ضد الأضرار النسيجية لكبد الفئران المغذاه على زيت القلي المغلي

السيد نصر وأمل هاشم

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فى هذا البحث تمت دراسة تأثير التغذية لمدة 12 أسبوعا على الزيوت المغلية بشده وآثارها على أنسجة الكبد في الفئران البيضاء. فبعد تغذية مجموعتين من الحيوانات كل منهما تحتوي على 15 فأراّ لمدة 12 أسبوعا على وجبات غذائية أحتوت اولاهما على زيت قلي مغلي 25٪ و الثانيه على زيت زيتون + زيت قلي مغلي . أظهر الفحص المجهري للأنسجة تغييرات كثيرة في أنسجة الكبد. وتمثلت معظم التغيرات المرضية النسيجيه في تشوهات خفيفة الى شديدة للبنية الطبيعية في خلايا الكبد واتساع في الأوردة المركزية، تحلل دهني لسيتوبلازم الخلايا القريبه من الأوعية الدموية، كما ظهرت بؤرمتحلله من خلايا التهابيه ولمفاويه وتنخرذات أحجام متفاوته. أما الفحص المجهري الدقيق فقد أوضح تدهوراّ كبيراّ في الشبكة الإندوبلازمية،وتشوه في الميتوكوندريا، تموج في الغشاء النووي مع تكتل للجسيمات الكروماتينيه على سطح الغشاء النووي وظهور حبيبات دهنيه متفاوتة الاحجام مع نضوب تدريجي لعضيات الخلية جميعها. على الرغم من أن تناول زيت الزيتون قد خفض الكثير من هذه التشوهات الكبديه، فإنه عمل على تنشيط خلايا كوفر.

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