Histomorphometric Evidence of Hepatic Recovery of Rats Fed Repeatedly Heated Palm Oil

Original Article

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ABSTRACT

Background: Repeated heating of vegetable oils especially palm oil that is commonly and commercially used in frying of food at homes and restaurants has been investigated for its risks on health status. It seemed to carry potential hazards on the liver tissue.

Aim: To investigate the impact of eating foods fried with 5-times heated palm oil on liver of adult male rats for 8 weeks and the possibility of recovery of histomorphometric changes after stoppage of use of reheated palm oil for additional 4 weeks.

Material and Methods: Thirty adult male albino rats were divided equally into 3 groups; control group (received normal balanced diet for 8 weeks), H5 group (received 5-times heated palm oil enriched diet for 8 weeks) and Recovery group (received five times heated palm oils for 8 weeks and then left on balanced diet for another 4 weeks). At the end of the experiment, animals were anesthetized with ether then sacrificed. Specimens taken from the liver were processed for histological evaluation. Morphometric studies were conducted and collected data undergone statistical analysis.

Results: Histological examination showed alterations in liver tissue of H5 group including fat droplets, mononuclear cellular infiltration, dilatation of central vein and blood sinusoids, collagen deposition and degeneration and necrosis of hepatocytes. Ultrastructural examination showed lipid droplets, irregular mitochondria, cytoplasmic vacuolation, irregular nuclear envelop and dilatation of endoplasmic reticulum. Recovery group showed decreased lipid droplets, less dilatation and congestion of central vein and blood sinusoids, decreased inflammatory cells but few hepatocytes still show degeneration. Ultrastructure of hepatocytes of recovery group became more or less normal with restoration of cytoplasmic organelles. Morphometric results support the histological findings.

Conclusion: Consumption of repeatedly heated palm oil is injurious to the liver tissue, and those injuries show possible recovery after stoppage of its consumption.

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Key Words: Histomorphometry; liver; recovery; repeatedly heated palm oil.

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INTRODUCTION

Palm oil is an edible vegetable oil derived from the fruit of palm tree. It is a common cooking oil in tropical area of Africa, South East of Asia and parts of Brazil. It is used in commercial industries and preparation of food in other parts of the world[1]. Palm oil (Elaeis guineensis) is considered as unique edible oil in its fatty acid composition because it is the only cooking oil which contains equal amount of saturated (50%) and unsaturated fatty acids (50%) making it suitable for frying purposes[2]. During the frying process, oil or fat is often reheated several times allowing moisture and air to be more mixed into the frying oil. As a result, these fats and oils undergo thermal and oxidative decomposition. Polymers formed led to physical and chemical changes in heated oil and the fried food, which appear to be harmful to health[3]. Some studies on rats fed reheated palm oil showed swollen hepatocytes, microgranules and chronic inflammatory cells without necrosis of hepatocytes[4,5]. Ilyas 2018[6] observed liver cellular damage and necrosis in Wistar rats received repeatedly heated palm oil. Some researchers observed no significant histological changes in liver of rats fed reheated edible oils[7]. This discrepancy between results of those studies as well as the few ultrastructural studies of precise implications of reheated palm oil on the liver motivated the conduction of the present work. Recovery of injuries that might occur in the structure of the liver as a result of consumption of reheated palm oil were scarce.

The aim of this study was to investigate effects of feeding rats with reheated palm oil for 8 weeks on the liver and evaluation of potentials of recovery of rats after stoppage of administration of the repeatedly heated palm oil for 4 weeks.

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MATERIAL AND METHODS

Palm oil

The fresh palm oil was obtained from Erma Company for Edible Oils, 10Th of Ramadan city, Egypt. The palm oil used in the present study is in the form of Refined Beached Deodorized (RBD) palm oil without addition of synthetic antioxidants. RBD palm oil was obtained by refining of the crude palm oil.

Experimental Animals

After approval of the ethical committee, thirty adult male albino rats weighted (180 – 200 g) were selected for the present study. Rats were obtained from animal house at Faculty of Medicine of Assiut University. Rats were housed in individual stainless steel cages and received tap water and standard balanced diet for rodents. Animals were kept under controlled conditions of adequate ventilation, room temperature 25˚C ± 5ºC, artificial illumination (12 h. light-dark cycle) and proper humidity in the animal house.

Experimental protocol

After one week of acclimatization, the rats were classified randomly into three equal groups; 10 rats in each group:

Group I (control group): Rats in this group received normal balanced diet and tap water for 8 weeks.

Group II (H5 group): Rats of this group received 5-times heated palm oil enriched diet (20 grams/day for each rat) for 8 weeks. The mixed diet was formed of (3 gm heated palm oil + 17 gm normal balanced diet), i.e. (15% of the mixed diet formed of reheated palm oil and the remaining 85% of mixed diet formed of normal balanced diet). A kilogram of potato slices was fried in an opened stainless steel pot containing two liters of palm oil heated at 180◦C for 15-20 minutes[8]. To obtain 5-times heated palm oil, frying process was repeated 4 times. A fresh kilogram of potato slices was used each time and no fresh palm oil was added between the frying processes.

Group III (Recovery group): Rats of this group received 5-times heated palm oil enriched diet (3grams of 5-times heated palm oil mixed with 17 grams of normal balanced diet) for 8 weeks and then left on balanced normal diet for another 4 weeks.

At the end of the experiment, animals were anesthetized with ether. The abdomen was opened and the liver was dissected and excised. The specimens taken from the liver were processed for histological examination and morphometric analysis.

Light microscopic examination (H&E stain)

Small pieces (0.5 cm) of the liver were fixed in 10% buffered neutral formalin for 4 days. Specimens washed, dehydrated in ascending grades of alcohol and cleared by 2 changes of xylol. Specimens embedded in paraffin. Paraffin blocks were cut as coronal sections of 5 µm thickness which then stained with hematoxylin and eosin stain[9].

Light microscopic examination (Masson trichrome stain)

After fixation of samples of hepatic tissue in 10% formalin, they were dehydrated in graded series of ethanol through 70, 80, 90, 95 and 100% with two changes for 1 h each. Three changes of xylene as clearing reagent for 30 min each were performed. Hepatic tissues were then embedded in paraffin, sliced into 5 µm sections, and stained with Masson's trichrome at room temperature for 2 h[10].

Electron microscopic examination

Specimens from the liver were fixed by immersion into fresh 3% buffered glutaraldehyde at 4°C for 24 hours. Then washed 3 times in 0.15 mol/l phosphate buffer (pH 7.4) for 20 minutes each. Then postfixed in 1% osmium tetroxide for 1 hour at 4°C. Then dehydrated in ascending grades of alcohol and then embedded in epoxy resin by routine protocol. Semithin sections (0.5-1 µm thickness) were obtained and stained with 1% toluidine blue and examined by light microscopy to detect the area of interest. Ultrathin sections (50-60 nm) were obtained by ultra-microtome and stained with uranyl acetate and lead citrate[11]. The sections were then examined and photographed by (JOEL – JEM 100 – CXII - Japan) at the Electron Microscopic Unit of Assiut University.

Morphometric study

Slides prepared for light microscopy were photographed using optical Olympus microscope (CX 41) with digital camera attached to and interfaced to a computer with image analyzer software (Leica DLMB light microscope, Qwin 500 LTD, Wetzlar, Germany) in the Anatomy Department, Faculty of Medicine, Assiut University. Ten random slides from each animal of each group were chosen. From each slide, five random non-overlapping fields were analyzed. Each field equal 100.000µm². All measurements were calibrated against a micrometers slide that enables us to obtain the measurements in µm instead of pixels. In each field image, hepatocyte nuclear area (at magnification ×200), central vein diameter (at magnification ×100) and area percentage of collagen deposition (at magnification ×400) were measured.

Statistical analysis

The obtained morphometric data were represented as mean ± SD (standard deviation) and analyzed using one-way analysis of variance (ANOVA) to compare between more than 2 means while repeated measured ANOVA test was used to compare more than two means in different follow up period by using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 24). The level of significance (*p value*) ≤ 0.05.

RESULTS

Light microscopic results (H&E stain)

Liver of rats of control group showed normal architecture of hepatic lobules and portal tracts. Each

lobule formed of hepatic cords radiating from the central vein. Each cord consisted of rows of hepatocytes which had polygonal shape, acidophilic cytoplasm and large vesicular nucleus with prominent nucleolus. Some hepatocytes were binuclear. Hepatocytes cords were separated by blood sinusoids, which lined by endothelial cells and Kupffer cells (Figure 1a). In addition, portal tract at periphery of each hepatic lobule contained a triad formed of branches of portal vein, hepatic artery and bile duct (Figure 2a).

Liver of rats of H5 group showed multiple vacuolations and degeneration of hepatocytes in the form of small darkly stained and pyknotic nuclei with vacuolated cytoplasm of hepatocytes (Figure 1bI), as well as dilatation and congestion of central vein and blood sinusoids with disturbed normal architecture of hepatic cords (Figure 1bΠ). Portal tract showed massive mononuclear cellular infiltration around dilated congested portal vein and bile ducts with degenerated hepatocytes (Figure 2b).

Liver of rats of recovery group showed improvements toward normal architecture with less vacuolations. Central vein and blood sinusoids became less dilated and less congested with restoration of trabeculated appearance of hepatic cords (Figure 1c). In addition, portal vein became less dilated and less congested with mild periportal inflammatory cellular infiltration (Figure 2c).

Light microscopic results (Masson trichrome stain)

The liver of rats of control group showed faint normal distribution of collagen fibers around central vein (Figure 3a) and around portal tract (Figure 4a). Liver of rats of H5 group showed apparent increase in deposition of collagen fibers around dilated congested central vein (Figure 3b) as well as around portal tract but the collagen deposition in-between hepatocytes was mild (Figure 4b). Liver of rats of recovery group showed less collagen fiber deposition around central vein (Figure 3c), around portal tract and between hepatocytes (Figure 4c).

Transmission electron microscopic results

Ultrastructure of liver of rats of control group showed a polygonal hepatocyte with rounded euchromatic nucleus with prominent nucleolus, small peripheral chromatin and regular nuclear envelope. Cytoplasm of hepatocytes possess numerous ovoid or spherical mitochondria, endoplasmic reticulum, glycogen granules and lysosomes (Figure 5a).

Ultrastructure of liver of rats of H5 group showed small irregular hepatocyte nucleus with condensed chromatin, irregular shape of mitochondria, and dilatation of rough endoplasmic reticulum (Figure 5bI). In addition, increased lipid droplets and dissolution of cytoplasm of hepatocyte with degenerated mitochondria were observed (Figure 5bΠ).

Ultrastructure of liver of rats of recovery group showed that the histological architecture of most of hepatocytes became similar to that of the control group. Nucleus became more or less euchromatic with regular nuclear membrane and the cytoplasmic organelles as rough endoplasmic reticulum, lysosome, mitochondria and glycogen particles were present. However, few lipid droplets were still seen (Figure 5c).

Morphometric results

There was non-significant decrease in the nuclear area of the hepatocytes of H5 group compared with control group. In addition, the nuclear area of hepatocytes of recovery group showed non-significant increase compared to H5 group but did not reach the control value (Table 1, Figure 6).

Diameter of the central vein was increased significantly in H5 group when compared with that of control group. In addition, diameter of the central vein of reversibility group showed significant decrease compared with H5 group but still greater than control level (Table 1, Figure 7).

Area percentage of collagen deposition was increased significantly in H5 group compared to the control group, whereas the area percentage of collagen deposition of recovery group showed significant decrease when compared to H5 group but did not reach the control values (Table 1, Figure 8).

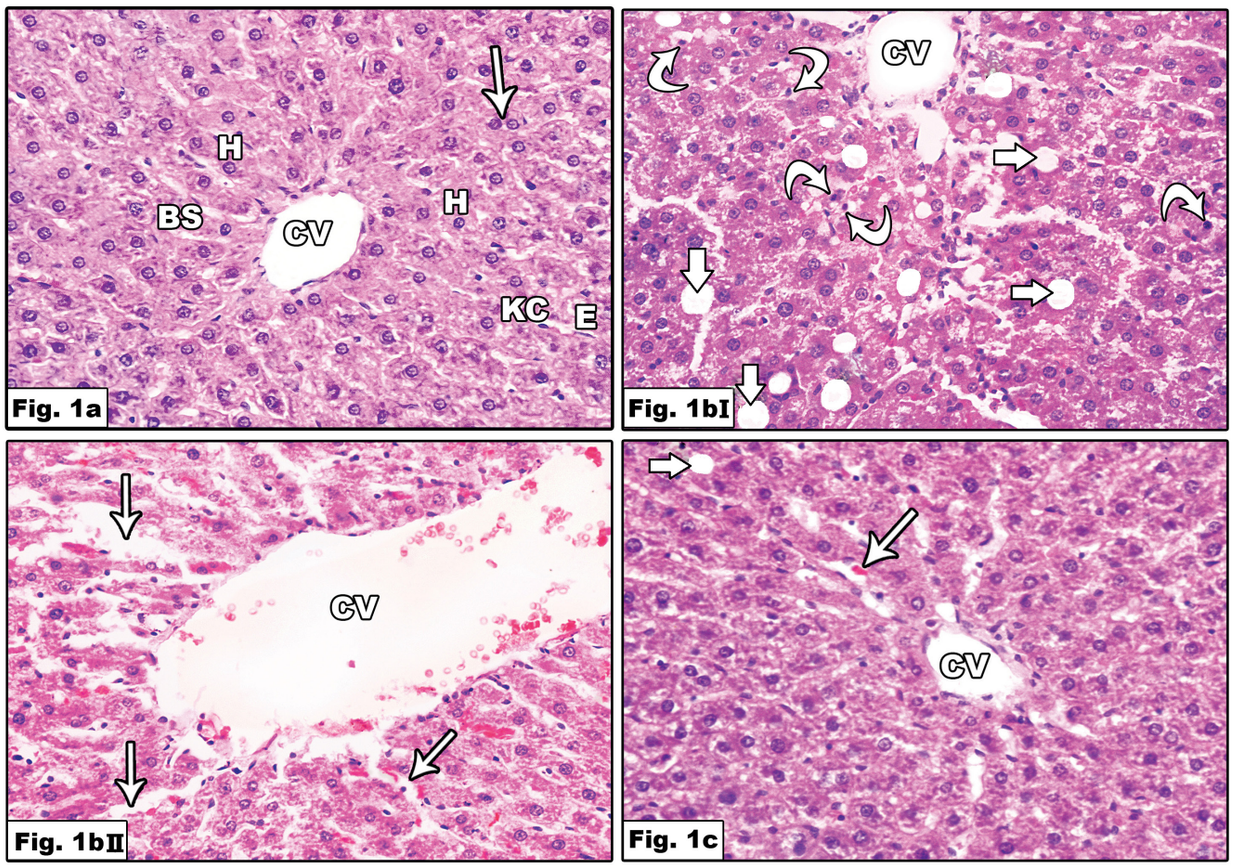

Fig. 1: Photomicrographs of hepatic lobule of rat of a): control group showing central vein (CV), hepatic cords (H), blood sinusoids (BS), endothelial cell (E), Kupffer cell (KC) and binuclear hepatocyte (arrow). bI): H5 group showing disorganized hepatic lobule, multiple vacuolations (thick arrows) and small dark nuclei of hepatocytes surrounded by cytoplasmic vacuolation (curved arrows); bII): H5 group showing dilatation and congestion of central vein (CV) and blood sinusoid (arrows) with disturbed normal architecture of hepatic cords. c): recovery group showing improved hepatic architecture, central vein (CV), vacuolation (thick arow) and congested blood sinusoid (arrow) (H&E X 400).

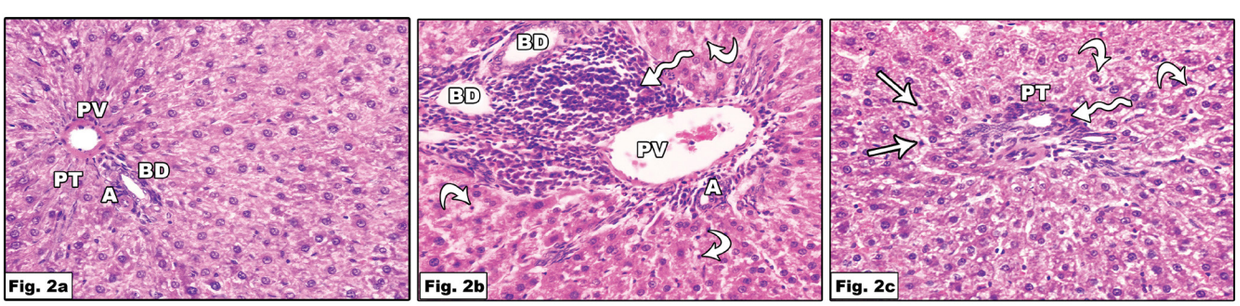

Fig. 2: Photomicrographs of portal tract of rat of a): control group showing portal tract (PT); portal vein (PV), hepatic artery (A) and bile duct (BD). b): H5 group showing massive mononuclear cellular infiltration (wavy arrow), dilated congested portal vein (PV) and dilated bile ducts (BD) with pyknotic nuclei and vacuolated cytoplasm of some hepatocytes (curved arrows). c): recovery group showing mild inflammatory cell infiltration (wavy arrow) around portal tract (PT). Some hepatocytes showing vacuolated cytoplasm (curved arrows) and fewer pyknotic nuclei (arrows) (H&E X 400).

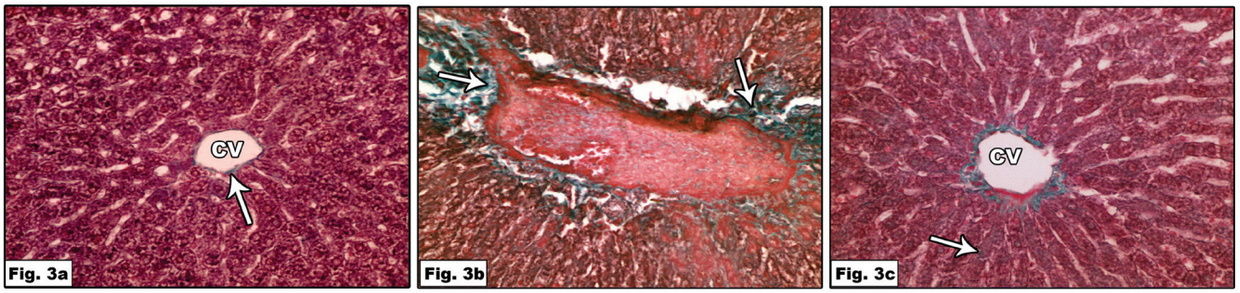

Fig. 3: Photomicrographs of hepatic lobule of a): control rat showing faint normally distributed collagen fibers (arrow) around central vein (CV). b): H5 group showing apparent increased deposition of collagen fibers around dilated congested central vein (arrows). c): recovery group showing less deposition of collagen fibers around central vein (CV) and between hepatocytes (arrow) (Masson trichrome X 400).

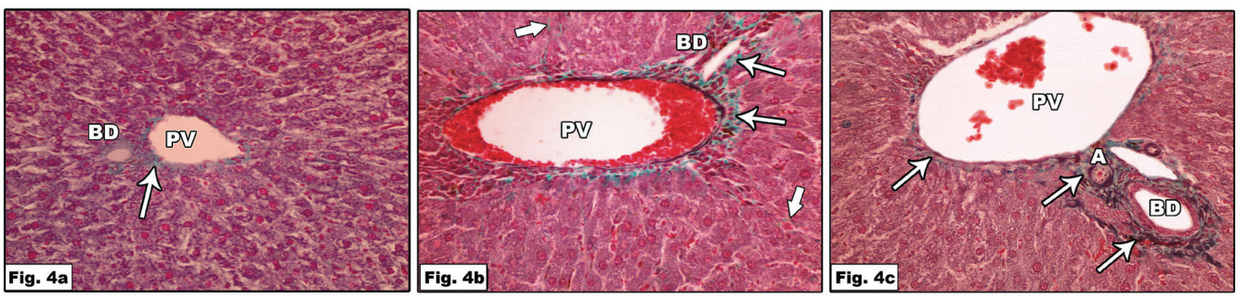

Fig. 4: Photomicrographs of portal tract of a): control group showing normal distribution of collagen fibers (arrow) around portal vein (PV) and bile duct (BD). b): H5 group showing increased deposition of collagen fibers (arrows) around congested portal vein (PV), bile duct (BD) and between hepatocytes (thick arrows). c): recovery group showing less deposition of collagen fibers (arrows) around portal tract (PV, A and BD) which still appeared dilated and congested (Masson trichrome X 400).

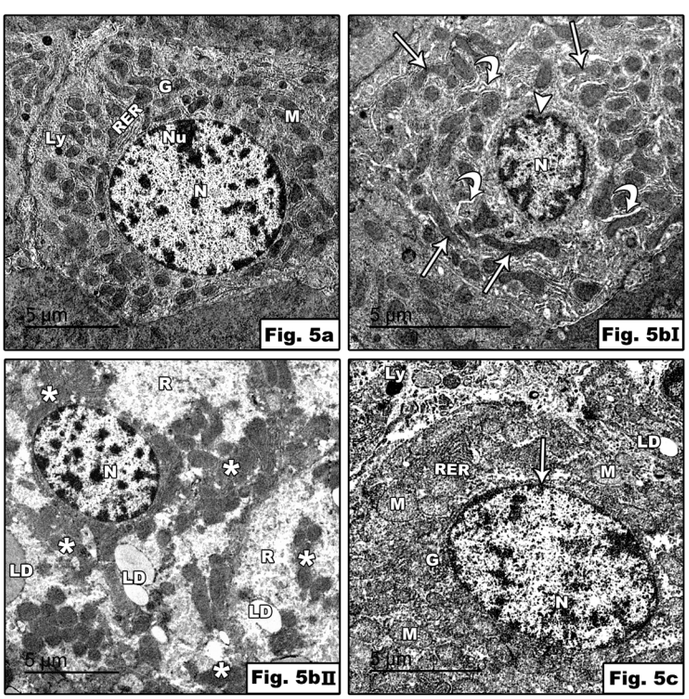

Fig. 5: Electron micrographs of hepatocytes of a): control rat showing nucleus (N) with prominent nucleolus (Nu), mitochondria (M), rough endoplasmic reticulum (RER), lysosomes (Ly) and glycogen granules (G) (Scale bar 5µm) (TEM X 1000). bI): H5 group showing small nucleus (N) with irregular envelope (arrow head), dilated rough endoplasmic reticulum (curved arrows) and deformed mitochondria (arrows) (Scale bar 5µm) (TEM X 1500); bII): H5 group showing small sized nucleus (N), fat droplets (LD), degenerated and coalesced mitochondria (asterisk) and rarified cytoplasm (R) (Scale bar 5µm) (TEM X 1000). c): recovery group showing more or less normal nucleus (N) with regular nuclear membrane (arrow), mitochondria (M), rough endoplasmic reticulum (RER), lysosome (Ly) and glycogen granules (G). A lipid droplet (L) still seen (Scale bar 5µm) (TEM X 1000).

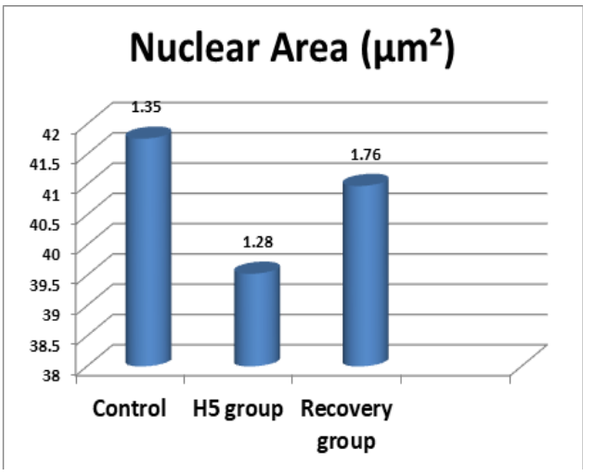

Fig. 6: Histogram showing the mean nuclear area of hepatocytes of different studied groups.

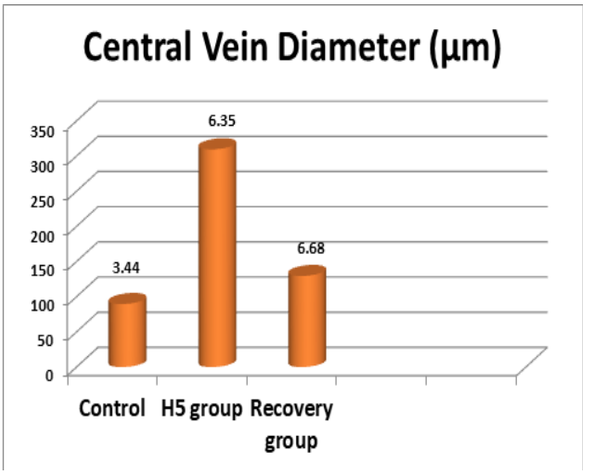

Fig. 7: Histogram showing the mean central vein diameter of different studied groups.

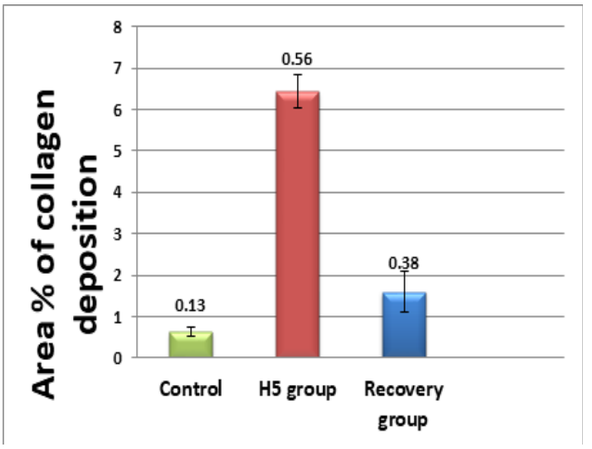

Fig. 8: Histogram showing area percentage of collagen deposition of the different studied groups.

Table 1: Comparison between the different studied groups as regard hepatocyte nuclear area (µm²), central vein diameter (µm) and area percentage of collagen deposition.

Liver	Control	H5 group	Recovery group	ANOVA *p-value*
Nuclear Area (µm²)	41.76±1.35	39.53±1.28	40.98±1.76	F=10.08 *P*=≤0.1
Central Vein Diameter (µm)	89.66±3.44	309.49±6.35[a]	129.46±6.68[d]	F=95.16 *P*=≤0.001*
Area % of collagen deposition	0.63±0.13	.44±0.56[a]	1.60±0.38[d]	F=568 *P*=≤0.001*

DISCUSSION

In the present study, light microscopic examination of the liver of rats showed that diets fortified with 5-times heated palm oil caused lipid droplet accumulation, dilatation and congestion of blood sinusoids and central vein, collagen deposition, marked periportal inflammatory cellular infiltration and degeneration and necrosis of multiple hepatocytes. These findings are in accordance with Ani *et al*., 2018[12] and Morshed *et al*., 2018[13].

This liver injury is caused by decreased antioxidants content of the heated palm oil as well as release of toxic compounds as free fatty acids aldehydes, ketones, alcohols and reactive oxygen species (ROS). Those compounds caused decrease of liver cell glutathione level and initiate lipid perioxidation that produce malondialdehyde (MDA). MDA resulted in injury of the membranous system of the hepatocyte and thus degeneration and necrosis of hepatocytes[20,21].

The mechanism of lipid accumulation in hepatocytes is not clearly understood but it may be due to overproduction of triglycerides due to increased delivery of dietary fatty acids into the liver[14]. Also, impaired transportation of triglyceride out from the liver due to reduction in biosynthesis of apolipoprotein B may be considered in this regard[15].

Inflammatory cellular infiltration in the liver tissue which is noticed in the present study is most probably due to mediation by cytokines produced by neutrophils and activated monocytes. These inflammatory cytokines include, monocyte chemoattractant protein (MCP), interleukins and tumor necrosis factor (TNF-α)[16]. However, recent studies have reported that the hepatocytes are able to produce these cytokines related to medical co-morbidities of obesity[17].

Vascular congestion and dilatation could be explained by activation of Kupffer cells with subsequent increase in

the production of nitric oxide which is a vasodilator[18]. It may be attributed to inflammatory changes or ischemia and hypoxia following high fat diets[19].

The released cytokines stimulate the production of prostaglandins E2 and collagenase that are involved in collagen deposition with subsequent fibrosis[22]. Moreover, in fatty liver, the transforming growth factor-beta (TGF-β) is elevated and causes activation of hepatic stellate cells (HSCs) which change their phenotype into extracellular matrix producing myofibroblasts (MFBs) with concomitant deposition of collagen type I[23].

On the contrary, Narasimhamurthy and Raina 2000[7] concluded that there were no significant changes in histology of rats fed thermally oxidized edible oils for 20 weeks. The authors claimed that to low concentrations of toxic materials produced during frying or the diet was nutritionally balanced as regard macro- and micronutrients and thus overcame the possible toxicity.

In the current study, electron microscopy of hepatocytes of rats fed with diet containing 5-times heated palm oil for 8 weeks showed deposition of lipid droplets, irregular small sized nuclei with condensed chromatin, multiple disrupted mitochondria, and dilatation of endoplasmic reticulum. The present ultrastructural findings agree with those of Hassan *et al*., 2018[19]. The nuclear changes could be attributed to mitochondrial dysfunction with accompanying decrease in oxidative phosphorylation leading to decrease in ATP level which causes structural disruption of protein synthetic apparatus followed by cellular degeneration[26]. Cytoplasmic vacuolations might be due to oxidative stress and lipid peroxidation that compromise membranes of the cell organelles thus increasing their permeability[27]. Moreover, rarefaction of the cytoplasm may be due to proliferation of smooth endoplasmic reticulum and glycogen accumulation[28]; while dilatation of rough endoplasmic reticulum cisternae resulted from lipid perioxidation or protein retention due to reduced secretory activity[29]. Mitochondrial abnormalities that is observed in the present study may be caused by decreased intra mitochondrial protein synthesis, increase of cytosolic calcium caused by oxidative stress and respiratory chain dysfunction[28,30].

Morphometric data of the current study showed reduction in nuclear area of hepatocytes of H5 group indicating that consumption of repeatedly heated palm oil resulted in liver degeneration; however, the reduction in nuclear area was non-significant. The central vein diameter was significantly increased in H5 group than the control group. The mean area percentage of collagen fibers deposition was significantly increased in H5 group when compared to control group. Similarly, Hassan *et al*., 2018[19] found that the rats of the experimentally induced fatty liver group showed a statistically significant increase in the mean area percentage of collagen fibers deposition when compared with the control group. Morphometric results reinforce the histological findings of the present study.

To the best of knowledge, no previous studies observed the effects of stoppage of consumption of repeatedly heated palm oil on the liver of rats. The liver of the recovery group of the present study showed improvement of the histological changes of the liver of rats with restoration of the trabeculated appearance of hepatic cords. However, there were still few inflammatory cellular infiltrations, traces of collagen depositions and degeneration of small number of hepatocytes. The restriction of consumption of the heated palm oil might result in decrease in lipid peroxidation and inducible nitric oxide in the liver as well as decrease in liver triglyceride levels.

Tanaka and Miyajima 2016[31] emphasized that the liver has extraordinary regeneration capacity upon various injuries and the most effective treatment of early stages of liver fibrosis is elimination of the causative agent but it is not sufficient to restore its original picture in many cases. They postulated that hepatocytes damaged by free radicals produce damage-associated molecular patterns (DAMPs) which induce inflammation and activation of non-parenchymal cells that contribute to regeneration. The inflammation stimulates hepatic stellate cells (HSCs) to initiate liver regeneration by proliferation of hepatocytes and by secreting hepatocyte growth factor (HGF) while the activated Kupffer cells secrete interleukin-6 (IL-6) which induces hepatic expression of multiple genes associated with acute phase proteins, redox, cell-cycle and anti-apoptosis to stimulate the proliferation of remnant hepatocytes[32]. In addition, liver sinusoidal endothelial cells (LSECs) play an important role in hepatic sinusoidal remodeling after liver injury[33].

In addition, histological assessment of biopsy tissue from patients with liver fibrosis and from animal models of fibrosis indicated that recovery with remodeling of the excess collagens was possible[37]. This explains the decreased collagen deposition in liver of recovery group of the present work.

Electron microscopy of the recovery group of our study showed that the ultrastructural picture of hepatocytes became more or less normal with restoration of most cytoplasmic organelles. This could be explained by decreased oxidative stress following stoppage of consumption of heated palm oil. So that, the normal level of mitochondrial protein synthesis was restored and lead to improvement of structure of mitochondrial cristae and thus regain its ability of division and respiratory chain function that produce energy needed for cellular activity and regeneration[36]. Moreover, most of liver cells have two nuclei indicating extreme capacity for regeneration and lysosomes can get rid of debris of degenerating particles.

In addition, Fan *et al*. 2003[24] found that switching of rats fed high fatty diet for 10 weeks and developed non-alcoholic steatohepatitis to low caloric diet for 2 weeks leads to reversibility of obesity and hepatic steatosis but does not exert significant effect on the hepatic inflammatory changes. In the same context, Lieber *et al*., 2004[25] showed

that features of steatohepatitis produced by the high fatty diets are significantly diminished by dietary restriction but the pathology is not fully abolished. The authors explained that by decrease of tumour necrosis factor – alpha which is a part of the inflammatory response that decreases strikingly after dietary fat restriction.

In the same context, Tamada *et al*., 2016[34] stated that switching of male rats fed high fat diet for 8 weeks to control diet for 6 weeks leads to improvement of hepatic steatosis but does not completely eliminate it. Similarly, Siersbæk *et al*., 2017[35] reported that mice which were fed high fat diet for 7 weeks followed by normal chow for another 5 weeks, present normal body weight and the hepatosteatosis is reversed.

Moreover, the morphometric measurements of the recovery group of the present work including hepatocyte nuclear area, central vein diameter and area percentage of collagen fibers deposition were improved as compared with H5 group. Thus, the reversal of injurious changes resulting from feeding repeatedly heated palm oil could emphasize the good effects of restriction of heated palm oil consumption for 4 weeks.

CONCLUSION

The current study confirmed the injurious effects of consumption of repeatedly heated palm oil on liver tissue. The damaging effects range from mononuclear cellular infiltration, vascular congestion, increased deposition of collagen fibers to signs of cellular degenerations and necrosis. On stoppage of consumption of reheated palm oil, the liver showed impressive amelioration of the damaging effects where its normal architecture was mostly regained. Thus, people should be encouraged to reduce their usage of repeatedly heated palm oil in frying purposes.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدليل النسيجي الشكلي على الانتعاش الكبدي للجرذان التي تم تغذيتها بزيت النخيل المسخن بشكل متكرر

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الخلفية: تم التحقق في التسخين المتكرر للزيوت النباتية وخاصة زيت النخيل الذي يستخدم بشكل شائع وتجاري في قلي الطعام في المنازل والمطاعم لمعرفة خطورته على الحالة الصحية. يبدو أنه يحمل مخاطر محتملة على أنسجة الكبد.

الهدف: دراسة تأثير تناول الأطعمة المقلية بزيت النخيل المسخن ٥ مرات على كبد ذكور الجرذان البالغة لمدة ٨ أسابيع وإمكانية استعادة التغيرات النسيجية بعد التوقف عن استخدام زيوت النخيل الساخنة لمدة ٤ أسابيع.

المواد والطرق المستخدمة: تم اختيار ثلاثين جرذا من الذكور البالغين وقسموا بالتساوي إلى ٣ مجموعات. مجموعة التحكم (تلقت نظامًا غذائيًا طبيعيًا متوازنًا وماء الصنبور لمدة ٨ أسابيع)، مجموعة H٥) تلقت نظام غذائي غني بزيت النخيل المسخن ٥ مرات لمدة ٨ أسابيع) ومجموعة التعافي (تلقت نظام غذائي غنى بزيت النخيل المسخن ٥ مرات لمدة ٨ أسابيع ثم تركت على نظام غذائي عادي متوازن لمدة ٤ أسابيع أخرى). في نهاية التجربة، تم تخدير الحيوانات بالإثير ثم التضحية بها. تمت معالجة العينات المأخوذة من الكبد للتقييم النسيجي. أجريت قياسات مورفومترية وخضعت البيانات المجمعة لتحليل إحصائي.

النتائج: أظهر الفحص النسيجي العديد من التغيرات في أنسجة الكبد لمجموعة H٥ بما في ذلك قطرات الدهون، والتسلل الخلوي وحيد النواة، وتمدد الوريد المركزي والجيوب الدموية، وترسب الكولاجين وتنكس ونخر خلايا الكبد. أظهر الفحص الدقيق لخلايا الكبد وجود قطرات دهنية، ميتوكوندريا مشوهة، تفريغ في السيتوبلازم، عدم انتظام الغلاف النووي، وتمدد الشبكة الإندوبلازمية مقارنة بمجموعة التحكم. ولوحظ ان القياسات المورفومترية تدعم النتائج النسيجية. أظهرت مجموعة التعافي تحسنًا ملحوظا في التغيرات النسيجية والشكلية.

الخلاصة: استهلاك زيت النخيل المعاد تسخينه في أغراض القلي يضر بأنسجة الكبد وهذه الإصابات تظهر إمكانية التعافي بعد التوقف عن تناوله.