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RESEARCH ARTICLE

Potential hazards of feeding albino rats on diet containing repeatedly boiled cooking oil: Clinicopathological and Toxicological studies

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Boiling of vegetable oils repeatedly leads to formation of a class of toxic substances. The potential clinicopathological and toxicological effects of repeatedly boiled oil (RBO) in albino rats were investigated in this study. The results showed a significant increase in body weight gains in fresh oil (FO) and one time boiled oil (OBO) groups while it was significantly decreased in RBO group. The liver, kidney and brain-relative weights showed a significant increase in RBO group. The hematological parameters implicated a significant increase in total leukocyte count, neutrophil and basophil percentages while lymphocyte percentage showed a significant decrease. The lipid profile showed a significant increase in serum triglycerides, total cholesterol and LDL levels while HDL level decreased significantly particularly in OBO and RBO groups. A significant decrease in the concentrations of total protein and albumin and a significant increase in the levels of glucose, BUN, creatinine and serum enzymatic activities of ALT and AST in OBO and RBO groups were recorded. The liver, kidneys and brain of treated groups implicated presence of inflammatory and degenerative changes. It is concluded that, repeat boiling of dietary oils should be avoided as this results in significant adverse clinicopathological, toxicological and pathological effects with weight losses in experimental animals.

INTRODUCTION

Lipids make up one of the most essential components of food. They decide not only of taste, sent, color or the texture of food product, but they also give the feeling of satiety **(Frankel, 1998)**. First of all, fats are indispensable substances in human nutrition **(Keller et al., 2004).** Vegetable oils supply energy-dense calories to diet. They may also contain essential fatty acids and serve as a vehicle for absorption of fat soluble vitamins **(Jan, 2014)**. Deep frying is one of the most common and oldest methods of food preparation worldwide and deep fried foods are increasingly being consumed by public **(Chacko and Rajamohan, 2011).** Upon frying, heating causes the oil to undergo a series of chemical reactions like oxidation, hydrolysis and polymerization which produce volatile and non-volatile components having important physiological effects. During this process, many primary and secondary oxidation products are produced, which lead to undesirable taste and decomposing nutritional quality as degradation of tocopherols, essential amino acids and fatty acids present in food. Fried products absorb a large quantity of its frying oil, thus eventually accumulating degradation products to become part of the diet **(Vlachos et al., 2006; Choe and Min, 2007; Halvorsen and Blomhoff,2011; Ghidurus et al., 2011).** The final products of oil frying are dependent on the time/temperature of frying, the frying method (deep-fat frying or shallow-frying), the type of oil,

the saturation ratio of the oil, and the presence of a catalyst/antioxidant and oil family **(Van de Voort et al., 1994; Gupta, 2005; Kamal.-Eldin, 2006; Halvorsen and Blomhoff, 2011).** Unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids and for this reason they are good source of free radicals **(Chacko and Rajamohan, 2011).**

Toxic and harmful free radicals of thermally oxidized oil may be enzyme inhibitors, vitamin destroyers, gastrointestinal irritants or lipid oxidation products which induce oxidative stress **(Clark and Serbia, 1991;Srivastava et al., 2010)**. This oxidative stress is thought to be involved in the development of many harmful and pathological effects in many tissues after consumption of heated oil (**Isong et al., 1992; Narasimhamurthy and Raina, 1999; Shastry et al., 2011).** For example, long term consumption of oxidized oils has been reported to cause growth retardation, atherosclerosis, thrombosis, fatty livers, essential fatty acid and nucleic acid deficiencies and micronutrient malnutrition resulting in deactivation of key metabolic enzymes **(Hill et al., 1982; Izaki et al., 1984; Golden and Ramdath, 1987; Isong et al., 1992; Osim et al., 1992).**

Sunflower, soybean oils are highly consumed in Egypt. They are used as a blend of commercial cooking oil and there is a tendency for the oil to be used repeatedly in frying and cooking. Such practice appears to cut the cost of cooking but unfortunately without consideration of its effect on health. To evaluate quality and safety of food products during high temperature processing or deep frying, it is important to understand how thermal changes in the oil at frying can cause risk effect to consumer, so the present study was designed to investigate possible hazard effects of reused cooking oils on albino rats.

Materials and Methods

Oil

The most common commercial oil in Egypt is a mixture of soybean oil and sunflower oil. Both are vegetable oils containing polyunsaturated fatty acids (FA) mainly oleic and linoleic acid. The oil was used as fresh unheated oil (FO); one time boiled oil (OBO) and repeatedly boiled oil (RBO). Fresh oil was purchased from a local market while oil boiled once and repeatedly boiled oils were obtained from a restaurant uses the same type of oil in Giza governorate, Egypt after one time of food frying and at the end of the working day respectively.The repeatedly boiled oil was deep or very dark brown in color, viscous with rancid taste and odor.

Experimental animals: housing and grouping

Forty healthy male and female Sprague Dawley rats weighing 110–150 g were used for this study. The animals were housed in plastic cages at room temperature with 12 hours day light cycle. They were allowed to acclimatize for 10 days before the commencement of the experiment and fed on standard diet (Table 1) formulated according to NRC recommendation (NRC, 1995). Access to food and water was *ad libitum*. Animal rearing and handling and all experimental design were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, University of Sadat City, Egypt. The rats were randomly divided into four experimental groups each of ten (5 males and 5 females, in separate cages). The first group was fed on the basal diet (control) while the other three groups fed on the basal diets mixed with 15% g/g of FO, OBO and RBO respectively **(Shastry et al., 2011)**. The treatments were added daily to ration till the end of experiment (3 months).

Samples collection:

At the end of the experiment, the animals were fasted overnight, weighed (to calculate body weight gains) and then anaesthetized; blood samples were collected from the median canthus of the eye using heparinized capillary tube. Blood sample of each rat was received in two tubes (tube containing disodium ethylene diaminetetracetic acid (EDTA) for hemogram and plain centrifuge tube for serum separation). Serum samples were divided into aliquots in Epindorf tubes so that each was used for one time. Serum samples were stored at -20°C until assayed for biochemical parameters. After blood collection, rats were sacrificed and the organs of each rat (liver, kidneys, brain, spleen and heart) were collected and weighed separately to calculate relative organ weight. Tissue samples of the liver, kidneys and brain were collected in 10% formalin for the histopathological studies.

Evaluated Parameters:

Hematological parameters:

The evaluated hematological parameters included estimation of red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leukocytic (TLC) and differential leukocyte counts. These parameters were done according to the routine hematological procedures adopted by **Feldman et al. (2000)**.

Serum Biochemical parameters:

Serum samples were assessed for lipid profile parameters including triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL) and high density lipoproteins (HDL). Serum levels of glucose, total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cr) and serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also recorded.All biochemical parameters were determined by spectrophotometric method using commercially available test kits supplied by Biomed diagnostics (Egypt) and following the manufacturer's instructions.

Histopathological alterations:

Tissue samples of the liver, kidneys and brain intended for histopathological examination were kept in 10% formalin, then prepared and stained according to **Bancroft et al. (1996).**

Statistical analysis

All values are expressed as the mean ± SEM (standard error of mean). All results were analyzed by one way analysis of variance (ANOVA) with the aid of the SPSS version 16 software (SPSS: statistical package for social sciencesInc, Chicago, USA). $P < 0.05$ was regarded statistically significant.

Table 1. Ingredients and nutrient composition (% DM) of growing rat ration by NRC (1995).

Ingredient	(%)
Sorghum	39.00
Yellow corn	31.60
Barley	8.00
Poultry by-product meal	8.00
Corn cob	7.30
Vegetable oil	4.00
Dicalcium phosphate[1]	0.20
Premix[2]	0.50
Limestone	0.40
DL-Methionine[3]	0.40
L- Lysine-HCL[4]	0.30
Starch	0.30
Nutrient	
ME(kcal/kg)	3400
Crude protein	12
Crude fiber	4.5
Ether Extract	7.5

[1]Dicalcium phosphate, 18% granular phosphate and 23 % calcium. [2]Each 3 kilograms contain: vitamin A 12000000 IU, vitamin D3 3000000 IU, vitamin E 40000 mg, vitamin K3 3000 mg, vitamin B1 2000 mg, vitamin B2 6000 mg, vitamin B6 5000 mg, vitamin B12 20 mg, niacin 45000 mg, biotin 75 mg, folic acid 2000 mg, pantothenic acid 12000 mg, manganese 100000 mg, zinc 600000 mg, iron 30000 mg, copper 10000 mg, iodine 1000 mg, selenium 200 mg and cobalt 100 mg. [3]DL-Methionine, Met AMINO® (DL-2-amino-4-(methyl-thio)-butane acid, DL-methionine, α-amino-Y-methyl-oily acid) by Feed Grade 99% (EU). [4]L-Lysine HCL 99% (Feed Grade) L-Lysine: 78.0% Min (Indonesia).

Results

Body weight gains (BWG) and relative organ weights:

The data in Table 2 showed that the body weight gain values of the rats maintained on fresh non-heated oil and one time boiled oil diets for 3 months were significantly higher than those of the control and the animals fed on diet contained repeatedly boiled oil. The latter group showed significant reduction in BWG compared to control. The relative weights of liver, kidneys and brain of rats maintained on repeatedly boiled oil diet showed a significant increase in comparison to control and other treated groups. Relative weights of spleen and heart showed insignificant changes in all treated groups (Table 2).

Table 2: Body weight gains (BWG) (g) and relative organ weights (%) of rats fed on fresh non-heated (FO), one time boiled (OBO) and repeatedly boiled (RBO) oils compared to the control group (C). Data are represented as mean±SEM, values of six determinations

Parameter	C	FO	OBO	RBO
BWG (g)	103.43±4.08 ^{b}	118.86±6.67 ^{a}	124.43± 6.1 ^{a}	65.43±2.53 ^{c}
Relative liver weight	2.93±0.13 ^{b}	2.94±0.13 ^{b}	3.01±0.12 ^{ab}	3.61±0.17 ^{a}
Relative kidneys weight	0.60±0.03 ^{b}	0.66±0.01 ^{b}	0.64±0.03 ^{b}	0.80±0.04 ^{a}
Relative spleen weight	0.34±0.00	0.39±0.06	0.38±0.00	0.32±0.02
Relative heart weight	0.34±0.01	0.36±0.01	0.39±0.03	0.39±0.01
Relative brain weight	0.75±0.02 ^{b}	0.74±0.02 ^{b}	0.71±0.04 ^{b}	0.86±0.02 ^{a}

Means within rows with different superscripts differ at P < 0.05.

***Hematology*:**

With respect to hematological parameters, data shown in Table 3 indicated that there was no significant difference in red cell parameters in the different treatment groups compared to control.

For white blood cells, in comparing to control as well as to the fresh oil groups, there was a significant increase in TLC in groups treated with OBO and RBO but the values of increase were significantly higher in RBO group (Table 4). All treated groups showed an increase in basophil and neutrophil percentages. Lymphocyte percentage showed a significant decrease in OBO and RBO groups. However, there was no significant difference in the percentage of reduction among the twogroups (Table 4).

Table 3: Red blood cell parameters of rats fed on fresh non-heated (FO), one time boiled (OBO) and repeatedly boiled (RBO) oils compared to the control group (C). Data are mean±SEM values of six determinations.

Parameter	C	FO	OBO	RBO
RBC ($x10^6$)	5.89±0.24	5.94±0.19	6.33±0.33	6.10±0.25
PCV (%)	35.33±1.20	35.66±1.15	36.00±2.00	36.66±1.52
Hb (g/dl)	11.75±0.62	11.88±0.38	12.66±0.66	12.21±.54
MCV (fl)	59.93±0.81	60.04±0.17	60.03±0.98	60.09±0.24
MCH (pg)	19.94±0.44	20.00±0.50	20.00±0.69	20.01±0.28
MCHC (%)	33.25±0.17	33.31±0.20	33.31±0.17	33.30±0.14

Means within rows with different superscripts differ at P < 0.05.

Table 4: White blood cell parameters of rats fed on fresh non-heated (FO), one time boiled (OBO) and repeatedly boiled (RBO) oils compared to the control group (C). Data are mean±SEM values of six determinations.

Parameter	C	FO	OBO	RBO
TLC (x103)	6.40±0.20 ^{c}	6.17±0.21^{c}	7.30±0.26^{b}	8.60±0.37^{a}
Neutrophil (%)	13.00±3.02 ^{c}	14.60±2.00b^{c}	16.05±1.52^{ab}	18.01±1.52^{a}
Eosinophil (%)	2.10±0.41	2.00±0.34	2.23±0.27	2.33±0.42
Basophil (%)	0.10±0.01^{b}	0.30±0.07^{a}	0.31±0.05^{a}	0.31±0.01^{a}
Lymphocyte (%)	82.05±2.12^{a}	80.23±1.78^{ab}	78.23±1.64 ^{b}	77.20±1.52 ^{b}
Monocyte (%)	2.50±0.33	2.21±0.53	2.45±0.57	2.22±0.77

Means within rows with different superscripts differ at P < 0.05.

Blood lipids:

The results of Table 5 concerning lipid profile showed gradual significant increase in triglycerides in FO, OBO and RBO treated groups compared to control. There was a significant increase of total cholesterol and LDL and a significant decrease in HDL levels in groups fed OBO and RBO, whereas fresh oil group did not show any significant changes.

Table 5: Lipid parameters (mg/dl) of rats fed on fresh non-heated (FO), one time boiled (OBO) and repeatedly boiled (RBO) oils compared to the control group (C). Data are mean±SEM values of six determinations

Parameter	C	FO	OBO	RBO
Triglycerides	163.42±4.24 ^{c}	179.14±6.19 ^{b}	191.33±8.33 ^{ab}	207.10±4.25 ^{a}
Total cholesterol	129.33±2.20 ^{c}	146.66±3.15 ^{bc}	165.00±6.80 ^{b}	191.26±2.52 ^{a}
LDL	33.72±4.62 ^{b}	36.41±5.38 ^{b}	57.76±1.16 ^{a}	63.51±3.54 ^{a}
HDL	62.93±2.31 ^{a}	59.57±6.17 ^{ab}	58.03±3.18 ^{b}	53.79±4.24 ^{c}

Means within rows with different superscripts differ at P < 0.05.

Serum biochemistry:

The data of serum biochemical parameters presented in Table 6 showed a significant decrease in the concentrations of total protein and albumin in groups fed on one time boiled and repeatedly boiled oils compared to control. On the other hand, the two groups showed a significant increase in the levels of glucose, BUN and creatinine in addition to serum enzymatic activities of ALT and AST. Animals fed on fresh oil did not show significant changes in these biochemical variables

Table 6: Serum biochemical variables of rats fed on fresh non-heated (FO), one time boiled (OBO) and repeatedly boiled (RBO) oils compared to the control group (C). Data are mean±SEM values of six determinations.

Parameter	C	FO	OBO	RBO
TP (g/dl)	6.10±0.30[a]	6.17±0.51[a]	5.03±0.26[b]	5.01±0.17[b]
Alb (g/dl)	3.40±0.32[a]	2.43±0.10[ab]	2.01±0.61[bc]	1 .81±0.42[c]
Glucose (mg/dl)	87.65±3.17 [b]	89.56±1.10[b]	204.52±9.48[a]	218.07±10.25[a]
BUN (mg/dl)	12.35±2.41[c]	14.18±1.57[bc]	17.22±1.74[b]	22.04±0.82[a]
Cr (mg/dl)	0.8±0.01[b]	0.7±0.07[b]	1.10±0.05[a]	1.21±0.03[a]
ALT (U/l)	65.05±4.12[b]	59.20±6.78[b]	86.11±7.44[a]	94.26±5.52[a]
AST (U/l)	58.41±4.31[c]	61.21±3.53[c]	91.45±8.57[b]	104.22±4.33[a]

Means within rows with different superscripts differ at P < 0.05.

Histopathological findings:

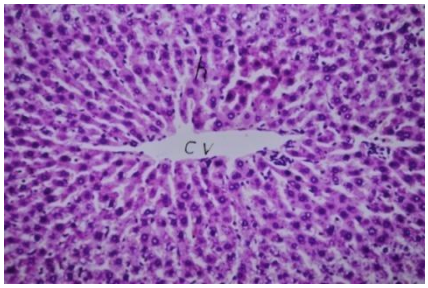
With regard to liver, no histopathological alterations were noticed in control rat liver (Fig.1). Rats fed on fresh oil diet showed slight fatty degenerative change and sporadic Kupffer cells proliferation (Fig.2).In groups maintained on oil boiled once and repeatedly boiled oil diets, the portal area showed severe dilatation and congestion in the portal vein, edema, hyperplasia of bile ducts, newly formed bile ductules and fibrosis in the surrounding tissue (Fig.3). Degeneration and vacuolation of hepatocytes (Fig.4) and focal Kupffer cells proliferation (Fig.5) were also observed in rats fed on RBO diet.

For renal tissue, normal histological structure of the glomeruli and tubules was recorded in kidneys of rats in control group (Fig.6). In fresh oil diet group, vacuolation was noticed in the lining endothelium of the glomerular tuft (Fig.7) while, focal fibrosis between renal tubules were recorded in group fed on one time and repeatedly boiled oil diets (Fig.8). Also, in the repeatedly boiled oil group, rats showed focal inflammatory cells infiltration in between the tubules associated with dilatation in the blood vessels, the glomerular tuft showed vacuolization in the lining endothelium (Fig.9). The lining epithelium of the tubules had many fat vacuoles (Fig.10).

Considering brain tissue, normal histological structure of the meninges, cerebral cortex was recorded in control group (Fig.11). In both fresh and boiled groups, the deep cerebrum had fat vacuoles in the matrix (Fig.12) as well as focal gliosis (Fig.13). Brain of rats fed on repeatedly boiled oil showed focal gliosis (Fig.14) The medulla oblongata showed also vacuolation in the matrix (Fig.15).

Figures (1-15)

1



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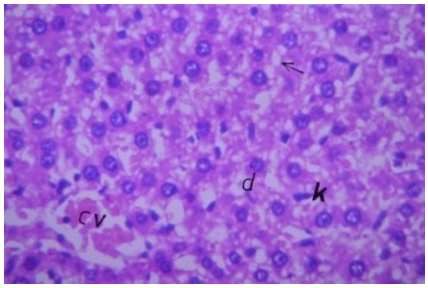

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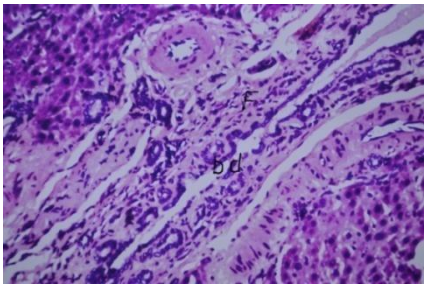

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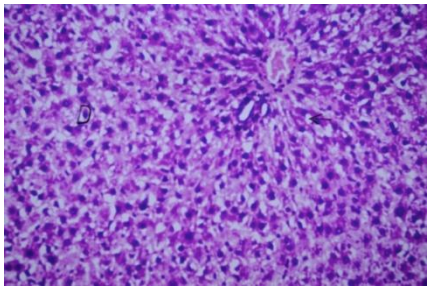

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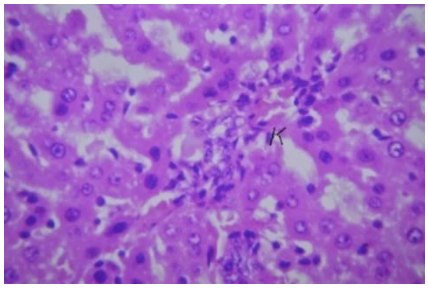

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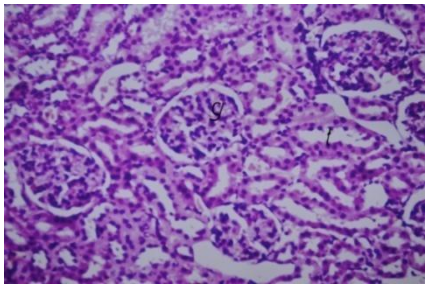

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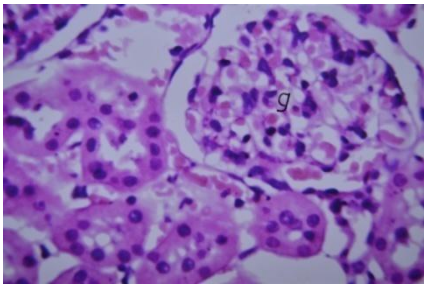

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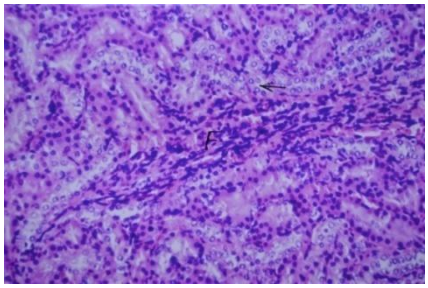

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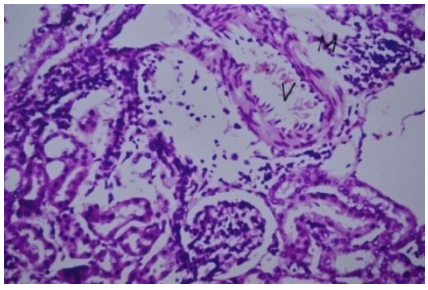

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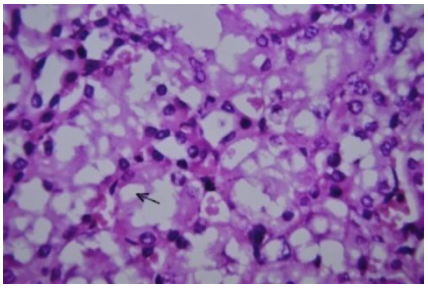

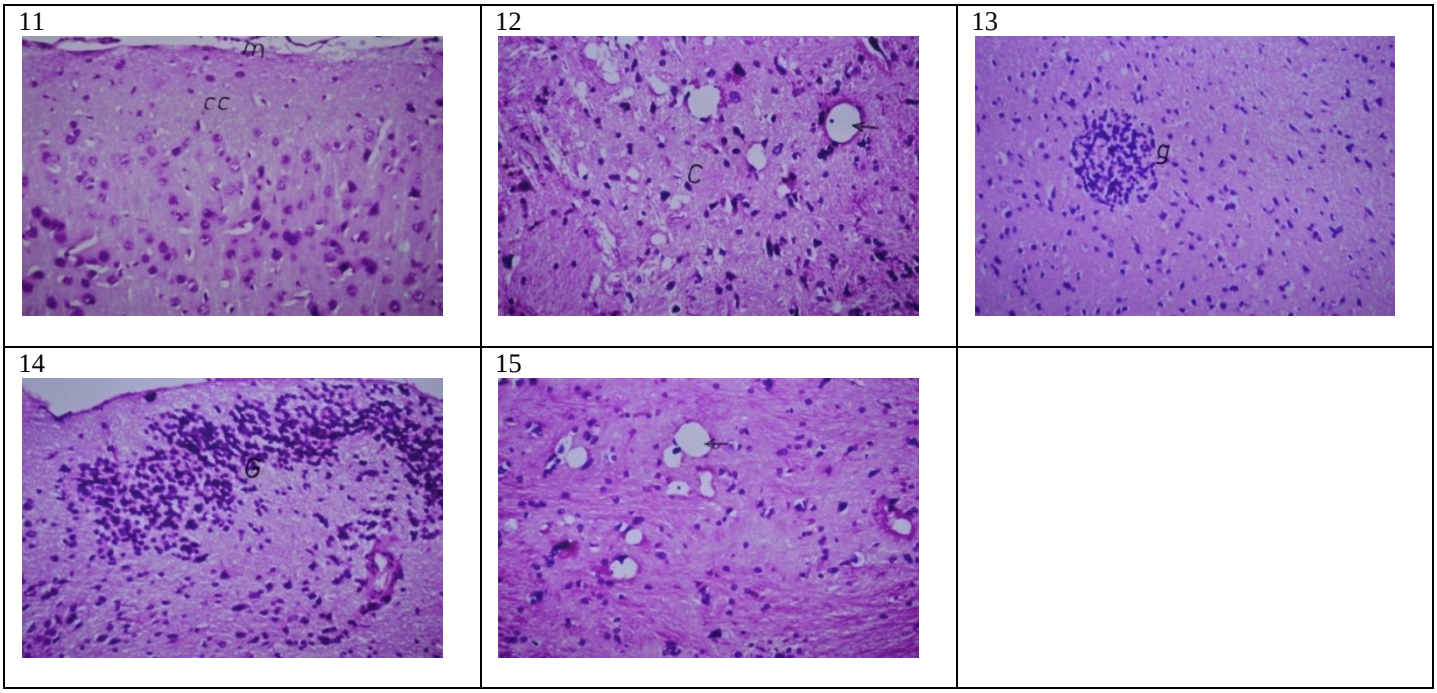

Control rat liver, normal histopathological structure of hepatocytes (h) around central vein (cv) (Fig.1). Rats fed on fresh oil diet showed slight fatty degenerative change (d) and sporadic Kupffer cells proliferation (k) (Fig.2).In groups maintained on oil boiled once and repeatedly boiled oil diets, the portal area showed severe dilatation and congestion in the portal vein, edema, hyperplasia of bile ducts (bd), newly formed bile ductules and fibrosis (f) in the surrounding tissue (Fig.3). Degeneration and vacuolation of hepatocytes (d, arrow) in rats fed on RBO diet (Fig.4) and focal Kupffer cells proliferation (k) in rats fed on RBO diet (Fig.5) (stained with H&E, X20, 40)
Normal histological structure of glomeruli (g) and tubules (t) of kidneys in rats of control group (Fig.6). In fresh oil diet group, vacuolation was noticed in the lining endothelium of the glomerular tuft (g) (Fig.7). Focal fibrosis between renal tubules was recorded in group fed on one time and repeatedly boiled oil diets (f) (Fig.8). Focal inflammatory cells infiltration (m) in between the tubules associated with dilatation in the blood vessels (v), the glomerular tuft showed vacuolization in the lining endothelium (Fig.9) and presence of many fat vacuoles in lining epithelium of the tubules (arrow) (Fig.10) in the repeatedly boiled oil group (stained with H&E, X20, 40)
Normal histological structure of the meninges (m), cerebral cortex in brain of control rats (cc) (Fig.11). In both fresh and boiled oil groups, the deep cerebrum had fat vacuoles in the matrix (arrow) (Fig.12) as well as focal gliosis (g) (Fig.13). Brain of rats fed on repeatedly boiled oil showed focal gliosis (g) (Fig.14) The medulla oblongata showed vacuolation in the matrix (arrow) (Fig.15) (stained with H&E, X20, 40)

Discussion

Recently and to prevent atherosclerosis in human, saturated animal fats were replaced byvegetable oils, due to their high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However during deep-frying, cooking oils are repeatedly used at elevated temperatures in the presence of atmospheric oxygen leading to oxidative degradation of these oils. Some of these degradation products can have adverse effects on human health **(Martin and Ames, 2001; Schiller et al., 2002).**
In the present study, sunflower and soybean oils were evaluated when used as fresh, boiled once or repeatedly boiled oils. These oils are highly consumed in Egypt and are used as a blend of commercial cooking oil. Both sunflower and soybean oils contain vitamins E and C which are antioxidants that can scavenge free radicals. These vitamins are destroyed by hydroperoxides generated during peroxidation when frying oil is repeatedly heated thus the antioxidant effect of these vitamins can no longer prevent the oxidation of fatty acids in these oils. The reduction in the vitamin E content of frying oils may contribute to the increased production of reactive oxygen species (ROS) and may cause oxidative damage**(Mensink and Katan, 1990; Andrikopoulos et al., 2002; Oyewole and Olayinka, 2007).**

Effects of dietary fats including vegetable oils on body weight gains and various biological body functions have been reported. In this study, both fresh and oil boiled once increased body weight gain which could imply that these oils support the growth of the rats. This may be referred to presence of long chain fatty acids in fresh sunflower and soybean oils. These long chain fatty acids were documented to be incorporated into chylomicra and transported through lymph not through the bile, so β-oxidation by the liver was suggested to be decreased with prolongation in postprandial energy expenditure and hence elevation in body weight **(Bach et al., 1996; Papamandjaris et al., 1998; St-Onge, 2005).**

In rat fed on repeatedly boiled oil diet, significant decrease in body weight gain was recorded compared with control. Loss of appetite, diarrhea, and disruption of skeletal muscle enzyme activities may be behind growth retardation in this group **(Odutuga and Oyewole, 2006; Shastry et al., 2011).** Moreover, when oxidized oils are fed in large quantities to animals, the taste and odor characteristics of the diet may deteriorate with a subsequent decrease in food intake and growth retardation. In addition, long chain polymers that are produced during oxidation process might cause the fat to be less absorbable and could interfere with the absorption of the other fat-soluble nutrients in the diet. Irritation of intestinal mucosa by peroxides could be another possible reason which might interfere with nutrient absorption **(Kirk, 1984, Chow, 1989; Miller and Long, 1990; Ma´ rquez-Ruiz et al., 2008).** Consumption of repeatedly boiled oil in the diet not only decreases the body weight gain but also induces nutrient deficiencies. This referring to thermal destruction of essential vitamins and fatty acids in the oxidized oil with decreased protein digestibility and absorption due to cross-linking reactions of secondary lipid oxidation products with proteins **(Kirk, 1984).**

Relative organ weights may be used as an index of organ swelling, atrophy, or inflammation **(Oladiji et al., 2009)**. In the present work, The recorded growth in liver, kidneys and brain in rats treated with repeated boiled oil was concomitant with the histopathological alterations as congestion, inflammatory cells infiltration, edema, hypertrophy and fattydegeneration **(Totani and Ojiri, 2007; Shastry et al., 2011).**

Growth of the liver and an increase in kidney volume were previously reported, following the use of degraded oils**(Paul and Mittal, 1997; Owu et al., 1998; Totani and Ojiri, 2007; Oladiji et al., 2009).Billek, 2000**stated that, certain fractions of the heated fats, Total Polar Compounds (TPC) can result in growth retardation, increased liver and kidney weights, and disorders of the enzyme system.

Alterations in the concentrations of major lipids can give useful information on the lipid metabolism and predisposition of an animal to cardiovascular risk. The lipid profile study showed an increase in serum triglycerides levels in all treated groups and in serum total cholesterol and LDL levels in the animals treated with one time and repeatedly boiled oils. On the other hand serum HDL levels were found to be significantly decreased in the boiled oil groups compared to the corresponding control and fresh oil fed rats. Earlier studies reported that serum lipids levels of rats fed repeatedly heated oil were significantly increased (**Tawefik et al., 1998;Ammu et al., 2000; Narasimhamurthy and Raina, 2003; Kamsiah et al., 2006; Liu et al., 2007; Karl et al., 2008).** The increase in triglycerides levels after oil ingestion may be due to the increase availability of substrate free fatty acids for esterification **(Aruna et al., 2004; Shastry et al., 2011**).

Ingestion of oxidized lipids rich in linoleic acid causes profound alteration in membrane composition fluidity and function which is likely to be associated with an enhanced cholesterol turn over **(Aruna et al., 2004; Shastry et al., 2011**). Others added that oxidation process of heated oil causes changes in fatty acid configuration from the cis isomer to the trans. Intake of trans fat was found to be correlated with the increase in serum total cholesterol and LDL levels and the decrease in HDL values **(Donfrancesco et al., 2008; Leong et al., 2008; and Soelaiman and Jaarin, 2008; Badlishah et al., 2013).**

It was proved that hydroxy fatty acids and other secondary lipid oxidation products of oil especially that contain PUSFA cause diarrhea, an impairment of liver function, elevation in serum liver enzymes with a slight hypertrophy and an increase in blood lipids and cholesterol **(Kanazawa et al., 1985; Oaradda et al., 1986; Alexander, 1996).**

Based on the reports regarding the harmful effects of heating oils at high temperature during deep frying, the safety of these oils must be studied from the point of view for their effects on blood and other organ functions. In this respect, the results of this study implicated that the heatingprocessdidnothaveanysignificanteffectsonred cell parameters.Thiswasprovenby similarvaluesincontrol and all treated groups that may referred to short duration of the experiment. Total leukocyte count showed a significant increase in OBO and RBO groups which could be explained by neutrophilia and basophilia. Irritation of intestinal mucosa by peroxides might result in some degree of inflammation as indicated by the diarrhea seen particularly in RBO group and could result in inflammatory response represented by neutrophilia**(Srivastava et al., 2010).**Neutrophilia could be also attributed to the stress to which the animal exposed during the experiment as a result of extensive oil treatment that result in endogenous release of corticosteroids which have major role in regulation of circulating leukocytes **(Duncan et al., 1994).** The significant

increase in basophil percent is suggested to be the result of hyperlipidemia.Basophils and mast cells are the only known sources of activators of lipoprotein lipase. So basophil count can be increased in hyperlipidemia due to any condition **(Duncan et al., 1994)**. Furthermore, the damage to the liver cells observed in this study in RBO group may have caused the insult that contributed to the observed increase in WBC count. In agreement, **Finlayson et al. (1999)** have reported that leukocytosis may occur in hepatic damage.Lymphopenia is a common stress response to corticosteroids which inhibit lymphocyte production or alter lymphocyte distribution in the body **(Duncan et al., 1994).**

Monitoring the serum values of total proteins and albumin revealed a significant decrease which could be attributed to decreased protein digestibility and absorption due to cross-linking reactions of secondary lipid oxidation products with proteins **(Shastry et al., 2011).**Anorexia, off food, and diarrhea may in part participate in the development of hypoproteinemia and hypoalbuminemia. As the liver is the major site of protein synthesis, liver dysfunction could be the cause of decreased serum protein concentrations **(Stockham and Scott, 2002).**

The mean values of serum glucose concentrations were significantly higher in groups fed on oil boiled once and repeatedly boiled oil. Elevated levels of free fatty acids and triglycerides in the blood stream and tissues have been found in many studies to contribute to diminished insulin sensitivity and development of insulin resistance which normally refers to reduced glucose-lowering effects of insulin**(Storlien et al., 1991; Roden et al., 1996; Koyama et al., 1997; Schinner et al., 2005).**Reduced peripheral use of glucose and a failure to suppress glucose production and release into the blood associated with insulin resistance all contribute to elevated blood glucose levels (**Duncan et al., 1994).**

Rats maintained on the oil boiled once and repeatedly boiled oil-based diet showed higher concentrations of BUN and creatinine which implicates renal dysfunction as was also confirmed by the histopathological findings of kidney suggesting renal damage**(Osim et al., 1994; Totani and Ojiri, 2007).**

Evaluation of serum activities of some enzymes demonstrated a significant increase in serum activities of ALT and AST in rats maintained on one time boiled and repeatedly boiled oil diets. Such elevation in these enzymes suggests liver dysfunction from reused edible oils which might be due to the presence of lipid oxidation products of heated oil with increased the chances of peroxidation of the cell membranes. This could result in structural and hence functional changes in membranes and thus leakage of enzymes out from cells**(owu et al., 1998; Oyewole and Olayinka, 2007; Chacko and Rajamohan, 2011; Shastry et al., 2011).**The histopathological study supports the above findings suggesting liver damage.

Overall repeated boiled oil revealed clear adverse effects than boiled oil as hydroperoxides produced at heating of sunflower oil at low temperature show very low absorption in the organisms via the gastrointestinal lumen than secondary oxidized products that are produced at heating of oil at higher temperature **(Frankel, 1998; Billek, 2000; Tres et al., 2010).** Further, after applying spectrophotometer and gas chromatography techniques, it was shown that sunflower is high in polyunsaturated fats and creates the most toxic aldehydes in short time **(Climaco Pinto et al., 2010; Guillén and Uriarte, 2012).**

In conclusion, we have opinion that consumption of food containing thermally heated soybean and sunflower oils may not be completely safe for consumption and may produce considerable damage to the vital organs of the rats. Practically and in veterinary field, there is tendency to add vegetable oil to animal and poultry ration as a source of energy in addition to decrease food consumption and this may have health hazard effects.

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