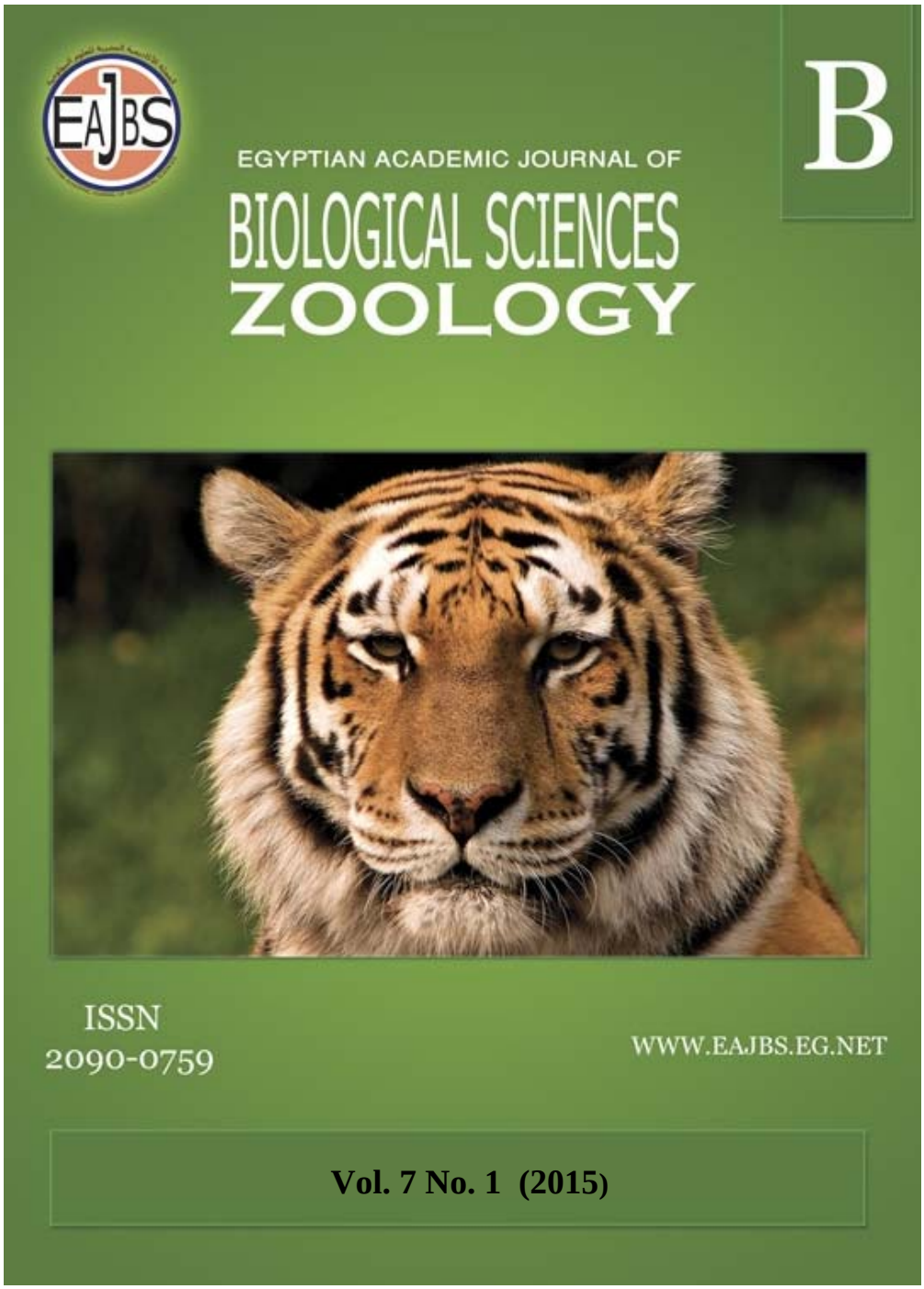

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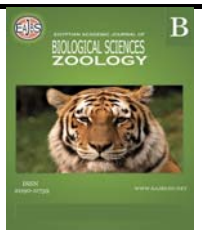

Effect of reused palm oil on biochemical and hematological parameters of mice.

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ABSTRACT

Objectives: the present study was carried out for more advances in understanding the effect of prolonged intake of reusing heated palm oil on hepatic tissue disorders developed in mice.

Methods. Twenty male mice were divided equally into five groups as follows: (a) Control group; (b) Fresh palm oil (FPO); (c) Palm oil heated one time (1HPO); (d) Palm oil heated five times (5HPO); (e) Palm oil heated ten times (10HPO). The amount of palm oil was 30% in all groups and treatment duration continued two months. Blood samples were used for estimation of lipid profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatin kinase (CK)..

Results: In repeatedly heated palm oil fed groups, serum parameters such as lipid profile, ALT, AST and CK as well as blood indices were significantly elevated whereas high density lipoproteins (HDL-ch) levels showed a reduction in all heated oil fed groups.

INTRODUCTION

Deep frying oil is the most common and one of the oldest methods of food preparation worldwide. This method is one of the most popular procedures for food preparation since it is rapid and develops desirable flavors and textures. However, using frying oils repeatedly can produce constituents that not only compromise food quality but also can promote the formation of compounds with adverse nutritional implications and potential hazards to human health (Sanibal and Mancini-Filho, 2004).

Cooking oil is sometimes reused due to its stability at high temperatures (Gupta *et al.*, 2005). During the frying process, various chemical reactions occur, such as thermal oxidation, hydrolysis, and polymerization, due to the exposure of the oil to high temperatures in the presence of air and moisture. As a result, cooking oil decomposes and forms volatile compounds and various monomers and polymers (Andrikopoulos *et al.*, 2002).

The repeated heating of oil at high temperatures (≥180°C) results in the thermal oxidation of the oil, which causes the configuration of the fatty acid to change from the *cis*isomer to the *trans*isomer.

This configuration change causes the polyunsaturated fatty acids (PUFAs) to acquire undesirable properties associated with saturated fatty acids (SFAs), such as their correlation with increased serum total cholesterol (TC) levels and low-density lipoprotein (LDL-ch) (Mensink *et al.,* 1990).

Several studies have also demonstrated the adverse effect of oxidized dietary fats on humans and experimental animals. These include hemolytic anemia, increased blood clotting time and hepatomegaly (Owu *et al.,* 1998). Reproductive toxicity, elevation of total cholesterol and free fatty acid levels of various tissues, thrombocytopenia and enhanced platelet aggregation levels have also been documented (Isong *et al.,* 1992; Narasimhamurthy and Raina, 1999). Other known effects of oxidized fats include essential fatty acid deficiency, nucleic acid deficiency and micronutrient malnutrition resulting in deactivation of key metabolic enzymes (Hill *et al.,* 1982 and Isong *et al.,* 1992).

Other studies reported that reactive oxygen species (ROS), also present in these oils, as inhibitors of mitochondrial function which may also affect health negatively (Costantini *et al.*, 2000 and Lin *et al.*, 2008).

MATERIAL AND METHODS

Experimental animals

This study was performed on male albino mice , initially weighing 20-27 g. Mice were obtained from Science Faculty, Sana'a University. They were housed in stainless steel cages at a well-ventilated animal house. Mice were permitted the following diet and given water *ad libitum* for one week of adaptation period prior to the experimental work.

Research design

A total of 20 albino mice were divided after adaptation into five groups of four animals each. The first group was considered as control group received diet formula without any supplementation according to (Ali *et al.,* 2012). (ii) Fresh palm oil (FPO). (iii) one time-heated palm oil (1HPO), (iv) five times-heated palm oil (5HPO), and (v) ten times-heated palm oil (10HPO) according to (Jaarin *et al.,* 2006). The amount of palm oil was 30% in all groups and treatment duration continued two months according to Ali *et al.* (2012).

Sample collection

At the end of this study, all mice were fasted overnight and sacrificed. From each muse, two blood samples were collected. The first blood sample was taken on EDTA as anticoagulant for the determination of hematological parameters (Gumaih, 2010). The second blood samples were withdrawn and serum was separated by centrifugation at 3000 rpm to estimation TC,HDL-ch, LDL-ch and TG. Also, serum was used for determination of AST, ALT and CK.

Biochemical analysis

Serum concentrations of TC, TG, LDL HDL, LDL, glucose, AST and ALT were determined calorimetrically according to Pisani *et al.* (1995); Siedel *et al.* (1993); Matsuzaki *et al.* (1996); Rifai *et al.* (1992); Kunst *et al.* (1984); Tietz (2006); Bergmeyer (1985 and1986), respectively.

Blood indices

This study examines the complete blood count (CBC) and differential parameters according to the method of de Beca *et al*. (2006). That were red blood corpuscles (RBCs) count, total white blood cells (T-WBCs) count, hemoglobin (Hb) ,

hematocrit (Hct%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis

All data are represented as means ± SE. One-way analysis of variance ANOVA followed by Least Significant Difference (LSD) test was used to determine differences among means of investigated groups. The differences were considered statistically significant at $P < 0.05$ (SPSS, 20 2013).

RESULTS

The present study investigates the impact of palm oil (PO) on the biochemical and hematological changes developed in male mice.

Body weight results

The data presented in Table (1) revealed that the weights of the mice fed reused palm oil for ten times were significantly increased than those of the control, fresh and one time groups at the seventh tested week. Similarly, the ingested ten times group had a significant increase in weight in comparison with control and five times groups. On the other hand there were non-significant differences between all groups in liver weight in all experimental weeks.

Table 1: Effect of repeatedly palm oil on the weight (g) of male mice.

Weeks Groups	1	2	3	4	5	6	7	8	9	Liver
Control	20.3± 0.25	22.5± 1.32	23.0± 1.47	25.5± 1.26	24.8± 1.31	25.0± 1.22	26.5± 1.50	26.3± 0.85	23.00± 0.82	1.55± 0.13
Fresh	24.0± 0.71^{a**}	25.3± 0.75	25.3± 0.75	26.5± 0.50	26.3± 0.25	26.3± 0.25	26.5± 1.71	29.0± 1.00^{a*}	24.50± 0.87	1.43± .050
One time	25.0± 0.58^{a***}	25.0± 1.00	25.3± 1.11	26.0± 1.08	25.5± 0.65	25.5± 0.65	26.8± 0.48	27.3± 0.48	25.50± 1.19	1.45± 0.13
Five times	25.0± 1.08^{a***}	26.6± 2.04^{a*}	26.5± 2.22^{a*}	26.8± 2.29	24.8± 1.79	24.8± 1.79	27.0± 1.58	27.0± 1.47	27.75± 1.11	1.58± 0.08
Ten times	25.5± 0.29^{a***}	25.8± 0.48a*	27.3± 063^{a*}	29.0± 0.82^{a*}	27.5± 0.96	27.5± 0.96d*	30.3± 1.11^{a*b*c*}	29.8± 1.11a*d*	25.50± 1.76	1.65± 0.12

Values are expressed as means ± SE.
a: significantly differences from the control group,
b: significantly differences as compared with the fresh group.
c: significantly differences when compared with the onetime group.
d: significantly differences in comparison with the five time group.

Biochemical results

In serum

Total cholesterol (TC) and triglycerides (TG) levels

As shown in Table (2) TC and TG in serum were found to significantly increase in mice fed with repeated use palm oil in comparison with control.

Table 2: Effect of repeatedly heated palm oil on serum TC and TG in male mice.

Parameters Groups	**T-Ch** mmol/l	**TG** mmol/l
Control	1.45±0.12	0.60 ±0.05
One time	4.61±0.38 ^{a***}	1.54±0.06^{a***}
Five times	3.09±0.44 ^{a**b**}	1.30±0.15^{a***}
Ten times	4.13 ±0.25^{a***c*}	1.53±0.11^{a***}

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in compari-son with the five time group.

High- density lipoprotein cholesterol (HDL-ch) and low-density lipoprotein cholesterol (LDL-ch)

The present data in Table (3) showed that serum HDL-ch levels were significantly elevated in all treated groups in comparison with control. On the other hand, LDL-ch was significantly increased in one time and ten times groups as compared to control.

Table 3 : Effect of repeatedly heated palm oil on serum HDL-ch and LDL-Ch in male mice.

Parameters	**HDL-ch**	**LDL-ch**
Groups	mmol/l	mmol/l
Control	1.21±0.31	0.45±0.09
One time	4.81±0.32 ^{a***}	1.18±0.25^{a*}
Five times	3.15±0.29^{a**b**}	0.90±0.28
Ten times	4.55±0.37 ^{a***c*}	1.14±0.25^{a*}

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in compari-son with the five time group.

Parameters of some ratios

As indicated in Table (4) serum levels of TG/HDL-ch and HDL-ch/LDL-ch were significantly lowered in all treated groups when compared with animal control group.

Table 4: Parameters of some ratios in serum of male mice.

Parameters Groups	**TG/HDL-ch**	**LDL-ch/ HDL-ch**
Control	1.21±0.16	0.42±0.09
One time	0.32±0.01^{a***}	0.24±0.05^{a*}
Five times	0.43±0.08^{a***}	0.22±0.05^{a*}
Ten times	0.36±0.03^{a***}	0.25±0.05^{a*}

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in comparison with the five time group.

Serum glucose level

As shown from the data in Table (5) serum glucose level appeared no significant differences between all experimental groups of male mice.

Table 5: Effect of repeatedly heated palm oil on serum glucose level of male mice.

Parameters	**Glucose**
Groups	mg/dl))
Control	4.35± 0.87
One time	3.98±0.34
Five times	4.90±0.39
Ten times	4.16±0.22

Values are expressed as means ± SE.
a :significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in compari-son with the five time group.

Aspartate aminotransferase (AST) activity and alanin aminotransferase (ALT) activity

The present data Table (6) showed that AST activity and ALT activi-ty were significantly increased in all treated groups in the serum of mal mice when compared with control group.

Table 6: Effect of repeatedly heated palm oil on AST activity and ALT activity in serum of male mice.

Parameters **Groups**	**ALT** u/l))	**AST** (u/l)
Control	140.70±6.83	25.73±1.23
One time	206.25±32.11[a*]	57.75±3.71[a**]
Five times	227.13±27.96 [a*]	66.00±10.34[a**]
Ten times	217.96±28.04 [a*]	54.75±7.31[a**]

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in compari-son with the five time group.

White blood cells (WBCs), red blood cells (RBCs) , he-moglobin (Hb) and hematocrit (Hct) %
Obtained data from Table (7) indicated that WBCs were in-creased in animals fed diet with one time and five times reused palm oil when compared with control group but significantly decreased with ten times group in comparison with control group.
On the other hand RBCs count, Hb and Hct were significantly decreased in five times and ten times groups as compared to control and one time groups.

Table 7: Effect of repeatedly heated palm oil on WBCs, RBCs, Hemoglobin (Hb) and Hematocrit (Hct%) in male mice.

Parameters **Groups**	**WBCs** (10^3/ul)	**RBCs** (10^3/ul)	**Hb** (g/dl)	**Hct%** %))
Control	3.84 ± 0.24	7.79 ± 0.55	13.63 ± 2.46	40.49 ±1.41
One time	4.74 ± 0.07	9.88 ± 1.26	16.21 ± 0.76	39.60 ± 10.39
Five times	5.20 ± 0.73 [a*]	5.45 ± 1.39 [a*b*]	11.28 ± 0.09 b*	34.65 ± 2.84
Ten times	2.99 ± 0.17 [b**c**]	4.29 ± 0.72 [a*b**]	9.95±0.17 [a***b***c***]	23.79±3.27[a*b*]

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group,
c: significantly differences in comparison with the five time group.

Mean corpuscular volume (MCV), mean corpuslar he-moglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC%)

In our study Table (8) showed that MCV had non-significant dif-ferences when control group was compared with treated groups, whereas, MCH and MCHC were increased in one time, five times and ten times in comparison with control group, except MCH in ten times group that had non-significant difference with control group.

Table 8: Effect of repeatedly heated palm oil on serum MCV, MCH and MCHC in male mice.

Parameters **Groups**	**MCV** (fl)	**MCH** (pg)	**MCHC** (g/dl)
Control	53.80 ± 2.05	14.73 ± 0.06	27.06 ± 1.15
One time	50.03 ± 1.78	17.06 ± 1.72[c*]	34.53 ± 2.69[a*]
Five times	51.28 ± 1.01	20.50 ± 1.79 [a*]	40.40 ± 3.04[a**b*]
Ten times	56.08 ± 1.79 [b*c*]	13.04 ± 1.84 [b*c**]	29.46 ± 0.25[c**]

Values are expressed as means ± SE.
a: significantly differences from the control group.
b: significantly differences when compared with the onetime group.
c: significantly differences in comparison with the five time group.

DISCUSSION

It was widely accepted that repeated use of oil for frying food caused changes in physical and chemical properties of oil leading to toxic compound occurrence and

palm oil was frequently used for frying, the objective of this study was to determine the adverse effects that probably influenced on repeated use of palm oil in cooking.

Nowadays, the consumption of deep-fried food has gained popularity which may cause increased risk of obesity (Sayon-Orea *et al.,* 2012). During frying, food is immersed in hot oil at a high temperature of 150°C to 190°C. The heat and mass transfer of oil, food and air that occurs during deep frying produces the unique and desirable quality of fried foods (Choe and Min, 2007). This heating process generates free radicals. The fried food absorbs this heated oil and free radicals thus it becomes part of our diet. The common practice of reusing these heated oils for frying may generate more free radicals that are harmful to tissues (Narasimhamurthy and Raina, 1999).

Palm oil is unique in terms of its ratio of saturated fatty acids (SFAs) to unsaturated fatty acids (USFAs), which is close to one. Furthermore, it is rich in the antioxidant vitamin E. Due to its availability and affordable price, palm oil is widely used as a dietary cooking oil in daily food preparation. Therefore, palm oil was chosen for our present study. Frying remains one of the most popular methods for food preparation. The various frequencies of frying were used simulate the cooking conditions used by street vendors and most households (Leong *et al.,* 2012).

Deep-frying oil contained relatively more SFA with less USFA. Initially, the USFA composition increased and then decreased as palm olein was repeatedly heated. Generally, heating at high temperatures has a negative effect on the fatty acid composition (Leong *et al.,* 2012). The presence of unsaturated bonds in the fatty acid chains render it accessible to attack from the free radicals produced during frying process. Fats with higher numbers of unsaturated bonds are prone to oxidation. The increased oxidative stress may be attributed to the destruction of double bonds by oxidation and polymerisation. Heat treatment causes oxidative rancidity, which may increase free fatty acids (Choe and Min, 2007).

Palm oil contains vitamin E, which is an antioxidant that can scavenge free radicals.

During the frying process, the oil is aged and becomes more oxidized when the natural antioxidants are depleted; thus, after repeated heating, the antioxidants can no longer prevent the oxidation of the fatty acids in the oil. Previous studies have reported that vitamin E is destroyed when frying oil is repeatedly heated (Andrikopoulos *et al.,* 2002 and Leong *et al.,* 2008). The reduction in the vitamin E content of frying oils may contribute to the increased production of ROS and may cause oxidative damage. Palm oil contains both tocopherol and tocotrienol compounds and tocotrienols have a stronger antioxidant activity than tocopherols (Adam *et al.,* 2008). So, the presence of tocotrienols in palm oil may contribute to the greater resistance to oxidation with repeated heating.

It was shown in the present study that the feeding mice on diet mixed with repeated use palm oil appeared an increase in total body weight (TBW) in five time group but decreased by administration of repeatedly heated palm oil ten times in the last week. The same results with rats were observed by Leong *et al.* (2008), who suggested that prolonged feeding with heated palm oil five times did not cause growth retardation whereas, administration of repeatedly heated palm oil ten times lead to decrease in food as a result of changes in the taste. It also appeared that the mice had a preference for the diet without additional of oil.

Serum and liver TG and TC were significantly increased. Besides, serum value of LDL-ch was also, increased, while HDL-ch was decreased. These results agreed with the results that was reported by Ebong *et al*. (1999), who attributed those

changes to transform double bonds in unsaturated fatty acids to the unhealthy saturated form. Moreover, increasing in TG was coincide with the findings of Rueda-Clausen *et al*. (2007) who reported that consumption of deep-fried palm oil increased serum TG level in humans. These results could be due to factors such as the oil preparation process, the subject's metabolic conditions and the duration of the study. On the other hand, it was contradictory to the findings of Staprans *et al.* (1996) who found that there was no difference in serum TG concentrations between control group. Therefore, increased levels of TG as detected in this study can be considered.

In contrast, this study disagreed with Kamsiah *et al.* (2004) who reported that both fresh and heated oil did not interfere with serum TC, TG, LDL-ch but reduced HDL-ch elevation.

Feeding rats on deep fried palm oil induced noTable elevation in the serum activities of AST and ALT. These results may be attributed to hyperlipidemia that leads to liver tissue injury. So, when cell membrane get damage, these enzymes which are normally located in the cytosol leak in the blood streams thus manifesting damage affected liver and other tissues. These results were in accordance with Shyamala *et al.* (2003), who reported that both ALT and AST enzymes activities were increased in rats fed with high fat diet. These results agreed with the result obtained by Chairello *et al*. (1998)

On contrast, dietary fresh palm oil was shown to attenuate oxidative stress and augment antioxidant enzymes activities in rat models (Narang *et al.,* 2005 and Hassan *et al.,* 2012). These findings disagreed with the present study where a significant increase in plasma lipid profile was observed in the oil-fed male mice. This positive effect may be attributed to the rich antioxidant content of the oils. On the other hand, high composition of saturated fats in palm oil confers it to withstand thermal oxidative changes, in addition to its rich content of tocotrienols (Shuid *et al.*, 2007).

In the present study the hematological indices were significantly decreased in five and ten times repeated heat. These results agreed with the result obtained by Mesembe *et al.* (2004) who showed that the RBC count was significantly lower in the rats fed thermoxidized palm oil diet when compared to the fresh palm oil and control groups. This result may be due to the suppressive effect of the hazardous constituents of thermally oxidized palm oil on the bone narrow.

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