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The Effects of Heated Oils Used in Fast Food Restaurants on Metabolic, Inflammatory and Oxidative Stress Markers, Blood Pressure, and Liver Histology in Sprague-Dawley Rats

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Abstract

Background: Long and repeated heating causes multiple physical and chemical changes in oil, which may result in serious biological damages upon consumption.
Objectives: This study investigated the effects of heated oils used in fast food restaurants on metabolic, inflammatory, and oxidative stress markers in rats.
Methods: The experimental clinical study was performed during summer 2016 in Shiraz, Iran. For 13 weeks, 32 Sprague-Dawley rats received one of the four diets: Group 1: basal diet mixed with 15% w/w heated oil containing total polar compounds (TPC) = 12.5% (TPC 12.5); Group 2: basal diet with 15% unheated oil used in group 1 (control TPC 12.5); Group 3: basal diet with 15% heated oil with TPC = 35% (TPC 35); and Group 4: basal diet with 15% unheated oil used in group 3 (control TPC 35). At weeks six and 13, blood samples were collected for determination of fasting glucose, lipid profile, liver enzymes, and inflammatory and oxidative stress markers. Blood pressure was measured on the 13th week. Histopathological examination of liver slices was performed after euthanization of rats. Statistical analysis was done using the SPSS software.
Results: On the 13th week, the TPC 35 group had higher plasma glucose (+40.4 mg/dL, $P < 0.05$), triglycerides (+13.6 mg/dL, $P < 0.05$), aspartate transaminase (+34.3 U/L, $P < 0.05$), interleukin-1β (+453 pg/L, $P < 0.01$), and blood pressure (+16/5 mmHg, $P < 0.05$) than the control and higher glucose (+59.3 ng/L, $P < 0.001$), aspartate transaminase (+55.5 U/L, $P < 0.05$), total cholesterol (+6.5 mg/dL, $P < 0.05$), and 8-isoprostane (+8.5 mg/dL, $P < 0.05$) than the values on week six. On the 6th week, Low Density Lipoprotein (LDL)-cholesterol was higher in the TPC 35 group than TPC 12.5 (+4.0 mg/dl, $P < 0.05$) and the level of serum malondialdehyde was higher in the TPC 35 than the control (+0.49 μmol/L, $P < 0.001$). On the 13th week, more histological changes were observed in rats of the heated oil groups.
Conclusions: Long-term consumption of fried foods from fast food restaurants may have detrimental impact on blood pressure, serum glucose and lipids, inflammatory and oxidative stress markers, and liver histology.

Keywords: Glucose, Inflammation, Lipids, Oils, Oxidative Stress, Rats

1. Background

Frying is one of the most popular ways of food preparation at home and in the food industry (1). In fact, it is a quick and easy method that enhances consumer satisfaction with color, texture, and taste of the food (2). However, at high temperatures, oil undergoes a number of physical changes, including foam formation, increased viscosity, and darkening of color that alter oil appearance and lower quality (3). In addition to physical changes, prolonged heating induces chemical reactions in oil, such as oxidation, hydrolysis, and polymerization (4), which produce oxidized fatty acids, polar compounds, polymers, hydroperoxides, and aldehydes (5) and upon consumption could cause serious health consequences in fried food consumers (6). These include increased adiposity (7), hypertension (8), cardiac damage (9, 10), diabetes (11), atherosclerosis (12), and cancer (13, 14).

However, to the best of our knowledge, previously performed studies reported detrimental effects of oils heated under laboratory conditions (7-9, 11, 15). For instance, administration of palm oil heated five and 10 times to rats

for 16 weeks elevated blood pressure (9). Similarly, heating soybean oil at 180°C for eight hours/day for four days elevated oxidative stress without elevation of blood pressure (15). Likewise, heating soybean oil for three hours increased fat mass while it reduced body weight of mice (7). Also, 8-week ingestion of high oxidized frying oil (205°C for four 6-hour periods) deteriorated insulin secretion from pancreatic islets (11).

Given that heating oil under laboratory conditions is not similar to real frying oil conditions in kitchens, bakeries, and fast food restaurants, the results of previous studies may not accurately show detrimental effects of heated oil consumption. In fact, under real circumstances, especially in fast food restaurants, oil is extensively and repeatedly heated, sometimes for more than 18 hours per day and for consecutive days. Therefore, the present study aimed at examining pathological effects of oils currently being heated and used in fast food restaurants of Shiraz.

2. Methods

This was an experimental and clinical animal study conducted during the summer of 2016 in Shiraz, Iran.

2.1. Collecting Oils

Oil samples were collected by stratified sampling from 42 fast food restaurants of Shiraz, Iran. The city was divided to three regions and from each region, 14 fast foods were randomly selected. Sampling was performed when oil was being used in the fryer at the peak of fast food restaurant activity, between 10 and 12 pm. Since in fast food restaurants, oil is heated for different purposes and considering that food particles that are released in the oil during frying can affect oil oxidation process, this work was limited to the oil that was used solely for frying potatoes in order to achieve a homogenous sample. After completion of the sampling, Total polar compounds (TPC) of the oils were determined. The median of the highest and the lowest tertiles of polar compounds of the collected oils was considered, as oxidized (TPC = 35%) and healthy (TPC = 12.5%) heated oil (16), respectively, and used for preparation of animals' food.

2.2. Oil Experiments

Total polar compounds of oils were measured to test oil health quality. The percentage of TPC was measured by the Testo 270 tester (Testo Company, Lenzkirch, Germany). To measure the percentage of polar compounds, the Testo sensor was placed in the oil at a temperature of 40°C to 200°C, and the percentage of polar compounds was recorded after 20 seconds. Before each measurement, the device was calibrated by the calibration oil, which was provided by the manufacturer. After each usage, the device was cleaned and dried with hot water and neutral detergents for the next measurement.

2.3. Diet Preparation

To prepare feeds, two types of heated oils (TPC 12.5% and 35%) were used. In addition, the unheated forms of the same oils were used to prepare feed of the control groups. All collected samples were used for the preparation of French fries. Feeds were prepared by mixing standard rat chow with 15% w/w oil. To obtain a better mixture, 0.5 L of water was added to each kilogram of feed. After uniform mixing, the prepared dough was cut using a stainless steel mold with dimensions of $1 \times 1 \times 0.5$ cm. Then, pellets were dried away from direct sunlight in a well-ventilated room for 2 days. The feeds were packed in plastic bags and kept at -22°C until the time of feeding. Feeds were prepared once, prior to the start of the study, and were used at maximum of 14 weeks from the preparation time.

2.4. Animals and the Study Plan

A total of 32 male Sprague-Dawley rats aged eight to nine weeks and weighing 200 to 250 g were used in this study. Rats were provided by comparative and experimental medicine center of Shiraz University of Medical Sciences. Animals were kept under standard conditions of light/dark cycles, temperature, and ventilation. During the study, all rats had free access to food and water. Animals were maintained and handled under humane care. The study was approved by Shiraz University of Medical Sciences' ethics committee on animal care (approval number 94-01-87-10498; dated on February 13th, 2016).

Animals were fed for two weeks with standard rat chow diet (Pars animal feed factory, Tehran, Iran) in order to be adapted to the environment. Then, they were randomly divided to four groups with eight rats each: Group 1: rat chow with 15% w/w heated oil with TPC = 12.5% (TPC 12.5); Group 2: rat chow with 15% w/w unheated oil of the oil used for group 1 (control TPC 12.5); Group 3: rat chow with 15% w/w heated oil with TPC = 35% (TPC 35); and Group 4: rat chow with 15% w/w unheated oil of the group 3 (control TPC 35). The method of randomization was to number the animals from 1 to 32 and to assign the animals to one of the four groups using a table of random numbers.

Food and weight of rats were recorded weekly from the 6th week of the study to the end. At week six and 13 (the end of the study), blood samples were taken after 12 hours of fasting. Serum was isolated from blood samples by centrifugation at 3000 rpm for 15 minutes. At the end of the

study, the animals were sacrificed and their livers were removed, weighed, and fixed in 10% neutral formalin for later histological assessment.

2.5. Biochemical Assessments

Serum lipids, glucose, alanine transaminase (ALT), and aspartate transaminase (AST) were measured by a BT 1500 auto-analyzer (ChemWell autoanalyzer, Awareness Technology Inc., USA) using commercially available kits (Pars Azmoon, Tehran, Iran). Malondialdehyde (MDA) was measured colorimetrically at 535 nm using the thiobarbituric acid reactive substance (Merck, Germany) method (17). Serum concentration of Interleukin-1β (IL-1) and 8-isoprostane was determined by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Zell-Bio GmbH, Germany) using an ELISA microplate reader (BioTek, Germany). All instruments were calibrated by expert laboratory technicians before use.

2.6. Blood Pressure

Systolic blood pressure was measured after warming rats to 40 $\pm$ 0.5°C for 10 minutes using the non-invasive tail-cuff method (PowerLab/4SP, ADInstruments, Australia). Systolic blood pressure was measured 3 times and the mean of the 3 measurements was used in the analysis (15).

2.7. Histopathology

Fixed livers were dehydrated in ethanol, embedded in paraffin, and cut to 5-μm thick slices. Sections were then stained with hematoxylin and eosin. For evaluation of histological changes, the number of spotty necrosis and portal inflammation was evaluated (18).

2.8. Statistical Analysis

Statistical analysis was performed using the SPSS version 19 software (SPSS Inc, Chicago, II, USA). Data were provided as means $\pm$ standard deviation (SD). Within group comparisons were determined by the paired t-test between weeks six and 13. Comparisons between TPC 12.5% and TPC 35% groups and between heated and unheated groups were performed by the independent t-test. Alterations in food intake and body weight were compared between the groups by repeated measures analysis of variance. The normality of the data was tested by the Kolmogorov-Smirnov test. All variables had a normal distribution. The significance level was set at $P < 0.05$.

3. Results

The study was initiated with 32 rats, yet four rats were lost after blood collection on the 6th week, one rat from Group 1 (TPC 12.5), two rats from Group 3 (TPC 35), and one rat from Group four (control TPC 35).

3.1. Body Weight and Food Intake

Rats in the TPC 35 group showed a significant increase in food intake compared to the control and TPC 12.5 group (Figure 1A). This increase was also seen in the TPC 12.5 group, yet the increase was not statistically significant. No significant difference in body weight elevation was observed between the groups (Figure 1B).

3.2. Serum Lipids

At the end of the study, serum cholesterol was significantly higher in the TPC 35 group when compared with the results on the 6th week ($P < 0.05$), yet no significant difference was observed in the TPC 12.5 group between week 13 and week 6, and between TPC 35 and TPC 12.5 groups on either week 6 or week 13 (Table 1). During week 6, Low Density Lipoprotein-cholesterol (LDL-C) of the TPC 35 group was higher than that of the TPC 12.5 group ($P < 0.05$), yet the reverse was observed for Triglycerides (TG). Also, at the end of the study, serum TG level of the TPC 35 group was significantly elevated compared to the control ($P < 0.05$). No significant difference was observed in serum levels of High Density Lipoprotein-Cholesterol (HDL-C) between the groups and time points.

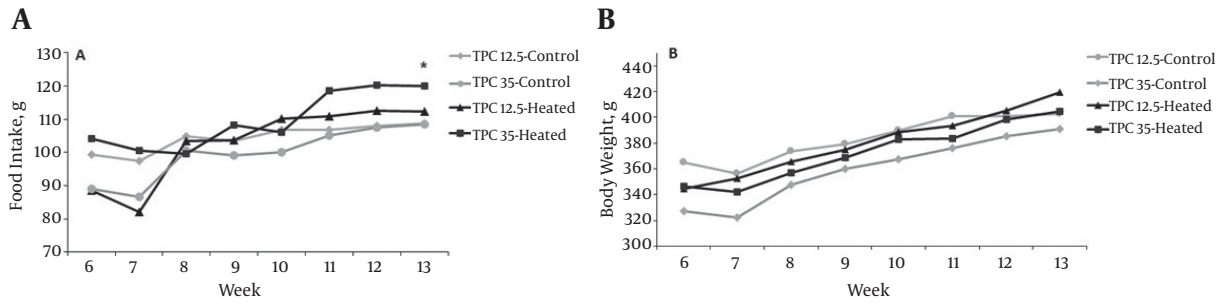
3.3. Serum Glucose

Fasting serum glucose (FSG) showed a significant difference in both heated oil groups (TPC 12.5 and TPC 35) at the end of the study compared to week six (Figure 2A). In addition, at the end of the study, FSG level was higher in the TPC 35 group compared to the control, yet the difference was not significant on the 6th week.

3.4. Blood Pressure

At the end of the study, a significant increase was observed in the mean systolic blood pressure in the TPC 35 group compared to the control (Figure 2B). Blood pressure was also higher in the TPC 12.5 group than the control, yet this difference was not statistically significant. No significant difference was observed between TPC 35 and TPC 12.5 groups.

Figure 1. Changes in Food Intake (A) and Body Weight (B) from Week 6 to the End of the Study



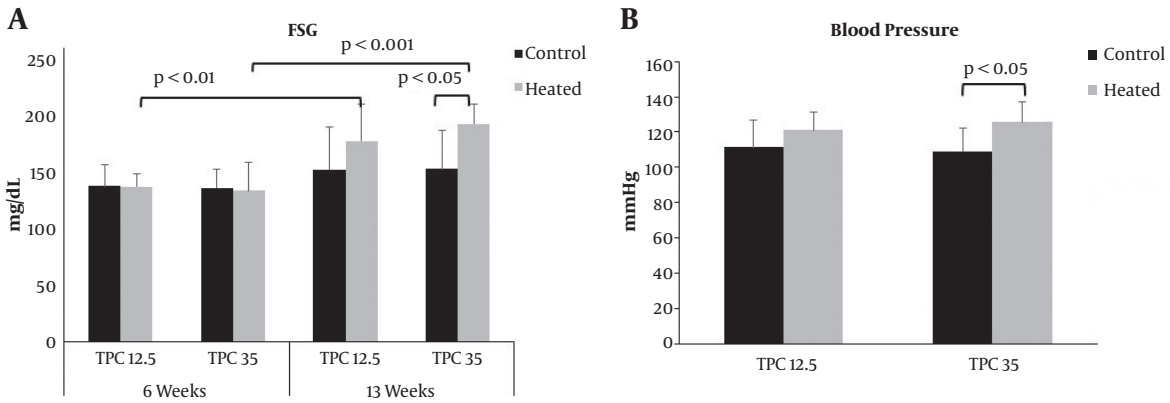
*Indicates $P < 0.05$ compared to other groups as assessed by repeated measures analysis of variance (ANOVA).

Table 1. Lipid Profile of Rats in the Experimental Groups on Weeks six and 13[a,b]

	TPC 12.5 Control		TPC 12.5 Heated		TPC 35 Control		TPC 35 Heated	
	6 w	13 w	6 w	13 w	6 w	13 w	6 w	13 w
TG	77.9 ± 7.3	77.9 ± 12.4	84.3 ± 14.6*	78.4 ± 15.9	67.9 ± 23.4	70.9 ± 10.4†	66.8 ± 8.8*	84.4 ± 10.1†
TC	62.3 ± 6.7	65.9 ± 14.5	69.3 ± 13.2	62.4 ± 17.0	68.3 ± 12.3	73.0 ± 9.2	69.8 ± 6.7*	76.2 ± 4.7*
LDL-C	14.9 ± 3.1	15.5 ± 2.9	15.4 ± 3.3*	16.6 ± 3.5	18.5 ± 3.0	18.9 ± 3.7	19.4 ± 2.9*	18.6 ± 2.2
HDL-C	45.1 ± 7.4	39.1 ± 13.8	44.6 ± 8.6	50.3 ± 6.6	43.8 ± 9.5	46.9 ± 5.3	46.4 ± 6.7	50.4 ± 4.8

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C; low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TPC, total polar compounds.
[a]Values are expressed as means ± SD.
[b]Paired t-test, $P < 0.05$ was considered significant.

Figure 2. Fasting Serum Glucose (FSG) (A) on Weeks 6 and 13 and Blood Pressure (B) on Week 13



The bars represent mean and SD. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C; low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TPC, total polar compounds. Paired t-test, $P < 0.05$ was considered significant.

3.5. Oxidative Stress and Inflammatory Markers

On week 6, MDA of rats in the TPC 35 group showed a significant increase compared to the control, yet no significant difference was found between heated TPC 12.5 and the control (Figure 3A). At the end of the study, MDA was not significantly different within (heated oils with their control) or between (heated oils) groups. On week six, serum 8-isoprostane level appeared to decrease in the TPC 35 compared to TPC 12.5, yet such difference was not observed on week 13 (Figure 3B). At the end of the study, 8-isoprostane level showed a significant increase in the TPC 35 group compared to that of week 6. The IL-1β showed no signifi-

cant difference between the groups on week 6, yet at the end of the study it was significantly higher in the TPC 35 group compared to its control and the TPC 12.5 group (Figure 3C).

3.6. Liver Enzymes

At the end of the study, the TPC 35 group demonstrated a significant increase in AST concentration compared to its control and also compared to that of week 6 (Figure 4A). No significant differences were observed in ALT concentration between groups and time points (Figure 4B). Although at the end of the study, liver weight was higher in heated oil groups, the difference between heated and unheated groups was not statistically significant (data not shown).

3.7. Histopathology

The extent of inflammation and necrotic spots in the liver of TPC 35 and TPC 12.5 rats was almost twice as that of their control, yet a statistical significant difference for both markers was observed only between the TPC 12.5 group and its control (Figure 5).

4. Discussion

Nowadays, with increasing consumption of fried and fast foods, heated oils threaten human beings more than ever before. The results of the present study showed that consumption of heated oils of fast food restaurants may lead to deleterious changes in blood pressure, blood glucose and lipid levels, liver aspartate transaminase, oxidative, and inflammatory markers, and histology of the liver. Prolonged consumptions of heated oils may increase the risk of these changes.

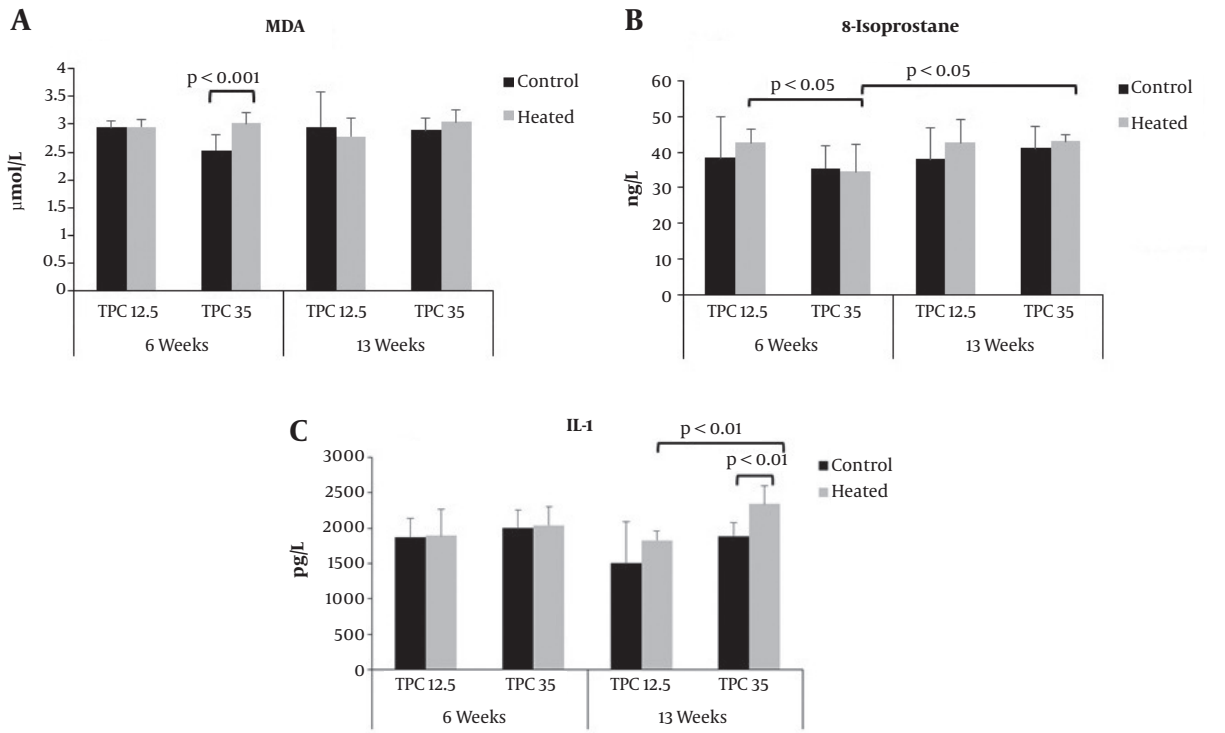
In this study, a significant increase in food consumption was observed in the TPC 35 group compared to the other groups. Since heated oil was used to fry potatoes, the oil may have gained some good flavor from potatoes, leading to increased food consumption by the TPC 35 group (4). Even though food intake was higher in the TPC 35 group, no significant difference was observed in weight gain between the groups. Toxic products derived from oil during heating may cause negative effects on energy metabolism, thus energy loss is intensified and prevents weight gain (19). It is also possible for polar toxic compounds that are produced under extreme temperatures to decrease food energy efficacy and impair fat calorigenic ability (20). Consistent with the results of this study, Bautista et al. (21) showed that although food intake in the group of rats that had consumed a diet containing 10 times heated oil was more than the other groups, yet no difference in weight gain was observed between groups after 10 weeks of intervention. Also, Penumetcha et al. (7) reported that a diet containing soybean oil heated for three hours increased adipose tissue weight yet decreased body weight in mice. The reduction that was seen in the energy and weight gain of rats on week 7 of this study was likely due to the effect of blood sampling at the end of week 6, which caused death of some animals.

Prolonged heated oil consumption caused an increase in fasting serum glucose. Similarly, in previous studies, heated oils by increasing production of reactive oxygen species caused glucose intolerance, pancreatic beta cell destruction, and reduced insulin secretion (11, 22). In this study, no significant difference was found in HDL-C level between the groups, TG and total cholesterol levels increased at the end of the study. Furthermore, on week six, LDL-C of the TPC 35 group was significantly higher than the TPC 12.5 group. Consistent with the results of the current study, Kamsiah et al. (23) showed that after 20 weeks of consumption of five times-heated palm oil, LDL-C levels increased, yet no significant change in HDL-C levels was observed. In this context, Garrido Polonio et al. (24) showed that negative alterations in lipid profile of rats fed heated sunflower oil may be due to decreased intake of unsaturated fatty acids, especially linoleic acid. Changes in lipid profile following consumption of heated oil could increase atherosclerosis risk as indicated by Adam et al. (25).

During the heating process, reactive oxygen species, such as hydroperoxides, are produced (26) and cause oxidative stress-induced cytotoxicity (27, 28). The MDA is one of the end products of lipid peroxidation that is considered as an index for cell damage (29). However, in the present study, on the 6th week, MDA was higher in the TPC 35 group compared to its control, yet at the end of the study, no significant relationship was observed between these groups. This unexpected result could be due to the increase of MDA in other groups as a result of consuming a fat-rich diet. Isoprostanes are other products of non-enzymatic lipid peroxidation. The current results showed that by prolonging consumption of TPC 35 oil, the level of 8-isoprostane increased. Oxidative stress is proposed as a stimulatory factor in increasing inflammatory mediators (30). Accordingly, at the end of the study, IL-1β in the TPC 35 group was higher than the other groups. This increase in IL-1 was associated with increased hepatic inflammation. This kind of hepatic inflammation was also observed in previous studies by repeatedly heated soy oil (31).

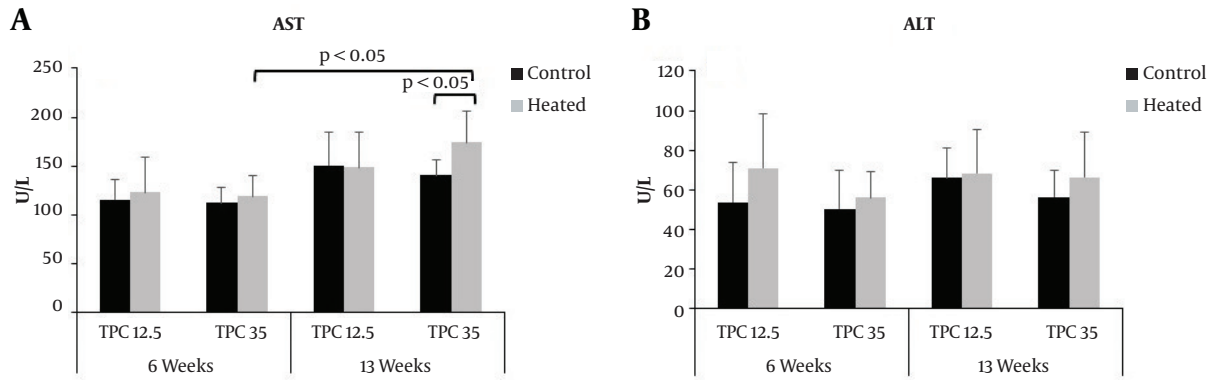
On the 13th week, the level of AST increased in rats consuming heated oils. This increase could be due to oxidative stress-induced damage of liver cells following consumption of oxidized heated oil (32, 33). Despite the increase in AST, no difference was observed in ALT levels. Although

Figure 3. Malondialdehyde (MDA) (A), 8-Isoprostane (B), and IL-1β (C) on Weeks 6 and 13



The bars represent the mean and SD. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C; low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TPC, total polar compounds. Paired t-test, P < 0.05 was considered significant.

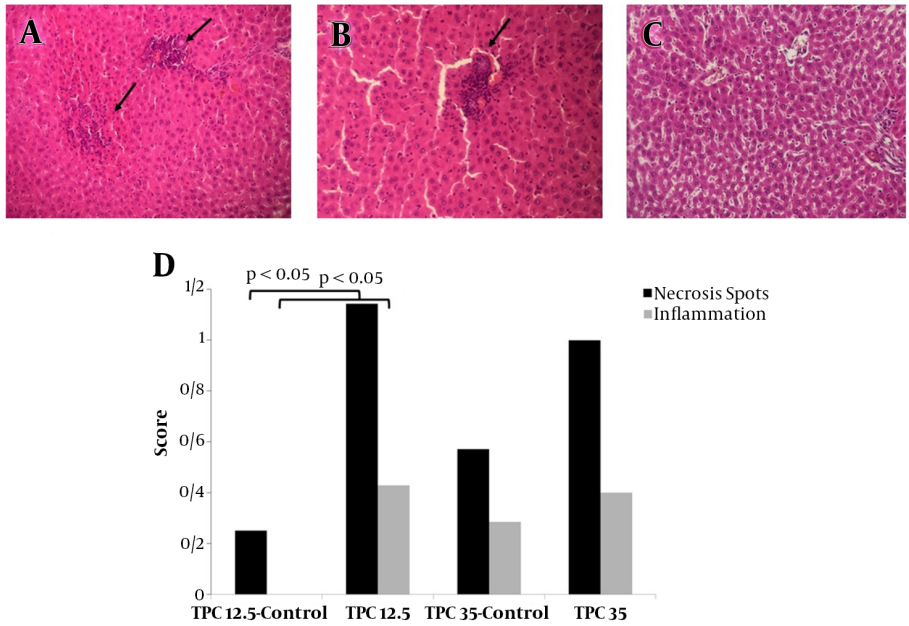
Figure 4. Aspartate Transaminase (AST) (A) Alanine Transaminase (ALT) (B) on Weeks 6 and 13



The bars represent mean and SD. Significance level was considered at P < 0.05. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C; low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TPC, total polar compounds. Paired t-test, P < 0.05 was considered significant.

both AST and ALT are liver enzymes that are increased in hepatocyte damage, the ratio of these two enzymes may indicate the type and degree of liver damage. For example, in non-alcoholic fatty liver disease, the ratio of AST/ALT was less than 1, while in more severe liver injury, the ratio increased to over 1 (34). In this study, with the increased AST and no change in ALT, the ratio of AST/ALT increased, which is an indication of liver injury, a finding which was

Figure 5. Histopathology analysis of rats' liver tissues of TPC 35 group (A) (Magnification = × 250), TPC 12.5 group (B) (Magnification = ×400), and TPC 12.5 Control (C) (Magnification = × 250)



Quantitative presentation of histopathological analysis is shown in D. Paired t-test, P < 0.05 was considered significant.

confirmed by histological examination.

Previous studies have reported that consumption of repeatedly heated oils increases blood pressure (35-38). In this regard, the results of blood pressure measurements at the end of the study were consistent with previous studies. Several mechanisms have been proposed for the increased blood pressure by oxidized oils. Leong et al. (37) reported that peroxides produced in the process of heating oil reduce heme oxygenase and increase angiotensin converting enzyme. Heme oxygenase plays an important role in regulating blood pressure through production of bilirubin and carbon monoxide and thus prevention of endothelial dysfunction (38). In addition, angiotensin-converting enzyme with increased production of angiotensin II increases blood pressure (37). The researchers did not find any significant difference in blood pressure between the TPC 12.5 group and its control, which could be due to low level of oxidation in TPC 12.5 oil.

The current study had some strengths and limitations. First of all, our study seems to be examining the biological effects of oils being heated in fast food restaurants. Previously, studies had tested the biological effects of oils that were heated under laboratory conditions and so could not accurately demonstrate the effects of heated oils in fast food restaurants. Eating fast foods is very popular in many countries throughout the world, including Iran and thus the results of this study may help highlight the importance of strict surveillance and vigilance over preparation of fast foods and probably other types of fried foods in restaurants. Nonetheless, this was an animal study and so the results cannot be easily generalized to humans. On the other hand, due to ethical issues, examination of the effect of heated oils on humans is not possible and therefore observational studies may be the only way to confirm these results. Another limitation was the loss of animals after the first blood collection (week six), thus it was not possible to have 8 animals in all groups at the time of statistical analysis.

4.1. Conclusions

All in all, consumption of heated oils from fast food restaurants could have a negative impact on consumer's health. Highly heated oils, which contain high-polar compounds, are more harmful than heated oils that contain permitted-polar chemicals. Also, longer use of oxidized oils disturbs the balance of biological systems more than their short-term consumption. Although heated oils are not directly consumed, a part of oil penetrates the food during the process of frying, and could thus harm the consumer. Strict supervision on the process of frying should

be compulsory regarding both the temperature and the duration of heating. The use of instruments, which measure total polar compounds of oil in real time should be obligatory for restaurant kitchens to notify the chefs to change oil when necessary.

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Footnotes

Authors' Contribution: Saeed Ghobadi collected the oil samples. Saeed Ghobadi and Fatemeh Mohammadian performed the experiments. Maral Mokhtari helped the histopathological evaluation. Masoumeh Akhlaghi contributed to the conception and design of the study and supervised the study. Masoumeh Akhlaghi and Saeed Ghobadi prepared the manuscript. All authors approved the final version of the manuscript. None of the authors had any conflict of interest to declare.

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