

The hepatotoxic effects of deep-fried sunflower oil on rat livers

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Objectives: To examine histopathological effects of deep-fried sunflower oil on rat livers.

Methods: Rats in the experimental group were fed for 5 weeks with deep-fried sunflower oil. Rats in the control group were fed for the same period with fresh sunflower oil. At the end of the study, the bodies and livers of the rats were weighed, and the livers were histopathologically examined.

Results: Hepatic parenchymal cells of rats fed with deep-fried sunflower oil showed diffuse hydropic and lipid degeneration, moderate but obvious levels of parenchymal necrosis, dense mononuclear cell infiltration in sinusoids and portal areas, and irregular piecemeal necrosis in the parenchymal borders of portal areas. There was not any histopathological change in the livers of the control group.

Conclusion: We think that sunflower oil used in deep fat frying is toxic to rat liver and, consequently, may be toxic to human liver.

Key words: Foxicity, rat, liver

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Introduction

Deep fat frying is a popular food preparation method worldwide; it produces a desirable flavor, a golden brown appearance and a crispy texture. Such fried foods are highly acceptable to all classes of social consumers, but especially to younger people.¹ The process of heating the oil to 18°C and even to more temperatures produces structural chemical, physical, changes which lead to compositional diversities as a result of degradation reactions that take place such as auto-oxidation, thermal polymerization, cyclisation and hydrolysis.²

The extent and exact nature of these degradation reactions are affected by the characteristics of the fried food, composition of the fat, frying conditions

(continuous or intermittent), temperature, exposure to oxygen, heating period, mode of heat transfer, frying capacity (kg food/hr), metal in contact with oil, cleanliness of the fryer, turnover rate and initial quality of the oil.³

The degradation products formed include both volatile and non-volatile compounds, although most of the volatiles are lost during the frying process.

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The non-volatile decompositional products are produced primarily by thermal oxidation and polymerization of unsaturated fatty acids.⁴

These compounds are of concern because they accumulate in the frying oil, promote further degradation, absorbed by the fried food and affect public health. Highly oxidized oils may also produce polycyclic aromatic hydrocarbons that are thought to have a carcinogenic effect.⁵ The nutritional consequences of ingesting deep-fried oils include a variety of symptoms ranging from allergic reactions of digestive tract, growth retardation, increase in liver and kidney weights, to other biochemical reactions.

As social, economic, and cultural conditions have changed over the twentieth century; significant alterations occurred in eating habits, including increased times of eating outside the home and especially at fast food restaurants which lead to increased consumption of oils used in deep-fat frying.⁶

Usually an experienced cook controls the quality of the oil by determining duration of time used for frying foods. The expiration date of the oils used in fast food or other restaurants is typically subjective, based on observations of the color, odor, smoking or foaming.¹ United States and some European countries have specific policies and arrangements designed to protect consumers from structurally changed frying oils. Up to now these efforts have yielded only a few significant improvements in the use of frying oils.⁷

In this study, we aimed to examine the short-term histological toxic effects of sunflower oil fried for 10 hours in deep-fat frying method. This is the duration of time which our institution's kitchen uses frying oils for vegetables.

Materials and Methods

Preparing grafts of defined cell populations This study was carried out on 20 mature Wistar Albino male rats. The rats, with an initial average weight of 203 ± 9.2 g, were randomly divided into two groups-intention and control group-. The rats were fed for 5-weeks with a diet including fried and fresh sunflower oil in intention and control group respectively. The composition of the diet is shown in Table 2. During the study, food and water were provided

ad libitum. After a five-week period of feeding, the rats were weighed and killed; the livers were immediately extracted, weighed and placed in a cup of boin solution prior to histopathological examination. The histopathological specimens were prepared at the laboratory of the Experimental Medicine and Research Institute, Istanbul University; pathological evaluations were conducted by the Department of Pathology in the Cerrahpasa Medical Faculty, also at Istanbul University.

Oil samples

The fried oil samples were obtained from our institution's kitchen that have a capacity of preparing lunch for 3000 people everyday. The fresh oil samples were obtained from the same trademark of sunflower oil used in deep-frying which is easily available from local supermarkets. The deep-fried oil sample was obtained after it was used for frying vegetables, primarily green peppers and eggplant. The frying process lasted for 10 hours at approximately 180°C. Every hour, fresh sunflower oil was added into the kettle to replenish the amount of oil that was absorbed by the frying vegetables. The fried oil sample consumed by the rats in intention group was obtained after the above-described frying process was completed. Analysis of the chemical characteristics of the fresh and used oil samples were done in the Advanced Techniques Laboratory of Istanbul University (Table 1).

Preparation of the diet

The diet was prepared in the form of a mixture of vitamin, mineral, wheat flour, sugar, carrot, bran, sunflower oil and Protifar 90 (Table 2). Fifty six per-

Table 1
*Characteristics of sunflower oils**

Oil characteristics	Sunflower oil	
	Fresh oil	Fried oil
Acid value	0.1	31.0
Lodine value Wijs 30'	123.7	7.5
Peroxide value meq/kg	3.81	7.4
Refractive Index 40°C	1.4668	1.4788

*: Goburdhun D, Jhaumeer-Laulloo, Musruck R. Int. J Food Sci Nutr 2001; 52(1): 31-41

Table 2
Composition of rat diet

Composition	g/ kg
Wheat flour	575.0
Sugar	120.0
Bran	60.0
Carrot	40.0
Sunflower oil	125.0
Protifar 90*	80.0
Total	1000.0

*: One scoop Protifar 90 is 2.5 g and contains 2.2 g protein, 0.04 g fat, 0.01 g lactose, 9.2 g calorie, 3.5 mg calcium, 1.75 mg phosphorous. Protifar 90 is a product of Nutricia.

cent, 15% and 29% of total energy was provided from carbohydrates, proteins and fat respectively. In order to prevent the peroxidation of the oils, the diets were prepared twice a week and stored in a closed container at a temperature below 5°C.

Statistics

Results were expressed as mean ±SD. Statistical analysis was performed using Mann-Whitney U test. Results are considered significant at p<0.05.

We used t-test and chi-square test for the statistical evaluations.

Results

Overall, the rats grew well with the specially prepared diets. Average food consumption, final body weight, weight gain and liver weight were statistically insignificant between two groups (Table 3). However, histopathological examination of the rat livers showed statistically significant differences between two groups (Table 4, Figure 1).

The parenchyma cells of all ten rats fed with the deep-fried sunflower oil exhibited widespread

Table 3
Food consumption, body weight and liver weight of the rats

Parameters	Groups		
	Experimental	Control	T-test
Food Consumption (g/day)	14.5±0.8	14.3±1.0	t=0.492, p>0.05
Final Weight (g)	293±15	294±17	t=-0.152, p>0.05
Weight gaining (g)	88±5.3	91±5.9	t=-0.231, p>0.05
Liver weight (body weight %)	3.52±0.16	3.47±0.20	t= 0.673, p>0.05

Table 4
Histological examination of rat livers

Histological changes	Groups		
	Experimental	Control	x2-test
Hydropic and lipid degeneration			
None	-	9	x ² =20
Focal	-	1	df= 2
Diffuse	10	-	P<0.001
Parenchymal necrosis			
None	1	9	x ² =13.07
Slight	5	1	df= 2
Severe	4	-	P<0.001
Portal inflammation (monocular infiltration)			
None	-	9	x ² = 17.33
Slight	2	1	df= 2
Severe	8	-	P<0.001
Piecemeal necrosis			
(-)	1	10	x ² =16.36
(+)	9	-	df= P<0.001

hydropic and lipid degeneration. Parenchymal necrosis (lobular degeneration) was found in nine of these rats and a dense mononuclear cell infiltration was seen at the sinusoids and portal areas in eight of them. Importantly, a great deal of irregular piecemeal necrosis was seen in the parenchyma borders of the portal area in nine of the rats fed with the deep-fried oil.

In contrast, only one of the rats that was fed with fresh sunflower oil exhibited slightly hydrophic swelling in its parenchymal vein; another of the control rats showed some focal necrosis and slight monocular cell infiltration was observed in the third.

Discussion

Fat is an important dietary component, which affects both growth and health. Previous studies have indicated that diets containing high levels of polyunsaturated fatty acids (PUFA) are effective in lowering serum cholesterol levels⁸ and are considered to be beneficial in preventing coronary heart disease.⁹ However, studies have also shown that excessive intake of vegetable oil containing PUFA is detrimental to health.¹⁰ It has been suggested that consumption of diets rich in PUFA render tissues more susceptible to free radical-mediated lipid per-

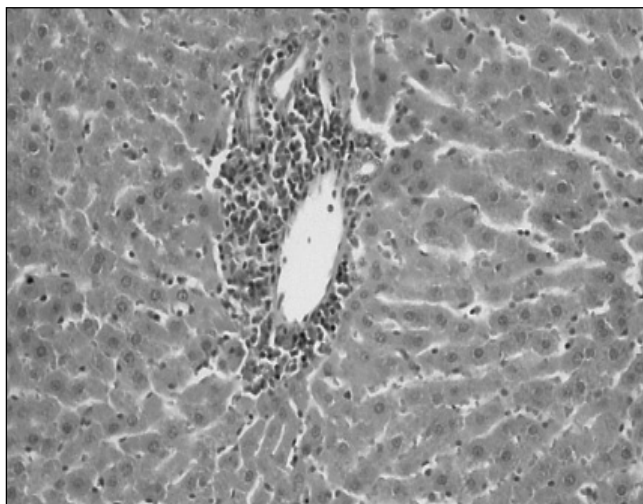


Figure 1a
Portal area

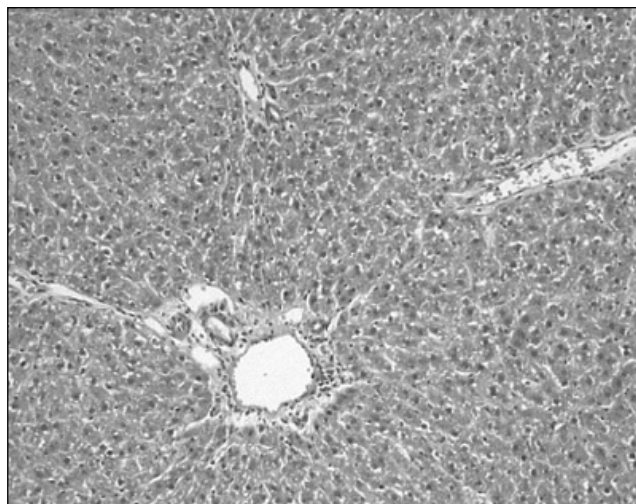


Figure 1b
Hydropic degeneration

oxidation, a process that has been implicated in tissue damage.¹¹

Heating of fats to certain temperatures leads to measurable changes in their physical and chemical properties. During deep-frying, when the fat is used repeatedly, oxidative and thermal effects result in the formation of many volatile and nonvolatile products, some of which are potentially toxic.¹²

A number of studies with a diversity of results regarding the effects of consumption of fried oils have been published.¹³ Some of them emphasize on the fact that consumption of the thermally oxidized oils results in decreased food consumption, growth retardation, weight gain in organs such as liver and kidneys, as well as mutagenicity and cellular damage in various organs of laboratory animals.¹²

Hageman et al found no significant difference in the food consumption, body weight gain and liver weight between rats fed with unheated sunflower oil for more than four weeks and rats fed with specially prepared diets of vegetable oils rich in unsaturated fatty acids and coconut oil in which various food had been deep-fat fried for 30 hours.¹⁴ Javadi et al showed that Feeding mice with conjugated linoleic acid (9 cis, 11 trans/9 trans, 11 cis-and 10 trans, 12 cis-CLA in equal amounts) resulted in triacylglycerol accumulation in the liver.¹⁵ Varela et al concluded that rats fed with sunflower oil (heated 75 times)

over a 25 day period had similar food intakes and liver weight as rats fed with fresh (not used for frying) sunflower oil but less body weight gain.¹⁶ In a similar study, Izaki et al, demonstrated that rats fed with sunflower oil obtained from a fast-food Japanese restaurant (used 66 days, 3 1/2 hr/day) for 13 weeks, had the same food intake and body weight gain as rats fed with fresh oil but increased liver and kidney weight.¹⁷ In contrast, all rats in both of our study groups have an apparently healthy growth pattern with no statistically significant difference in final body weight and liver weight.

During the study period, the rats fed water and food ad libitum consumed an average of 14.4 ± 0.9 g/day food. Comparing food consumption and calorie intake, weight gain between the groups is similar. Thus, these findings regarding growth and liver weight are similar to the results of Hageman. The possible explanation for why the weights of livers in our study did not change despite the histopathological changes may be attributed to duration of frying time. Use of fried oils with prolonged duration of frying process may lead to more liver weight gains.

The results of various studies investigating the pathogenesis of carcinogenesis strongly suggest the importance of nutrition, in addition to environmental factors like sunlight and ionizing radiation as

well as genetic factors. It has been reported that, in 35% of the cancer related deaths, nutrition plays an important role.^{2,5}

Carcinogenic effects of nutrition can be explained in terms of the physical and chemical alterations that can occur during different stages of preparation of food, from the cultivation and harvesting of a crop through storing, processing and cooking.¹⁸

Many studies report that the oxidative hazards that emerge under the influence of temperatures at 180°C and above in the course of deep-fat frying process (among the most popular institutional and commercial cooking methods) adversely affect both food quality and human health; which faces the public with serious health problems.¹⁹ This concern is supported by the experimental studies carried out on laboratory animals.^{3,4}

The combined oxidative and thermal effects of repeatedly using deep-fried oils results in the formation of many volatile and nonvolatile products, some of which are potentially toxic. During the course of frying, many of these products naturally disappear, but some remain in the frying oil and give flavor to the food. Many of the products, though only found in tiny amounts, have been shown to carry toxic characteristics in experimental studies. Evidence suggests that toxic characteristics of deep-frying oil changes depending on the duration of oxidation and temperature. The mutagenicity or harmful compounds of frying oil is positively correlated by temperature, duration of use and exposure of O₂.² Elevations in tissue levels of lipid peroxides are thought to be responsible for the initiation of the associated toxicities and tissue damage. Opinions still differ on whether such elevations in peroxide levels are causes or consequences of the associated tissue damage.²⁰ As a consequence of tissue damage, increase in the levels of lipid peroxide would occur as a late event during toxicity and tissue damage. This can occur at the point of cell death and lysis.²¹ Laboratory animals fed with repeatedly used frying oils exhibited moderate to severe forms of diarrhea, seborrheic dermatitis and hair loss depending on the duration of consumption. Pathologically, cellular defects or damages

have developed in the thymus, liver, heart and kidneys, and elevation of liver enzymes, damage to testes and epididymes have been noted; in some cases, complete cessation of spermatogenesis has been determined.²¹

In rats fed with fried corn oil and peanut oil, in 5 different groups and for 5 weeks, Alexander et al showed moderate lobular mosaic pattern due to periportal, perinuclear clear areas, and homogeneity of the pericentral cytoplasm.²² The portal area of a rat fed with heated peanut oil was composed of cells having irregular cytoplasmic basophilia with loss of hepatic cord pattern and marked anisokaryosis. Also, the perivenous area showed a reduction in cytoplasmic basophilia with mild anisokaryosis and nuclear hyperchromatism.

Izaki, Hageman, Paul and Mittal all showed cellular damage, increased thiobarbituric reactive substances (TBA-RS), reduced glutation content, peroxide formation and decreased tocopherol content in the livers of the rats fed with deep-fried fats and oils.^{14,17} Varela et.al noticed serious vacuolization and moderate fibrotic degenerative areas in the livers of rats fed with heated sunflower oils.¹⁶ Based on their results, the above authors suggest that frying sunflower oil is potentially toxic and should be evaluated as a risk factor. The histopathological changes in the livers of the rats in our group have shown similarities with the results of earlier studies.

The results presented here indicate that deep-fried sunflower oil have toxic effects on rat livers. We think that our experimental data can be applicable to human studies. This findings desire special consideration for human health in consuming frying oils. We believe that public should be warned to consume less deep-fried foods and more efficient arrangements should be done about food preparations. Further well-designed studies are needed in order to elucidate exact mechanisms of how deep fried oil exert toxic effects to various organs

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