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Alterations in P53 Gene Expression, Lipid Profile, Total Antioxidant Activity and Semen Picture Induced by Repeated Fried Palm Oil in Rats: Possible Protective Role of Ginger

KEYWORDS: repeated fried oil, ginger, thermally oxidized oil, total antioxidant activity, p53, semen picture, lipid profile

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ABSTRACT *Recurrent using of oils during frying of food is a common trend in houses and commercial sectors. The repeated heated oil which is thermally oxidized may have detrimental effects on biological tissues. This study was conducted to examine the effect of repeated fried palm oil alone or in combination with ginger on serum lipid profile, total antioxidant activity (AOA), semen picture and p53 gene expression in rat tissues. Twenty four male rats were divided into four groups (6 each); control (normal basal diet), FPO (diet+ 15% fresh palm oil), RFO (dietmixed with 15% repeated fried palm oil), RFO+ G (diet + RFO+ ginger 1%). The results showed that the high fat diet (FPO or RFO) cause significant elevation in total cholesterol, phospholipids, HDL-c and LDL-c. AOA increased in hepatic tissue but decreased in testicular tissue of rats consumed RFO indicating oxidative stress which seems to affect semen picture dramatically causing a decrease in sperm count, motility% and live-dead% with an increase in sperm abnormalities %. Moreover, P53 gene expression increased significantly in both liver and testis of rats fed RFO. Ginger succeeded to modulate this effect on p53 expression but failed to modulate the biochemical alterations. It could be concluded that using repeated fried oil have obvious harmful effect for vital tissues like liver and reproductive system but the protective role of ginger need further studies.*

Introduction

Frying food using vegetable oils is a common method for preparing food, highly desirable especially for children all over the world. Vegetable oils (palm, soya bean, corn, sunflower, etc.) were regarded as the healthier choice comparing to animal fats due to their unsaturated fat and cholesterol free substance. The mostly used frying oils in the world are palm oil and soy oil [1].

Palm oil is extracted from Elaeis guineensis tropical plant. It contains 50% unsaturated fat mainly monounsaturated fatty acids and 50% saturated fat [2] and rich in palmitic acid (44.86%) [3]. Fresh palm oil contains antioxidants which have also anticancer and cholesterol lowering effects. These antioxidants include carotenoids, tocopherols and tocotrienols [4, 5].

Tocotrienols is 40–60 times more potent antioxidant than tocopherols. Due to lower PUFA content beside antioxidant content of palm oil [6], it is considered as a good choice as a frying oil owing to its respectable resistance to oxidation at extended period of heat and elevated temperatures [7]. Therefore, high percentages of palm oil are used as frying oil for both performance and financial reasons (its low price).

However, heating of oils at high temperature and in presence of oxygen results in their oxidative deterioration. Oxygen from air and water from food being fried when mixed with heated oil accelerating the rate of its oxidation. The cooked food absorbs this oxidized oil so it becomes part of our diet [8].

The repeatedly use of the same frying oil occur usually in the household or in the commercial area foremost to save cost. The oils would only be thrown away if their characters has changed like produced smoke, foam with bad smell or darkened color [9]. Consequently, in developing countries, intake of highly oxidized fat through intake of deep fried food is high.

The recurrent heating of oil decreases its antioxidant properties due to oxidation progression where antioxidants (e.g. vitamin E) are highly sensitive and destructed by heat [10]. This may lead to increase formation of free radicals in oil as reported that recurrent thermal exposure of oil may do so [7].

Thermal oxidative breakdown of Frying oils results in formation of lipid peroxidation compounds, volatile and non-volatile decomposition compounds, enzyme inhibitors, anti-nutritional factors, mutagens and carcinogens [11]. The changes that occur depend on the degree of unsaturation, existence of unsaponifiables and antioxidants.

During the frying process, polyunsaturated fatty acids (PUFA) of oil become oxidized, hydrolyzed and polymerized. The non-volatile compounds formed in the oil are polymers and polar substances. When frying oils are heated at 70°C, polar compounds as hydroperoxides and aldehydes are formed but heating at 150 °C produce aldehydes, giving rice to rancidity [12].

In addition, other toxic substances are formed in repeated heating oils, polycyclic aromatic hydrocarbons (PAHs) which are well-recognized for their mutagenic or carcinogenic prospective [13], dioxin impurity (a family of polychlorinated tricyclic aromatic compounds [14]. Others are triacylglycerol dimers, trimers which reach up to 30% of recycled oils and cyclic monomers which present to a minor extent but their higher rates of absorption in the body give them greater toxicity compared with polymers [15] beside the occurrence of compositional changes of free-fatty acids content (e.g. formation of trans-fatty acids) [16].

Many studies reported that recurrently heated oils may cause destruction of vitamins and essential fatty acids [5], with an increase in the production of free-radicals [1, 7, 17]. Oil adsorbed during frying process may represent up to one-third of the dry weight of a deep-fried food, so ingestion of these degradation products occur and may cause numerous pathophysiological effects. The free radicals are highly reactive, may bind to lipids, proteins, carbohydrates, and DNA in body cells, leading to severe oxidative stress. Continuously, this might exaggerate lipid peroxidation, leading to a wide series of biological alterations ranging from poor appetite and slight decline in growth to severe organ injuries and detrimental health risks [8, 11, 15]. Also, it may induce increase uptake of lipid, increased lipoprotein oxidation, disturbance of the endothelial function, damage the arterial wall and, subsequently, develop atherosclerosis [18-20], beside hypertension [21] .

Other adverse effects of oxidized dietary oils on humans and experimental animals have been established in a lot of studies. These include elevation of total cholesterol and free fatty acid levels of various tissues with deficiency of essential fatty acid, hemolytic anemia, thrombocytopenia and increased blood clotting time but enhanced platelet aggregation levels; liver become enlarged and injured beside, reproductive toxicity [22, 23]. It has been stated that oxidized fats and oils induce deficiency of nucleic acid which in turn cause alterations of genetic material [24], micronutrient malnutrition leading to reducing activity or even inactivation of key metabolic enzymes [17] like isocitric dehydrogenase, carnitine palmitoyl transferase-1 and glucose-6-phosphate dehydrogenase or increase in the activity of cytochrome P450 and b5 which occur in rats fed heated soybean oil [16]. Furthermore, ingestion of the formed trans-fatty acids is documented as a risk factor for cardiovascular system and may be linked to chronic respiratory disease, neural degenerative diseases and development of cancer [16].

The tumor suppressor gene p53 assumes a critical part in controlling cell cycle and in the preservation of genomic integrity during initiation of genetic damage. It is commonly believed that the chief mechanisms ruling the activity of p53 take place at the protein level. The p53 tumor suppressor protein is a universal sensor of genotoxic stress that regulates the transcription of genes required for cell-cycle arrest, DNA repair, cellular senescence, differentiation and apoptosis in vertebrates as diverse as animals and humans [25]. Regulation of p53 in response to stress most ordinarily happens by avoiding ubiquitination and degradation of the p53 protein [26]. On the other hand, in some models, the tumor-suppressing effects are mediated to a limited extent through activating p53 transcription [27].

Ginger (Zingiber officinale Roscoe, Zingiberaceae) is well-thought-out as one of the best usually consumed condiments for food all over the world [28]. Ginger rhizomes have special distinctive odor and flavor, and well known for their antioxidant property [6, 29]. Such features exist due to many bioactive components present in the oleoresin (i.e., oily resin) extracted from the roots of ginger, as [6]-gingerol (1-[4′-hydroxy-3′- methoxyphenyl]-5-hydroxy-3-decanone; gingerols, gingerones and shogaols [6, 30]. Moreover, these ingredients supposed to exert a variety of significant pharmacological and physiological activities. Therefore, most recently, interest in ginger or its various components as valid preventive or therapeutic agents has extended uniquely.

The antioxidant activity of ginger ethanol extract in soybean oil thermally oxidized at 180 °C, for 20 h was studied by [6]. They observed that the ginger extract offered superior protection for oil during the process of thermal oxidation of oil and exposed noticeable efficiency against lipid oxidation, consequently, it is suggested to be used as a food additive to act as a natural antioxidant for food [6]. Therefore, we wondered if ginger could also be applied in the area of biological tissue for the protection against oxidative stress of repeated fried oil . The present study aimed to determine the effect of repeated fried palm oil on p53 mRNA levels in the liver and testes tissues of rats using real-time PCR technique in addition to lipid profile and semen picture. The study also evaluates ginger effectiveness in protection against some adverse effects of consuming frying oil.

Materials and Methods

1. Materials

Unpackaged palm oil and potatoes were acquired from local market in Ismailia governorate, Egypt to be used in this experiment. Palm oil was chosen because it is cheap and available to consumers, particularly in rural areas in Egypt. Ginger was obtained from MAPECO pharmaceutical, Egypt. Chemical kits for measuring total lipids, triglycerides, total cholesterol, phospholipids and HDL-c were obtained from Biodiagnostic Company, Cairo, Egypt

2. Animals

Twenty-Four sexually mature male Albino rats (weighing approximately 150±10 g) were used in this experiment. The rats were obtained from laboratory animal house of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Rats were acclimatized for two weeks before starting the experiment. During this period, they housed in metal cages at a laboratory temperature of 23±3°C, maintained under a 12 h light/dark cycle, fed on a standard diet and water ad libitum. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. All animal study plans were approved by the Experimental animal bioethics Committee of the Suez Canal University (Approval number 2016087)

3. Preparation of frying oil

For 2 liter palm oil, pan frying was done in an uncovered stainless steel pan fryer. Two batches, each containing around 400 g potatoes or tamea (reciprocal) were fried. The frying processes were repeated 16 times at 175 ± 5 °C (12.3 minutes each) twic e daily for 8 successive days. No renewal of oil was done. At the end of the experiment, oil was taken out, filtered and placed in a bottle in the refrigerator (4°C), then thoroughly mixed with the basal diet.

4. Experimental design

The animals were divided at random into four groups, 6 animals in each group, namely: Group I, the Control group (CO) where the animals were fed basal diet. Group II, the Fresh Palm Oil group (FPO) where the animals fed basal diet containing fresh pure palm oil (150 mL/kg diet). Group III, the Repeated Fried Oil group (RFO) where the animals fed the basal diet containing thermally oxidized palm oil repeatedly used for frying process with a dose of 150 mL/kg diet, while

Group IV (RFO+G), was given a combination of RFO (150 mL/kg diet) and ginger (1 g/100 g in diet) [31]. All rat groups were fed the corresponding previously mentioned diets for one month and body weight was monitored weekly.

At the end of the experiment, all animals were fasted for 12 hours and then blood samples were collected from venous plexus of eye under diethyl ether anesthesia and all efforts were made to minimize suffering. Blood samples were left to clot. Sera were separated from the clotted blood samples by centrifugation at 3000 rpm and stored at -20°C until used for biochemical analysis. Liver and testes tissue samples were taken, snap frozen in liquid nitrogen at -196°C, kept at -80°C for P53 gene expression analysis,. The cauda epididymis was collected in pettri dish containing 2 ml warm normal saline (37°C). To obtain epididymal content, cauda epididymis was macerated in saline. The obtained suspension was considered as semen [32] which used to determine epididymal semen picture.

5. Serum Lipid profile

Total lipids [33], triglycerides [34], total cholesterol [35], phospholipids [36] and HDL-c [37] were estimated calorimetrically using kits of Bio Diagnostics company. LDL-C was calculated using the Friedwald equation [L = C − H − 0.2 T; Where L is LDL –c, C is total cholesterol, H is HDL -c, , T are TG (mg/dl)] [38].

6. Measurement of total antioxidant activity (AOA)

AOA was measured in serum, testis and liver according to [39]. A standard solution of Fe-EDTA complex reacts with H_2O_2 leading to formation of OH^- radical which degrade benzoate resulting in release of thiobarbituric acid reactive species (TBRAS). Antioxidant from added sample causing suppression of the produced TBRAS. The inhibition of the developed color is defined as AOA and can be measured spectrophotometrically at 532 nm.

7. Realtime -PCR analysis

Real-time quantitative PCR was done using Fast Start Essential DNA Green Mater (Roche, 06402712001) and Light Cycler Nano system (Roche Applied Science) using β-actin cDNA as an internal control. Firstly, Reverse transcription was performed for the RNA samples isolated from liver and testes using PrimescriptTM 1st strand cDNA synthesis kit (Takara, Japan) according to the manufacturer's instructions. Primers for Rattus norvegicus tumor protein p53 (Tp53) and β-actin cDNAs (Table 1) were designed using Primer3plus software (www.bioinformatics.nl/primer3plus) [40]. Each PCR reaction consisted of 10 μl of Mater Mix 2X conc., 2 μl of c DNA template ,10 μM of each primer, and ddH2o to a final volume of 20 μl. Reactions were run under the following conditions: initial denaturation at 95°C for 10 min; 35 cycles of 95°C for 10 sec , 60°C for 10 sec, 72°C for 15 sec, one cycle of 95°C for 30 sec; and one cycle of 60°C for 20 sec, 95°C for 20 sec. Each PCR run included an RT negative control for each gene and a no-template control with water added instead of cDNA. All samples and standard curves were run in triplicates and all samples for each gene were run on the same plate. Quantification analysis of gene expression using the relative quantification calibrator normalized efficiency corrected analysis method was performed using the Roche Lightcycler Nano software.

Table 1. Real-time PCR primers of *Rattus norvegicus* *P53*and β-actin genes

Gene	Primer description	Sequence (5'-3')	Location	Product size
CYP1C 1	F	5'- AGG GAG TGC AAA GAG AGC AC -3'	1028-1047	136 bp
	R	5'- CCT CAT TCA GCT CTC GGA AC-3'	1145-1164	
β-actin	F	5'- AGCCATGTACG TAGCCATCC-3	468-487	115 bp
	R	5'- ACCCTCATAGA TGGGCACAG-3	563-582	

Results

Table 2 summarizes the effect of feeding palm oil (FPO, RFO, RFO+G) on serum lipid profile of rats. The results showed that the total lipids significantly increased in rat fed palm oil either fresh or repeated fried compared to normal control. With a higher significant level (P< 0.001) in those fed fresh oil than repeated fried group. However, the rise in the total lipid level in the RFO+G group than normal is not significant. No significant change occurred in the triglyceride level of all groups. Results of cholesterol, phospholipids, HDL – c and LDL-c showed significant increase in their levels in all oil treated groups comparing to control. But the elevation of the phospholipid level in RFO treated group comparing to control was not significant.

Table 2. Effect of feeding palm oil (FPO, RFO, RFO+G) on serum lipid profile of rat

Parameters	Control	Fresh palm oil (FPO)	Repeatedfried palm oil (RFO)	RFO+Ginger (RFO+G)
Total lipids (mg/dl)	355.9 ± 24.4^{c}	701.2± 37.6^{a}	574.1± 22.7^{b}	449.8 ± 37.9^{bc}
Triglycerides (mg/dl)	163.2 ± 5.0^{a}	147.1 ± 2.0^{a}	150.3 ± 4.8^{a}	150.9 ± 7.8^{a}
Total Cholesterol (mg/dl)	95.4 ± 4.7^{b}	139.1 ± 6.0^{a}	119.9 ± 6.1^{a}	128.3 ± 2.4^{a}
Phospholipids (mg/dl)	88.2 ± 1.5^{b}	97.8± 1.5^{a}	92.5 ± 1.4^{ab}	96.7 ± 2.5^{a}
HDL-c (mg/dl)	39.7 ± 1.1^{b}	53.5± 2.7^{a}	50.7± 1.4^{a}	54.3 ± 2.2^{a}
LDL-c (mg/dl)	24.8 ± 3.7^{b}	56.1± 5.6^{a}	45.1± 6.2^{a}	46.5 ± 1.7^{a}

For all tables data expressed as Mean ± SD at P< 0.05. Different letters means significantly different

Table 3 summarizes the effect of feeding palm oil (FPO, RFO, RFO+G) on total antioxidant activity (AOA) of rats. The results showed that there was no significant difference in serum total antioxidant activity (AOA) among all groups. AOA increased significantly in hepatic tissue of rats treated with RFO or RFO+G comparing to control. Hepatic AOA of FPO group showed a non-significant increase when compared with the control group and a non-significant reduction when compared with RFO and RFO+G groups. Our data showed that AOA levels in testicular tissue of RFO and RFO+ ginger groups were significantly lower than that of control and FPO groups. Testicular AOA of FPO group was significantly decreased than control.

Table 3. Effect of feeding palm oil (FPO, RFO, RFO+G) on total antioxidant activity (AOA) of rats.

	Control	Fresh palm oil(FPO)	Repeated friedpalm oil (RFO)	RFO + Ginger(RFO+ G)
Serum (mmol/L)	2.19 ± 0.05^{a}	2.26 ± 0.29^{a}	2.18 ± 0.30^{a}	1.99 ± 0.22^{a}
Liver (mmol/ 100 g tissue)	3.58 ± 0.19^{b}	4.02 ± 0.11^{ab}	4.23 ± 0.07^{a}	4.40 ± 0.06^{a}

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Testis (mmol/ 100 g tissue)	2.94 ± 0.14 [a]	1.88 ± 0.16 [b]	0.84 ± 0.11 [c]	0.75 ± 0.11 [c]

Table 4 summarize the effect of feeding palm oil (FPO, RFO, RFO+G) on epididymal semen picture. The results reported that there was a significant decrease in the sperm cell concentration and individual motility%, live-dead% with increase in sperm abnormalities in rats of the repeated fried oil (RFO) treated groups (with or without ginger) when compared to control group.

Table 4. Epididymal semen picture of control, fresh and repeated frying palm oil treated rats (Mean ± SE.).

	Sperm cellconcentration (125x104) / ml	Individual motility %	Live – dead %	Sperm abnormalities%		
				Primary	Secondary	Total
Control	51.94±3.73 [a]	72.64±0.21 [a]	83.30±0.92 [a]	3.51±0.13 [a]	9.51±0.46 [b]	13.25±0.48 [b]
Fresh palm oil (FPO)	47.56±4.3 [ab]	69.58±0.43 [a]	81.12±0.87 [a]	4.08±0.32 [a]	10.97±0.61 [b]	14.95±0.63 [b]
Repeated fried palm oil (RFO)	35.96±3.12 [c]	59.47±0.51 [b]	65.32±1.19 [b]	4.65±0.51 [a]	16.55±0.38 [a]	22.01±0.78 [a]
RFO + Ginger (RFO+ G)	38.52±3.07 [bc]	60.28±0.64 [b]	70.29±2.36 [b]	4.36±0.47 [a]	17.35±0.47 [a]	21.83±1.54 [a]

In the same column values having different superscript letter are significantly different (P<0.05).

P53 gene expression patterns using real-time PCR.
Table 5 and Fig 1 summarize the Amount of p53 mRNA, normalized to β-actin mRNA in the four studied groups. The results indicated that the gene transcripts (mRNA) of the p53 gene were successfully detected in the all liver and testes tissues within all control and treated groups (Fig 1, Table 5). Gene expression was normalized with the expression values of the β-Actin gene. The results revealed that p53 mRNA expression in the liver and testes tissues of the group fed RFO diet was higher (82.14 and 97.0 in liver and testes respectively) than the control group. On the other hand, the mRNA expression in the liver and testes in the group fed basal diet containing RFO + ginger were 5.24 and 31.34 in liver and testes respectively which indicate that ginger treatment was able to inhibit the up-regulation of the gene expression occurred by RFO exposure.

Table 5. Amount of p53 mRNA, normalized to β-actin mRNA.

Samplename	Rat P53 average Ct	B-actin geneaverage Ct	Ct	Ct	Ratio
L.C	34.2	30.21	3.99	0	1
L.FPO	33.32	30.81	2.51	-1.48	2.79
L.RFO	30.16	32.53	-2.37	-6.36	82.14
L.RFO+G	34.07	32.47	1.6	-2.39	5.24
T.C	33.74	30.98	2.76	-1.23	2.35
T.FPO	33.7	31.45	2.25	-1.74	3.34
T.RFO	28.67	31.28	-2.61	-6.6	97
T.RFO+G	29.7	30.68	-.098	-4.97	31.34

Where :
L.C: liver samples from rats in control group.

L.FPO: liver samples from rats fed basal diet containing the natural oil

L.RFO: liver samples from rats fed basal diet containing repeated fried, thermally oxidized palm oil

L.RFO+G: liver samples from rats fed basal diet containing repeated fried, thermally oxidized palm oil + ginger

T.C: Testes samples from rats in control group.

T.FPO: Testes samples from rats fed basal diet containing the natural oil

T.RFO: Testes samples from rats fed basal diet containing repeated fried, thermally oxidized palm oil

T.RFO+G: Testes samples from rats fed basal diet containing repeated fried, thermally oxidized palm oil + ginger

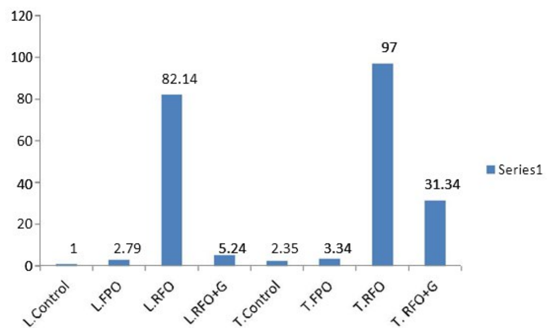

Fig 1. Amount of p53 mRNA, normalized to β-actin mRNA.

Discussion
Table 2 shows that adding 15% palm oil caused elevation in total-cholesterol, HDL-C, phospholipids and LDL-C in all oil treated groups compared to the control group. Fried heated palm oil did not appear to be more hyperlipidemic than fresh palm oil so the increase in lipid profile parameters may be due to high dietary lipid content (150 g palm oil / kg diet). The finding that fried oil with or without ginger shows the same rise in the parameters of the lipid profile as the fresh palm oil group, is in harmony with the finding of [17]. But these effects was in contrary with results of [41] who reported that both fresh and heated oil did not interfere with serum TC, TG, LDL-C but decreased HDL-C. Other study showed Elevation of plasma TC levels, with decrease in level of HDL and increase in level of LDL [42].

Elevation of total cholesterol may be explained by the finding of [43] that a palmitic acid-rich diet leads to a higher total plasma cholesterol concentration than an oleic-rich diet and it is well known that palm oil is rich source of palmitic acid [3].

Oxidative stress in biological systems is known to occur when there is an imbalance between the cellular antioxidant defense system and the factors responsible for creating oxidative conditions. Due to the significance of the deviations in the oxidant to antioxidant ratio in the development of pathological conditions, the measure of antioxidant capacity has progressively been used as a monitor of the antioxidant status of biological systems [44]. Plasma total antioxidant status (TAS) is a collection of several antioxidant defenses, including enzymatic and non- enzymatic systems [45].

Possible explanation for the elevation of AOA in hepatic tissue of RFO groups compared to control (Table 3) is the changes in antioxidants enzymes as catalase and GPx activities which were reported to be increased in rats liver fed heated or fried

palm oil [11]. Increased cytochrome p450 has been stated in rats fed heated oil [16]. It is probable that oxidized oil may lead to an escalation of $O_2.^-$ generation by cytochrome p450 and NADPH dependent microsomal source, and simultaneously increased formation of H_2O_2. It has also been shown that the destruction of H2O2 by catalase appears to be important only at higher concentrations of H_2O_2, while at lower concentration; it is reduced by glutathione peroxidase (GPx). However, catalase and GPx have been credited with the ability to detoxify peroxides and hydroperoxides and prevent lipid peroxidation [11].

Our data show that AOA in testicular tissue of RFO and RFO+ ginger groups decreased significantly than the control group because thermally oxidized oil generates harmful oxygen reactive species [46]. This may be a significant finding in view of the concurrent harmful effects of free radicals on testis [47] as it is well known that decreased AOA results in increased lipid peroxidation, which sequentially plays an important role in the pathogenesis of deleterious changes [48]. In the same respect with other reports which stated that feeding oxidized oils to rats induces peroxidation *in* vivo, destroys biological membranes leading to changes in fluidity and permeability [7, 49] and change the membrane bound enzymes [10]. All of this explained the alterations induced here by fried oil on the studied semen parameters (Table 4) as shown by decreased number and motility of sperms which is accompanied by increased abnormalities and % of dead sperms in rats fed fried oil with or without ginger and also substantiated the increased expression of p53 gene in testis of RFO group. It becomes obvious that Ginger failed to improve the harmful effects of fried thermal palm oil on all biochemical data; lipid profile, AOA or semen picture. However, the expressed level of p53 gene in testis of RFO+ginger group was decreased significantly comparing to RFO group.

In general, it is very hard to detect wt p53 protein in tissue since its level is very low because of a rapid turnover and low levels of expression [50-53] and high levels are only found in cells expressing mutant forms of p53 [54]. It is generally believed that mutant forms of p53 are protected and stabilized by a conformational change from the normal fast degradation that happens with wt p53. High levels of p53 may be induced in normal cells after exposure to certain genotoxic compounds or ionizing radiation. This is most likely because of stabilization of wt p53 by phosphorylation by specific kinases such as DNA–activated protein kinase [55].

Real-time results in this study shows a large increase in p53 gene expression in the group fed on repeated fried oil compared to control group. Although the exact mechanism underlying the RFO-dependent p53 induction remains unknown, results of this study may16indicate a cellular response to a genotoxic stress exerted by the RFO. Thus, the higher transcript levels of p53 may be a condition required to manage the stress and maintain genomic stability. [56] reported that different hepatocarcinogens induce high levels of p53 protein in the rat liver however, non-hepatocarcinogenic genotoxic compounds and cytotoxic compounds do not increase p53 in this organ.

In conclusion, current data show that the repeated fried oil cause a significant alterations in lipid profile, total antioxidant activity, semen picture and p53 gene expression; indicating the undesirable genotoxic effect of the repeated fried oil on tissues of rats. However, ginger partly modulates these alterations as shown only by its effect on the p53 gene expression but failed to modulate either biochemical changes or the alterations in semen picture. Due to limited protective effect of ginger, further studies are recommended to examine possible ginger protective effect using different protocols.

These data are alarming us to the demand of changing lifestyle in our society and trying to decrease using of repeated fried oil. With a considerable attention to the elevation in the lipid profile parameters in rats fed fresh palm oil, we concluded that using vegetable oils as heart friendly (because of their polyunsaturated fat content) is not freely safe and great attention must be considered to the daily allowance and amount of oil intake as well.

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