Genetic alterations induced by toxic effect of thermally oxidized oil and protective role of tomatoes and carrots in mice

Mariam G. Eshak[1], Inas S. Ghaly[1], Wagdy K. B. Khalil[1], Ibrahim M. Farag[1], Kadry Z. Ghanem[2]
[1]Cell Biology Department, [2]Food Science and Nutrition Department, National Research Centre.
12622 Dokki, Giza, Egypt
E-mail: wagdykh@yahoo.com ; Inas.ghali@yahoo.com

Abstract: The present study was designed to investigate the genetic alterations and sperm abnormalities in male mice fed diet containing thermally oxidized sunflower oil repeatedly used for frying processes (FO). Also, tomatoes and carrots were added to FO diet to test their protecting ability against potential hazards caused by oxidized oil rich foods. The genetic alterations including DNA fragmentation and chromosome aberrations as well as changes of mRNA expression of some lipid metabolism related-proteins were determined. The results revealed that rate of DNA fragmentation was significantly higher in animals fed FO diet than those of animals fed normal oil diet (NO). On the other hand, the rates of DNA fragmentation decreased in animals fed FO diets plus low (5%) or high (10%) concentration of tomatoes (FOT1 or FOT2) or carrots (FOC1 or FOC2) compared with those fed FO diet. Chromosome examination showed that total structural aberrations increased significantly in animals fed FO diet than those fed NO diet. On the other hand, animals fed diets containing FO plus low or high concentration of tomatoes or carrots had significantly lower frequencies of total structural aberrations than those fed FO diet. Sperm studies showed a significant increase in the number of morphologically abnormal sperms and a significant decrease in the sperm count in animals fed FO diet compared with those fed NO diet. However, the animals fed diets containing low or high level of tomatoes or carrots showed significant decreases of sperm abnormalities. The mRNA expressions of the lipid metabolism related-genes, RBP, H-FABP and C-FABP were significantly higher in liver tissues of mice fed FO diet than those found in mice fed NO diet. However, the expression of all tested genes was down-regulated in FOT1 and FOT2 or FOC1 or FOC2 groups compared with those detected in the FO group. In conclusion, the present study adds evidence for a link between prolonged feeding intake of FO diet and induced mutagenic effects in animal cells. However, tomatoes and carrots proved to be good protective agents against hazards of such mutagenic foods. [Journal of American Science 2010;6(4):175-188]. (ISSN: 1545-1003).

Keywords: Thermally oxidized oil, mice, tomato and carrot, genetic alterations, RT-PCR, sperm morphology.

1. Introduction

Several studies reported that using vegetable oils in prolonged frying processes may be a principle or strong reason for inducing or forming mutagenic or carcinogenic diets (Hamilton et al., 1997; Coultate, 2002; Gouveia De Souza et al., 2004; Pavia & Gordon, 2005; Khalil et al., 2009). The mutagenicity/ carcinogenicity of such diets may be due to the fact that the repeatedly used vegetable oils in frying processes undergo degradation by oxidative reactions that lead to form lipid peroxidation (Nagao et al., 1997; Lampe, 1999; Raloff, 1999).

High intakes of diets containing lipid peroxidation component may be associated with the generation of free radicals (Schut et al., 1997; Lampe, 1999; Coultate, 2002; Guardiola et al., 2008). If not quenched by antioxidants, these highly reactive compounds will react with and potentially alter the structure and function of several cellular components, such as lipid, lipoproteins, proteins, carbohydrates, RNA and DNA. These alterations have been considered as important factors which can affect the gene expression for a lot of genes causing variety of diseases, especially hyperlipidemia, atherosclerosis and cardiovascular diseases (Suganuma & Inakuma, 1998; Lampe, 1999; Nicolle et al., 2003).

Vegetables have been identified to be major dietary sources of antioxidants. Tomatoes and carrots are the main vegetables that have been found to contain a remarkable combination of antioxidant micro-constituents, such vitamins A, C, D and E, polyphenols and various carotenoids, including alpha – and beta – carotene and lycopene (Duthie et al., 1996; Lampe, 1999; Cramer et al., 2001; Nicolle et al., 2003; Gitenay et al., 2007; Devaraj et al., 2008). The activity of antioxidants has been shown to improve immune status, scavenge of free radicals, reduce the production of DNA adducts and could be effective means of preventing variety of diseases (Allard et al., 1994; Brown et al., 1994; Lampe, 1999; Van Breda et al., 2005; Devaraj et al., 2008). A significant decrease in endogenous oxidative base damage in the lymphocyte DNA was detected by Duthie et al. (1996) in both smokers and non-smokers with 20–wk daily supplement of micro-constituents of tomatoes such as vitamin C (100 mg), α–tocopherol (280 mg), and β–

carotene (25 mg). Also, Devaraj et al. (2008) tested the effects of different doses of purified lycopene supplementation on biomarkers of oxidative stress in healthy volunteers. Their results revealed a significant decrease in DNA damage by the comet assay and a significant decrease in urinary 8–hydroxyl deoxaguanosine (8–OhdG) at 8 weeks versus baseline, with 30 mg lycopene/ day. In an additional study, tomatoes were found to have a preventive effect on atherosclerosis by protecting plasma lipids from oxidation. This effect was observed by Suganuma & Inakuma (1998) who found that mice fed the atherogenic diet without tomato for 4 months had a significant increase in plasma lipid peroxide level than those fed the atherogenic diet containing 20% (w/w) lyophilized tomato powder.

On the other hand, Nicolle et al. (2003) investigated the effects of 3-week supplementation of the diet with carrots on lipid metabolism and antioxidant status in rats. They observed significant decreases of cholesterol levels together with reduction of the triglycerides levels in the liver. Also in this experiment, the results revealed that carrot consumption improved the antioxidant status by decreasing the urinary excretion of thiobarbituric acid reactive substances (TBARS), reducing TBARS levels in heart and increasing the vitamin E plastimatic level as compared to the control. The authors concluded that these effects could be interesting for the protection of cardiovascular disease.

Tomatoes and carrots (or its micro-constituents) have also been considered as anti-carcinogenic foods (Cramer et al., 2001). This study estimated the women consumption of carrots and tomatoes of 549 ovarian cancer and 516 control cases. They found that the consumption of such foods that have higher level of carotene and lycopene had reduced the risk of ovarian cancer. Gitenay et al. (2007) used epidemiologic studies and found that high consumption of tomatoes had a protective effect against prostate cancer. Furthermore, few studies have been investigated the effect of vegetables, especially carrots, on gene expression changes (Van Breda et al., 2005). They studied the effect of vegetables on the expression of genes involved in carcinogenic and anti-carcinogenic processes in the lungs of female mice using cDNA microarray technology. During their study, mice fed different diets: a control diet containing no vegetables, a diet containing mixture of vegetables and a diet containing carrots. They investigated the expression of 602 genes involved in pathways of anti–carcinogenesis. The results indicated that carrots were able to modulate most gene expressions and most of these effects occurred in processes that favored lung cancer risk prevention.

Although, humans usually use the vegetable oils in prolonged frying processes, there were no data regarding the possible effects of consuming diets containing repeatedly used frying oils (FO) on genetic alterations and sperm changes in male mice. Also, there were deficient data on the effect of FO diets on the expression of lipid metabolism–related protein genes. Therefore, the present study was aimed to investigate the DNA fragmentation, chromosome aberrations, sperm abnormalities and changes of mRNA expression of some lipid metabolism related–protein genes in mice fed diet containing thermally oxidized sunflower oil repeatedly used for frying process (FO). Also, some vegetables such as tomatoes and carrots were evaluated for their protecting ability against potential hazards caused by the thermally oxidized oil rich foods.

2. Materials and Methods

2.1 Animals: Forty eight adult male Swiss mice, weighting about 25 gm, were obtained from the Animal House Lab., National Research Centre, Giza, Egypt. Apparently healthy animals were randomly assigned into six groups (8 mice each) and housed in stainless steel wire mesh cages on a bedding of wood chips. They were kept in an ambient temperature of 25±3°C on a light/ dark cycle of 12/12 h and supplied with mice chew and fresh water *ad libitum*.

2.2 Diets: Six different diets were used in this study. The ingredients used were according to Phillip et al. (1993) to formulate these diets as shown in Table 1.

2.3 Preparation of frying oil: Sunflower oil was used for frying potatoes. The frying processes were repeated for about 15 times (20 min each). The frying oil was then placed in a glass bottle and mixed with the basal diets, as shown in Table 1.

2.4 Preparation of tomatoes and carrots: The Egyptian types of tomatoes and carrots were purchased from local market. These vegetables were sliced and then dehydrated, separately, by drying at 60°C for three days. Dried tomatoes and dried carrots were also ground, separately, using an electric mill. Five or ten percent of each protector (tomatoes or carrots) were added to the basal diet according to Phillip et al. (1993).

2.5 Experimental design: Mice were divided into six groups as follows: First group, as a negative control was fed basal diet containing the natural sunflower oil (NO) that was not used in frying process. The second group was fed the basal diet containing thermally oxidized sunflower oil repeatedly used for frying process (FO). The third and fourth groups were fed the basal diet containing FO plus dried ground tomatoes at levels of 5% (FOT1) or 10% (FOT2) of the diet. The fifth and sixth groups were fed the basal diet containing FO plus dried ground of carrots at levels of 5% (FOC1) or 10% (FOC2) of the diet. All mice groups were fed the corresponding previously mentioned diets for one month. Afterward, mice were sacrificed for genetic alteration and sperm abnormality studies.

Table 1: The composition of the different diets used.

Ingredient %	Types of diets					
	B+NO	B+FO	B+FOT1	B+FOT2	B+FOC1	B+FOC2
Casein	14	14	14	14	14	14
Sucrose	10	10	10	10	10	10
Salt mixture	5	5	5	5	5	5
Vitamin mix	1	1	1	1	1	1
Sunflower oil	4	-	-	-	-	-
Cellulose	5	5	5	5	5	5
FO	-	4	4	4	4	4
Tomato (5 %)	-	-	5	-	-	-
Tomato (10%)	-	-		10	-	-
Carrot (5 %)	-	-	-	-	5	-
Carrot (10 %)	-	-	-	-	-	10
Starch	61	61	61	61	61	61

B= Basal diet; NO= Normal sunflower oil (not used in frying processes); FO= Thermally oxidized sunflower oil (repeatedly used in frying processes); FOT1= FO + dried ground tomatoes at level 5%; FOT2= FO + dried ground tomatoes at level 10 %; FOC1= FO + dried ground carrots at level 5%; FOC2= FO + dried ground carrots at level 10%

2.6 DNA fragmentation:

Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 1.5 ml of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 min. The samples were centrifuged for 20 min at 10 000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 750 µl of 5% TCA, followed by incubation at 100°C for 20 min. Subsequently, to each sample 2 ml of DPA solution [200 mg DPA in 10 ml glacial acetic acid, 150 µl of sulfuric acid and 60 µl acetaldehyde was added and incubated at room temperature for 24 h (Gibb et al., 1997). The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA Fragmentation} = \frac{\text{OD of fragmented DNA (S)}}{\text{OD of fragmented DNA (S)} + \text{OD of intact DNA (P)}} \times 100$$

2.7 Cytogenetic analysis: For chromosomal analysis, both treated and control animals were sacrificed by cervical dislocation. Two hours before sacrifice, mice were injected with 5 mg colchicine /kg. b.w. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of **Preston et al. (1987)**. Fifty metaphase spreads per animals were analyzed, for scoring the different types of chromosomal aberrations.

2.8 Sperm analysis: For sperm-shape analysis, the epididimus excised and minced in about 8 ml of physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (aqueous), according to the methods of Wyrobek et al. (1983), Farag et al. (2002) and Hana et al. (2008). At least 2500 sperms per group were assessed for morphological abnormalities. Epididymal sperm count was also determined by hemocytometer.

2.9 Semi-quantitative RT-PCR:

The RT-PCR assay was conducted to verify the expression of fried oil-diet on the mRNA expression of the lipid metabolism related-protein genes: retinoid binding protein (RBP), heart fatty acid binding protein (H-FABP), and cutaneous fatty acid binding protein (C-FABP), in the liver tissues. The effect of tomatoes and carrots on the expression of these genes was also examined using the following steps:

2.9.1 RNA extraction: Immediately after animal sacrifice, liver tissues were frozen in liquid nitrogen and stored at −80 °C prior to extraction. Total RNA was extracted from 50 to 100 µg of each tissue by the standard TRIzol extraction method (Invitrogen, Paisley, UK). The solution of extracted RNA was recovered in 100 µl molecular biology grade water. Then, the total RNA samples were pretreated using DNA-free™ DNase treatment to remove any possible genomic DNA contamination. These steps were performed according to manufacturer's protocol (Ambion, Austin, TX, USA).

2.9.2 Reverse transcription: The complete Poly(A)$^+$ RNA isolated from the mice samples was reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo (dT) primer. The composition of the reaction mixture, termed as master mix (MM), consisted of 50 mM $MgCl_2$, 200 U/ µl reverse transcriptase (RNase H free), 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, and 50 µM of oligo (dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards, the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through polymerase chain reaction (PCR) (Brun et al., 2006; Khalil et al., 2009).

2.9.3 Polymerase chain reaction (PCR): The first strand cDNA from different mice samples was used as templates

for RT-PCR with a pair of specific primers. The sequences of specific primers and product sizes are listed in Table (2). The reaction mixture for RT-PCR in a total volume of 20 µl was consisted of 10 mM dNTP's, 50 mM MgCl2, 1 U/ µl taq polymerase,10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3), and autoclaved water. The PCR cycling parameters were one cycle at 95 °C for 4 min, 50 cycles at 94 °C for 30 s, 55 to 60 °C for 30 s, 72 °C for 60 s, and a final cycle at 72 °C for 7 min. The PCR products were then loaded onto 2.0 % agarose gel, with PCR products derived from β-actin of the different mice samples (Kronmiller et al., 1995, Khalil et al., 2009).

2.10 Statistical Analysis: Data for cytogenetic analysis were statistically analyzed by ANOVA. Wilcoxon′ s Signed – rank sum test was used for the statistical analysis of the sperm data. Furthermore, gene expression data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System (SAS, 1982) followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significant were based on probability of $P < 0.05$.

Table 2: Primers and reaction parameters in RT-PCR

Target cDNA	Primer name	Primer sequence (5'–3')	Annealing temperature (°C)	PCR product size (bp)
β-Actin	Act-F	CCC CAT CGA GCA CGG TAT TG	57	189
	Act-R	ATG GCG GGG GTG TTG AAG GTC		
RBP	RBP -F	GC AAG GCT CGT TTC TCT GG	55	178
	RBP -R	GAC TCG TCC CTT GGC TGT AG		
H-FABP	H-FABP -F	CTA GCA TGA GGG AAG CAA GG	55	138
	H-FABP -R	TGC TTC ATC CAG ACA AGT GG		
C-FABP	C-FABP-F	GGG CTG GCT CTT AGG AAG AT	60	100
	C-FABP-R	AAA ACA CGG TCG TCT TCA CC		

3. Results

3.1 DNA fragmentation

As shown in Table 3, the rates of DNA fragmentations were significantly higher (P< 0.001) in animals fed diet containing FO than those in control group. On the other hand, the rates of DNA fragmentations decreased significantly (P< 0.05 or P< 0.01) in animals fed diets containing tomatoes (FOT1 or FOT2) compared with those observed in FO group, in which FOT2 group had the lowest rate of DNA fragmentations. The rates of DNA fragmentations also decreased in animals fed diets containing carrots (FOC1 or FOC2) than those of FO group, however, these decreases were not statistically significant.

Table 3: Rates of DNA fragmentation in male mice fed different diets.

Treatment	DNA fragmentation	
	Rang	Mean± S.E
NO	15.2 - 20.1	17.6 ± 2.4^{c}
FO	37.9- 38.2	38.3 ± 0.1^{a}
FOT1	24.1-30.3	27.2 ± 3.1^{b}
FOT2	18.6- 29.6	23.4 ± 3.2^{bc}
FOC1	33.4 - 37.5	35.5 ± 2.0^{ab}
FOC2	32.7 - 36.0	34.4 ± 1.6^{ab}

[a,b,c,]: Means followed by different superscripts, within columns, differ significantly ($P< 0.05$), NO= Normal oil, FO= Thermally oxidized oil (repeatedly used in frying processes), FOT_1 = FO + dried tomato at level 5%, FOT_2 = FO + dried tomato at level 10%, FOC_1 = FO + dried carrot at level 5%, FOC_2 = FO + dried carrot at level 10%.

3.2 Chromosome examination

Examination the mice chromosomes showed structural aberrations (Table 4), however, numerical aberrations were absent. The structural aberrations of the chromosomes included chromatid gaps, deletions, chromatid breaks, centromeric fusions (C.F), end to end association (E.E) and rings. The mice fed FO diet had higher frequencies of chromosomal aberrations than the control group. Deletions were more frequent than other types of chromosome aberrations. Statistical analysis showed that there were highly significant (p< 0.01) differences between FO and NO groups in the frequencies of total structural aberrations, especially deletions.

The animal fed FO diet plus low level (5%) of tomato (FOT1) had decreased in the frequencies of gaps, deletions, C.F, E.E and total structural aberrations. These decreases were highly significant (p< 0.01) for the frequencies of the deletions and significant (P< 0.05) for the frequencies of the total structural aberrations (Table 4). The frequencies of chromatid breaks were similar in the two mentioned groups. However, the frequencies of rings were non-significantly decreased in the FOT1 group compared to those found in the FO group. Regarding to high concentration of tomato, FOT2 group had the lowest percentages of all structural aberrations. The only exception to this percentage was in the frequency of chromatid breaks which was similar in the FO group. Statistical analysis showed that there were significant

(P< 0.05) differences of each of deletions and total structural aberrations between FO and FOT2 groups (Table 4).

On the other hand, the frequencies of chromosome aberrations were lower (except for chromatid gaps and rings) in the animals fed diet with low level of carrots (FOC1) compared with those of the FO group (Table 4). Statistical analysis showed significant differences (P< 0.05) for the frequencies of deletions and total structural aberrations between FOC1 and FO groups. However, the frequencies of the chromatid gaps and the rings were raised, non-significantly, in the FOC1 group compared with the FO group. Also the frequencies of chromosome aberrations decreased (except for the rings) in animals fed diet with high level of carrots (FOC2) than those of the FO group. Statistical analysis showed that there were significant (P< 0.05) differences in the frequencies of deletions and total structural aberrations between these two groups. However, the ring aberrations were raised, non-significantly, in the FOC2 group compared to those of the FO group (Table 4).

3.3 Sperm examination

3.3.1 Sperm-shape analysis: Sperm examination showed that the abnormalities of sperms (head and tail) were more frequent in mice fed FO diet than those of control group (Table 5). Statistical analysis showed that the differences of the frequencies of head abnormalities (such as amorphous, without look, banana shape and big shape), tail abnormalities and total sperm abnormalities (head + tail) were significant (P< 0.05 or P< 0.01) between FO and control groups. However, the sperm abnormalities in head and tail decreased in animals fed FOT1 and FOT2 diets compared to those of the FO group. These decreases were significant (P< 0.01 or P< 0.05) in the frequencies of banana shape, big shape, tail and total abnormalities (Table 5). The frequencies of sperm abnormalities (banana shape, big shape and total abnormalities) were significantly lower in animals fed FOC1 diet compared to those fed FO diet. Also, the animals fed FOC2 diet had significantly decreases in all sperm abnormalities (amorphous, banana shape, two head and total head abnormalities) compared with that fed FO diet (Table 5).

3.3.2 Sperm counts: Sperm counts decreased significantly (P < 0.01) in animals fed FO diet than those of the control group. In contrast, sperm counts significantly increased (P< 0.05) in animals fed FOT1 diet compared to those of the FO group. The animals fed FOT2 diet had increases in sperm counts than those fed FO diet. However, these increases were not statistically significant. Sperm counts were similar in the FOC1 and FO groups. While, the animal fed FOC2 diet had significant increases (P< 0.05) in sperm counts compared to those fed FO diet.

3.4 Gene expression Patterns

The gene transcripts (mRNAs) of the three genes RBP, H-FABP and C-FABP were successfully detected in all liver tissues within all treated groups (Fig. 1, 3 and 5). The gene expression was normalized with the expression values of the β-Actin gene. The results revealed that RBP, H-FABP, and C-FABP mRNA expression in the liver tissues of the group fed the FO diet was significantly higher (P < 0.0001) than the control group (Figures 2, 4 and 6). On the other hand, tomato treatment was able to inhibit the up-regulation of the gene expression occurred by FO exposure. Where, the mRNA expression of RBP, H-FABP and C-FABP genes in the liver of the FO group was significantly higher (P < 0.001 or P < 0.0001) than those occurred in the FO plus tomato groups (FOT1 or FOT2), respectively (Figures 2, 4 and 6).

Regarding to carrots treatment, the results revealed that the expression of the RBP gene was significantly higher (P < 0.05) in the liver tissues of the FO group compared to those observed in the FO plus carrot groups (FO-C1 or FO-C2) (Figures 1 and 2). The expression levels of the H-FABP and the C-FABP genes were significantly higher (P < 0.05) in the liver of the FO group compared to those found in the FO-C2 group. However, the expression levels of the H-FABP and the C-FABP genes were somewhat higher (P = 0.061) in the liver of the FO group than those detected in the FO-C1 group.

4. Discussion

This study indicated that FO food has a mutagenic effect on the genomic materials of male mice. Mice fed FO diet had more frequent of DNA fragmentation and structural chromosome aberrations than those fed NO diet. This mutagenic effect may be due to the formation of oxidative compounds of lipidperoxid or heterocyclic aromatic amines (HAAs), that have been found to be mutagenic or carcinogenic (Hamilton et al., 1997; Starvic et al., 1997; Raloff, 1999; Coultate, 2002; Guardiola et al., 2008). Lipidperoxid compounds may be associated with generation of free radicals causing DNA fragmentation or DNA mutation (Lampe, 1999; Coultate, 2002; Nicolle et al., 2003; Guardiola, et al., 2008). Also the mutagenic and possibly carcinogenic products of HAAs are metabolized and activated by enzymes of cytochrome system–mediated N-hydroxylation to a number of hydroxylated metabolites which react with cellular DNA to induce fragmentations or mutations (Davis & Snydermine, 1995; Schut et al., 1997; Lampe, 1999; Nicolle et al., 2003; Jackson & Pereira-Smith, 2006; Devaraj et al., 2008). Hydroxyl free radicals are generated due to the cardiotoxic effect of HAAs and

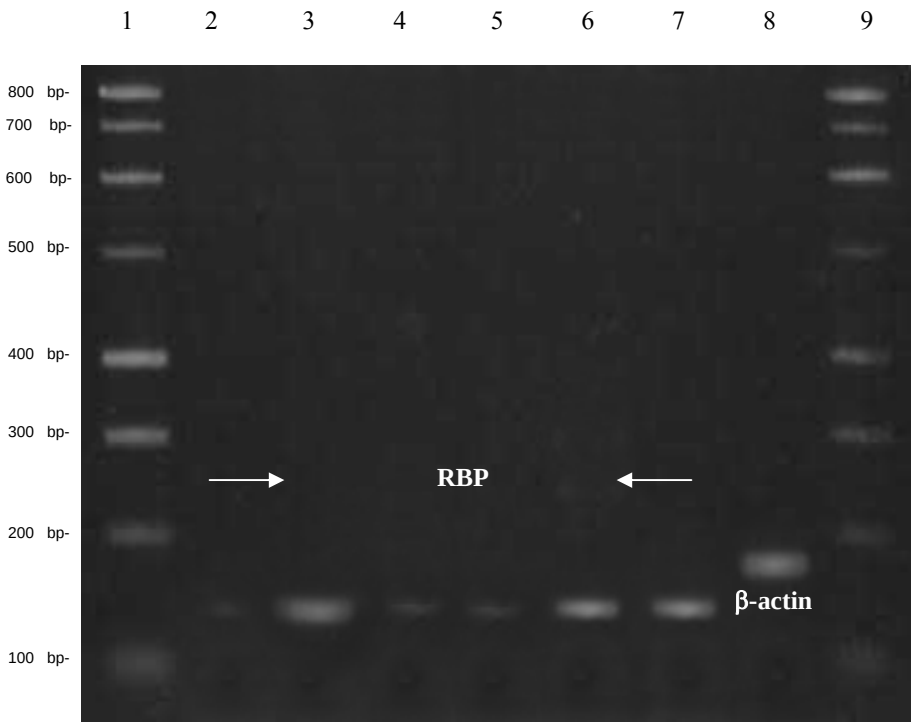

Fig. 1: Semi-quantitative RT-PCR analysis of RBP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10% of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity RBP gene and β-actin gene.

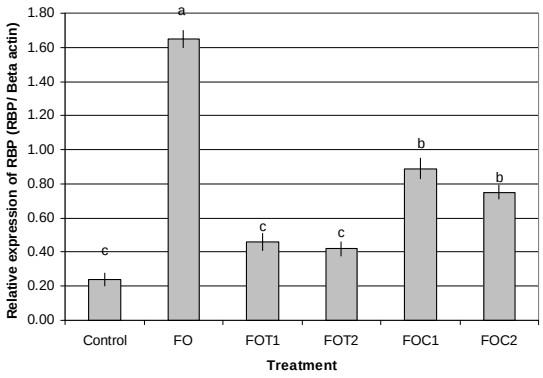

Fig. 2: RBP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

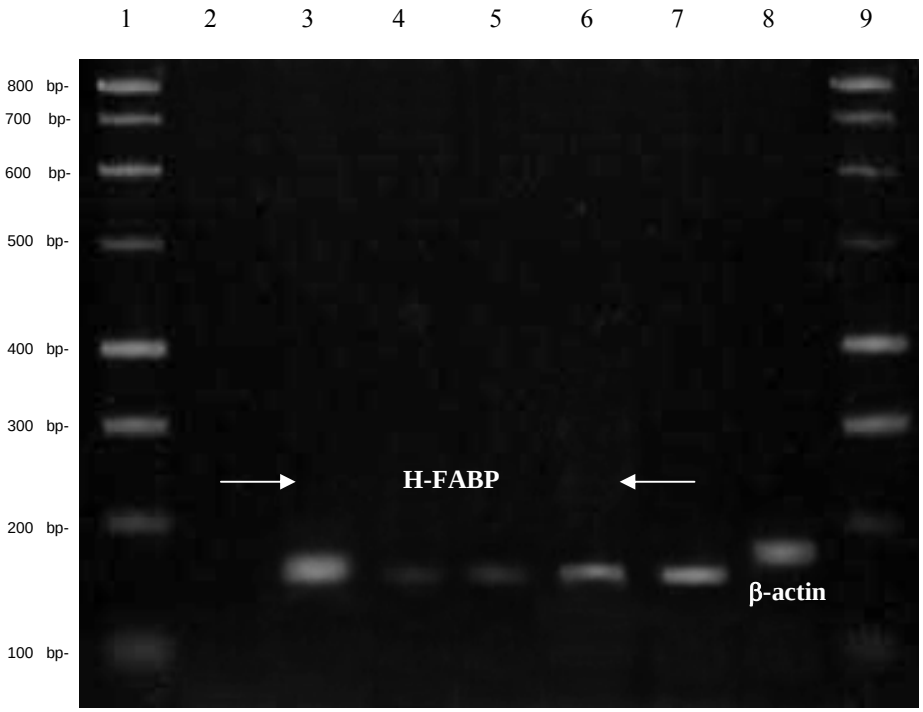

Fig. 3: Semi-quantitative RT-PCR analysis of H-FABP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10% of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity H-FABP gene and β-actin gene.

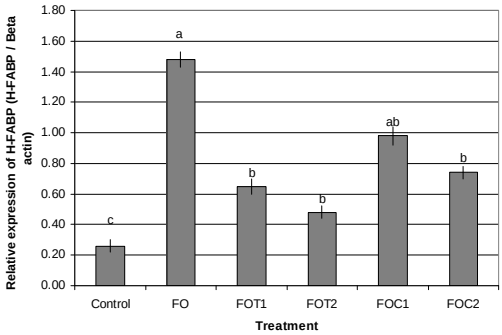

Fig. 4: H-FABP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

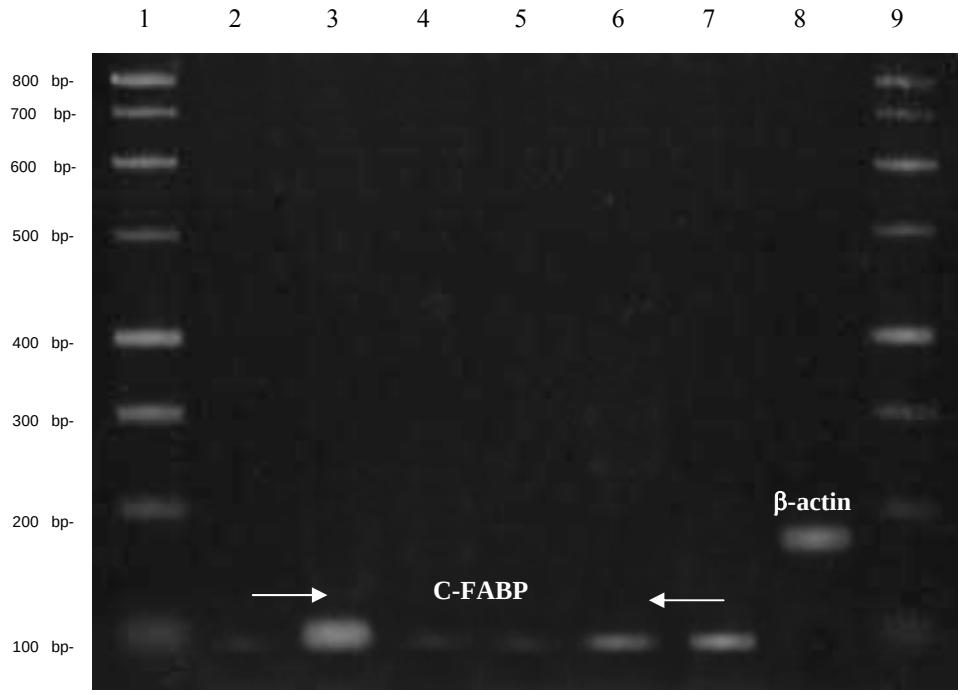

Fig. 5: Semi-quantitative RT-PCR analysis of C-FABP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10%of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity C-FABP gene and β-actin gene.

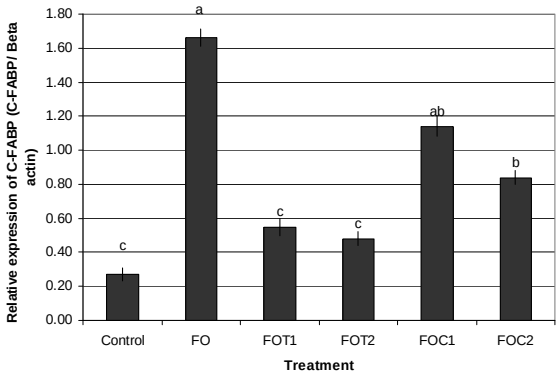

Fig. 6: C-FABP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

Table 4: Chromosome aberrations (Mean percentages± SEM) in male mice fed different diets.

Treatment	Chromosome abnormalities						Total structural chromosome aberrations
	Gaps	Deletions	Breaks	Centric Fusions	End to End association	Rings	
NO	1.7±0.3^{A}	2.0±0.0^{A}	0.3±0.3^{A}	0.7±0.3^{A}	0.1±0.0^{AB}	0.0±0.0^{A}	5.6±0.3^{A}
FO	2.7±0.5^{A}	5.5±0.3^{C}	1.0±0.0^{A}	1.5±0.3^{A}	1.8±0.3^{B}	0.1±0.4^{AB}	13.5±0.6^{C}
FOT1	2.7±0.4^{A}	2. 3±0.3^{A}	1.0±1.0^{A}	0.7±0.3^{A}	1.7±0.3^{B}	1.6±0.3^{B}	10.0±0.6 ^{B}
FOT2	2.0±0.0^{A}	3.0±0.6^{AB}	1.0±0.6^{A}	0.3±0.3^{A}	1.3±0.3^{AB}	0.0±0.0^{A}	7.6±1.3^{AB}
FOC1	3.0±1.0^{A}	3.5±0.5^{B}	0.5 ±0.5^{A}	0.5±0.5^{A}	0.5±0.5^{A}	1.0±0.0^{AB}	9.0±0.0^{B}
FOC2	2.0±0. 6^{A}	3. 0±0.0^{AB}	0.0±0.0^{A}	0.3±0.3^{A}	1.6±0.3^{B}	1.0±0.6^{AB}	8.0±1.0^{AB}

^{A,B,C}: Means followed by different superscripts, within columns, differ significantly (P< 0.05), NO= Normal oil (not used in frying processes), FO= Thermally oxidized oil (repeatedly used in frying processes), FOT_1 = FO + dried tomato at level 5%, FOT_2 = FO + dried tomato at level 10%, FOC_1 = FO + dried carrot at level 5%, FOC_2 = FO + dried carrot at level 10%.

Table 5: Sperm abnormalities in male mice fed different diets.

Groups	Types of sperm head abnormalities						Total tail	Total abnormal sperms (head and tail)	(Sperm count x 100)
	Amorphous	Without hook	Banana shape	Two head	Big shape	Total head			
NO	12.0±1.0^{A}	4.5±0.5^{A}	0.0±0.0^{A}	0.0±0.0^{A}	2.0±1.0^{A}	18.5±0.5^{A}	11.5±1.5^{A}	30.0±1.0^{A}	23.6±0.4^{A}
FO	18.8±0.9^{B}	7.5±0.9^{B}	3.5±0.3^{C}	0.3±0.3^{A}	8.5±0.6^{C}	38.5±1. 9^{C}	19.3±0.8^{C}	57.8±1.7^{C}	16.5±0.8^{C}
FOT1	17.5±1.0^{B}	6.3±0.5^{AB}	1.0±0.4^{A}	0.3±0.3^{A}	1.5±0.3^{A}	26.5±1.4^{B}	15.3±1.4^{B}	41.8±1.8^{B}	19.2±1.2A^{B}
FOT2	11.0±1.1^{A}	6.3±0.8^{AB}	0.5±0.3^{A}	0.3±0.3^{A}	6.0±0.8^{B}	24.0±1.1^{B}	14.8±0.6^{AB}	38.8±1.5^{B}	18.4±0.8^{BC}
FOC1	17.0±0.9^{B}	7.0±0.6^{B}	0.3±0.3^{A}	0.0±0.0^{A}	0.3±0.3^{A}	24. 5±1.4^{B}	14.8±0.3^{AB}	39.3±1.3^{B}	16.5±0.6^{C}
FOC2	13.5±1. 0^{A}	6.5±0.3^{AB}	2 .3±0.3^{B}	1.0±0.0^{B}	0.3± 0.3^{A}	23.5±1. 3^{B}	14.0±1.3^{AB}	37.5±0.5^{B}	21.0±0.6^{B}

$^{A,B,C:}$ Means followed by different superscripts, within columns, differ significantly (p< 0.05), number of sperm examined per each animal was 2500, NO= Normal oil (not used in frying processes), FO= Thermally oxidized oil (repeatedly used in frying processes), FOT1=FO+dried tomato at level 5%, FOT2= FO+dried tomato at level 10%, FOC1= FO+dried carrot at level 5%, FOC2=FO+dried carrot at level 10%.

subsequent single and double strand scissor of DNA are produced (Davis et al., 1994; Davis & Syndrmine, 1995). In addition, N-guanine adduct was found in various organs of mice (Tada et al., 1994) and rats (Turesky et al., 1996) fed HAAs containing diets.

The induction of DNA fragmentation or DNA mutation leads to anomalies in the chromosome as a result of disturbance of DNA replication (Sinha & Prasad, 1990; Director et al., 1996; Breneman et al., 1996). To our knowledge, there were no data available regarding the cytogenetic effects of feeding fried oil (FO) to animals. However, in similar studies, Farag et al. (2002) found that prolonged feeding intake of meat (fish or beef) previously exposed to high heat treatment greater than cooking temperature caused induction of chromosome aberrations in rats. On the other hand, Khalil et al. (2009) reported that feeding FO diet induced abnormalities in the reproductive materials and liver enzyme activities of female mice. In a previous study, Breneman et al. (1996) showed a significant response of sister chromatid exchange (SCE) and micronuclei in mice fed MeIQX (2-amino-3, 4-dimethylimidazo (4, 5- f) quinoline) a kind of HAAS diet and suggested that the increase of micronuclei and SCE confirm that MeIQx and/or its metabolites reached peripheral lymphocytes of quantities sufficient to induce chromosome aberrations. Also, Director et al. (1996) reported a significant increase in whole-animal toxicity and the induction of SCE in mice fed PhIP [a kind of HAAs (2- amino-1methyl-6-Phenylimidazo (4, 5-b) Pyridine). Another study by Ohgaki et al. (1987) suggested that the induction of tumor in liver, lung and intestine, in female mice, after receiving MeIQX, may be an indication for observable chromosome damages. In the present study, it was noted that the increase of deletions, in mice bone marrow cells, was more than any other type of structural aberrations. This means that FO induced the chromatid deletion in mice bone marrow cells. Similarly, in another study, high proportion of chromatid deletion, than other structural ones, was observed in mice (Klimova et al., 1990; Khlusova et al., 1992) and in rat (Balabel, 2006) treated with adriamycin.

In the present study a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count occurred in animals fed FO diet. The consistently high incidence of DNA fragmentation and chromosome aberrations, as a result of potential formation of oxidative compounds of lipidperoxid or HAAs (or its metabolites), may be indicative of a general susceptibility of these animals, in the present study, for inducing DNA fragmentation and consequently chromosome derangements of gonadal cells causing abnormalities in sperm shape and reduction in sperm count (Nordenson et al., 1984; Devi and Reddy, 1985; Sailer, et al., 1995; Sakkas et al., 1999; Aitken & Krausz, 2000). Evidence that sperm shape abnormalities, induced by selected mutagens and carcinogens, have been reported by Bruce & Heddle (1979); Wyrobek et al., (1983) and Sinha & Prasad (1990). DNA fragmentation of human genomic of gonadal cells was due to an excessive production of reactive oxygen species (ROS) by oxidative stress and lead to damage in sperm morphology (Twigg et al., 1998; De Lamirande & Gagnon, 1999; Aitken & Krausz, 2000; Muratori et al., 2003). Our results were similar to those reported by Farag et al. (2002) who found a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count in rats fed diets containing meat (fish or beef) previously exposed to high heat treatment, greater than cooking temperature.

Some vegetables such as tomatoes and carrots were tested, in the present study, for their protective action against the mutagenic effects of FO diet. The current results showed that the animals fed diet containing FO plus low or high levels of tomatoes or carrots (FOT1, FOC1 or FOT2, FOC2) had lower DNA fragmentation, chromosome aberrations and morphological sperm abnormalities with an increase in sperm count. These results indicated that tomatoes and carrots have a protective role, in body cells, against the observed mutagenic effect of FO diet. There is an evidence indicated that some components of tomatoes (such as vitamin C, α–tocopherol and β–carotene) have anti-mutagenic effects against smoke induced-lymphocyte DNA damage of smokers (Duthie et al., 1996). Also, many components of tomatoes and carrots (such, polyphenols, various carotenoids and lycopene) were found to improve the immune system, scavenging of free radicals and reduce the production of DNA mutations in different mammalian cells that were previously exposed to variety of oxidative conditions (Nicolle et al., 2003; Van Breda et al., 2005; Srinivasan et al., 2007; Devaraj et al., 2008). Moreover, tomatoes and carrots (or its micro-constituents) were considered to be anti-carcinogenesis. They were able to reduce the risk of ovarian cancer, lung cancer and prostate cancer in human and mice (Cramer, 2001; Van Breda et al., 2005; Gitenay et al., 2007). To our knowledge, there are no published data regarding the effects of tomatoes and carrots on the cytogenetic level in animal. However, the reduction of DNA mutations induced by anti-mutagenic materials such tomatoes and carrots (or its micro-constituents) lead to a reduction in chromosome abnormalities as a result of a decrease in disturbance of DNA replication (Sugimura & Wakabayashi, 1990; Sinha & Prasad,

1990; Breneman et al., 1996; Starvic et al., 1997). Consequently this lead to a reduction in sperm abnormalities (Sailer et al., 1995; Sakkas et al., 1999; Aitken & Krausz, 2000; Farag et al., 2002). The mode of action of these vegetables (tomatoes or carrots) or their constituents against the mutagenic foods (FO) may be due to binding with the mutagens or inhibition of activation of cytochrome systems-mediated N-hydroxylation enzymes with a consequent reduction of genetic material (DNA or chromosomes) and sperm abnormalities (Aitken & Krausz, 2000; Farag et al., 2002; Wang et al., 2004; Devaraj et al., 2008).

Regarding the gene expression alterations our results indicated that RBP, H-FABP, and C-FABP mRNA expression in liver tissues of the group fed the basal diet mixed with FO was significantly higher than the controls. The up-regulation of the RBP mRNA in the present study was compatible with a previous study by Kushiro et al. (2007). Also, Afolabi et al. (2007) reported that a low fat diet reduces plasma RBP in men. Another study showed that there were positive correlations between plasma RBP and total cholesterol in both men and women (Qi et al., 2007). So, our findings of up-regulation of RBP mRNA expression by the FO-diet in male mice is similar to those changes in RBP seen in the above clinical nutrition trials.

In the current study, we also found that mRNA expression of H- and C- FABP was increased in liver tissue of mice fed the FO-diet. These results are similar to those reported by Margareto et al. (2001). They found that FABP mRNA expression increased in the tissues of rats fed a high-fat diet for 30 days. Moreover, Kushiro et al. (2007) found that C-FABP mRNA expression in peri-renal white adipose tissue is high in obese rats.

On the other hand, we found that expression of all genes tested was down-regulated in the liver tissues collected from mice fed the basal diet mixed with frying oil with low or high concentration of tomatoes (FOT1 or FOT2). The mRNA expression of RBP, H-FABP and C-FABP genes in the liver of FO was significantly higher than the FO-T1 and FO-T2 groups. No study was performed yet to investigate the effect of tomatoes or carrots on the expression of the lipid metabolism related-genes, RBP, H-FABP, and C-FABP. However, Bub et al. (2005) reported that tomato juice intake reduced lipid peroxidation in healthy volunteers carrying the R-allele of the PON1-192 genotype.

Concerning carrot treatments, the results revealed that the expression of RBP, H-FABP and C-FABP genes were also significantly down-regulated in the liver tissues of carrot fed groups (FOC1 and FOC2) compared to the FO group. In agreement with our results, Parveen et al. (2000) reported that carrot residue fibers significantly decreased the serum cholesterol, triglycerides, LDL-cholesterol and carcass fat in albino rats. They concluded that carrot residue fibers may be used for the dietary management and control of hyperlipidaemia. We can also suggest that the action mechanism of carrots on lipid metabolism may be attributed to the alteration in the expression of the lipid metabolism related- genes tested in the present study.

The effect of tomatoes or carrots on the expression of the lipid metabolism related-genes, RBP, H-FABP, and C-FABP, require further studies to understand how tomatoes and carrots affect gene expression of the lipid metabolism related-genes in liver and other organs.

Conclusion: the present study adds evidence for a link between prolonged feeding intake of FO diet and induced mutagenic effects in the genomic material and increase the sperm abnormalities in male mice. However, tomatoes and carrots proved to be good protective agents against hazards of such mutagenic foods.

5. References

Afolabi, P. R., Jahoor, F., Jackson, A. A., Stubbs, J., Johnstone, A. M., Faber, P., Lobley, G., Gibney, E., Elia, M. 2007. The effect of total starvation and very low energy diet in lean men on kinetics of whole body protein and five hepatic secretory proteins. *American Journal of Physiolog. Endocrinology and Metabolism* 293: 1580-1589.

Aitken, R. J., Krausz, C. 2000. Oxidative stress, DNA damage and Y chromosome. *Reproduction*, 122: 497-506.

Allard, J. P., Royall, D., Kurian, R., Muggli, R., Jeejeebhoy, K. N. 1994. Effects of β – carotene supplementation on lipid peroxidation in humans. *American Journal of Clinical Nutrition* 59: 884-890.

Balabel, E. A. M. A. 2006. The protective role of royal jelly against chromosomal and DNA changes induced in rats after treatment with adriamycin and cobalt gamma radiation. *Master Thesis. Department of Zoology, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt.*

Breneman, J. W., Briner, J. F., Ramsey, M. J., Director, A., Tucker, J. D. 1996. Cytogenetic results from a chronic feeding study of Me IQx in mice. *Food and Chemical Toxicology* 34: 717-724.

Brown, K. M., Morrice, P. C., Duthie, G. G. 1994. Vitamine E supplementation suppresses indexes of lipid peroxidation and platelet counts in blood of smokers and nonsmokers but plasma lipoprotein concentrations remain unchanged. *American Journal of Clinical Nutrition* 60: 383-387.

Bruce, W. R., Heddle, J. A. 1979. The mutagenic activity of 61 agents as determined by the micronucleus, salmonella and sperm abnormality assay. *Can J Genet Cytol* 23: 319- 333.

Brun, M. E., Gasca, S., Girard, C., Bouton, K., De Massy, B., De Sario, A. 2006. Characterization and expression analysis during embryo development of the mouse ortholog of MLL3. *Gene* 371: 25–33.

Bub, A., Barth, S. W., Watzl, B., Briviba, K., Rechkemmer, G. 2005. Paraoxonase 1 Q192R (PON1-192) polymorphism is associated with reduced lipid peroxidation in healthy young man on a low- carotenoid diet supplemented with tomato juice. *Br J Nutr* 93: 291-297.

Coultate, T. P. 2002. Food: The chemistry of its components. The Royal Society of Chemistry. 4th edn., *Cambridge*, PP: 73-125.

Cramer, D. W., Kuper, H., Harlow, B. L., Titus-Ernstoff, L. 2001. Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenoposal women. *Int J Cancer* 94: 128-134.

Davis, C. D., Farb, A., Thorgeirsson, S. S., Virmani, R., Synderwine, E. G. 1994. Cardiotoxicity of heterocyclic amine food mutagens in cultured myocytes and in rats. *Toxicology and Applied Pharmacology* 124: 201- 211.

Davis, C. D., Snydermine, E. G. 1995. Protective effect of N-acetylcysteine against heterocyclic amine- induced cardiotoxicity in cultured myocytes and in rats. *Fd Chem Toxic* 33: 641-651.

De Lamirande, E., Gagnon, C. 1999. The dark and bright sides of reactive oxygen species on sperm function. In: Gagnon, C. (ed.). The male gamete: From Basic Science to Clinical Applications. Vienna, IL: *Cache River Press*; P.455-467.

Devaraj, S., Mathur, S., Basu, A., Aung, H. H., Vasu, V. T., Meyers, S., Jialal, I. 2008. A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. *Journal of the American College of Nutrition* 27: 267-273.

Devi, K. R., Reddy P. P. 1985. Bisazir induced cytogenetic damage and sperm head abnormalities in mice. *IRCS Med Sci* 13: 1245 -1246.

Director, A., Nath, J., Ramsey, M. J., Swiger, R. R., Tucker, J. D. 1996. Cytogenetic analysis of mice chronically fed the food mutagen 2- amino-1-methyl- 6- phenylimidazo (4, 5b) pyridine (PhIP). *Mutat Res* 359: 53-61.

Duthie, S. j., Ma, A., Collins, A. R. 1996. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res* 56: 1291-1295.

Farag, I. M., Abdou, H. S. A., Ayesh, A. M., Osfr, M. M. H. 2002. Chromosomal and sperm studies on the mutagenic effect of over heated meat and the protective role of green tea and ginseng on rats. *Al-Azhar Bull Sci* 13: 105-120.

Gibb, R. K., Taylor, D. D., Wan, T., Oconnor, D. M., Doering, D. L., Gercel-Taylor, C. 1997. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. *Gynecologic Oncology* 65: 13-22.

Gitenay, D., Lyan, B., Rambeau, M., Mazur, A., Rock, E. 2007. Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamine E deficient rats. *Eur J Nutr* 46: 468-475.

Gouveia De Souza, A., Oliveira Santos, J. C., Conceicao, M. M., Dantas Silva, M. C., Prasad, S. 2004. A thermo-analytic and kinetic study of sunflower oil. *Brazilian Journal of Chemical Engineering* 21(2): 265-273.

Guardiola, F., Ricard, B., Rafael, C., Alba, T., Tric, A. 2008. Determination of hydroperoxides in foods and biological samples by the ferrous oxidation- xylenol orange method: A review of the factors that influence the method's performance. *Analytical Biochemistry* 377: 1-15.

Hamilton, R. J., Kalu, C., Prisk, E., Padley, F. B., Pierce, H. 1997. Chemistry of free radicals in lipids. *Food Chemistry* 60: 193-199.

Hana, H. Y., Khalil, W. K. B., Elmakawy, A. I., Elmeeged, G. A., 2008. Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. *Journal of Steroid Biochemistry & Molecular Biology* 110: 284–294.

Jackson, J. G., Pereira- Smith, O. M. 2006. Primary compensatory role for RB family members at cell cycle gene promoters that are deacetylated and downregulated in doxorubicin- induced senescenceof breast cancer cells. *Molecular Cell Biology* 26: 2501- 2510.

Khalil, W. K. B., Abd El-Kader, H. A. M., Eshak, M. G., Farag, I. M., Ghanem, K. Z. 2009. Biological studies on the protective role of artichoke and green pepper against potential toxic effect of thermally oxidized oil in mice. *Arab J Biotech* 12: 27- 40.

Khlusova, M. I., Novitskii, V. V., Goldberg, E. D. 1992. The cytogenetic effects of antitumor preparations with different mechanisms of action. *Eksperimental'nain Klinicheskaia Farmakologiia* 55: 49-52.

Klimova, M. I., Bogdashin, I. V., Novitskii, V. V., Goldberg, E. D. 1990. Sensitivity of bone marrow cells damaged by antitumor drugs to cytotoxic action of effectors of a normal spleen. *Biulleten Eksperimental'noi Biologii I Meditsiny* 109: 60-62.

Kronmiller, J. E., Nguyen, T., Berndt, W., Wickson, A. 1995. Spatial and temporal distribution of Sonic

hedgehog mRNA in the embryonic mouse mandible by reverse transcription/polymerase chain reaction and in situ hybridization analysis. *Arch Oral Biol* 40: 831–838.

Kushiro, M., Takahashi, Y., Ide, T. 2007. Modulation of cutaneous fatty acid- binding protein mRNA expression in rat adipose tissues by hereditary obesity and dietary fats. *Journal of Oleo Science* 56: 533-541.

Lampe, J. W. 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *American Journal of Clinical Nutrition* 70: 475S-490S.

Margareto, J., Gomez- Ambrosi, J., Marti, A., Martinez, A. 2001. Time- dependent effects of a high- energy- yielding diet on the regulation of specific white adipose tissue genes. *Biochemical and Biophysical Research Communications* 283: 6-11.

Muratori, M., Maggi, M., Spinell, S., Filimberti, E., Forti, G., Baldi, E. 2003. Spontaneous DNA fragmentation in Swin- up selected human spermatozoa during long term incubation. *J Androl* 24: 253-262.

Nagao, M., Ushumia, T., Toyota, M., Inoue, R., Sugimura, T. 1997. Genetic changes induced by heterocyclic amines. *Mutat Res* 376: 161-167.

Nicolle, C., Cardinault, N., Aprikian, O., Busserolles, J., Grolier, P., Rock, E., Demigne, C., Mazur, A., Scalbert, A., Amouroux, P., Remesy, C. 2003. Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol - fed rat. *Eur J. Nutr* 24: 254-261.

Nordenson, L., Abramsson, I., Ducbek, M. 1984. Somatic chromosomal aberrations and male infertility. *Hum Hered*, 34: 240-245.

Ohgaki, H., Hasegawa, H., Suenaga, M., Takayama, S., Sugimura, T. 1987. Carcinogenecity in mice of a mutagenic compound (MeIQx) from cooked foods. *Carcinogenesis* 8:665-668.

Parveen, N., Akhtar, M. S., Abbas, N., Abid, A. R. 2000. Effects of carrot residue fibre on body weight gain and serum lipid fractions. *Int J Agri Biol* 2: 125-128.

Pavia, M. F., Gordon, M. H. 2005. Interaction of ferric ions with olive oil phenolic compounds. *Journal of Agricultural and Food Chemistry* 53: 2704-2709.

Phillip, G. R., Forrest, H., Nielsen, G. C., Fahey, J. R. 1993. AIN-93 purified diet for laboratory rodents: Final Report of American Institute of Nutrition Ad Hoc Writing Committee on The Reformation of The AIn- 76 A Rodent Diet. *J Nutr* 123: 1935-1951.

Preston, R. J., Dean, B. D., Galloway, S., Holden, H., Mcfee, A. F., Shelly, M. 1987. Mammalian in vivo cytogenic assay: Analysis of chromosome aberrations in bone marrow cells. *Mutat Res* 189: 157-165.

Qi, Q., Yu, Z.,Zhao, F., Huang, P., Hu, F. B., Franco, O. H., Wang, J., Li, H., Liu, Y., Lin. X. 2007. Elevated retinol- binding protein 4 levels are associated with metabolic syndrome in Chinese people. *The Journal of Clinical Endocrinology and Metabolism* 92: 4827-4834.

Raloff, J. 1999. New recipes for making serfously browned meats less of a cancer risk. *The Weekly Newsmagazine of Science* 155:17-24.

Sailer, B. L., Jost, L. K., Evenson, D. P. 1995. Mammalian sperm DNA susceptibility to in situ denaturation associated with the prescence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. *J Androl* 16: 80-87.

Sakkas, D.; Mariethoz, E.; Manicardi, G.; Bizzaro, D.; Bianchi, P. G.; and Bianchi, U. (1999). Origin of DNA damage in ejaculated human spermatozoa. Rev. *Reprod.*, 4: 31-37.

Schut, H. A. J., Wang, C. L., Twining, L. M., Earle, K. M. 1997. Formation and persistence of DNA adducts of 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) in CDFI mice fed a high w-3 fatty acid diet. *Mutat Res* 378: 23-30.

Sinha, S. P., Prasad, V. 1990. Effect of dietary concentration of crude aflatoxin on meiotic chromosomes, sperm morphology and sperm count in mice, Mus musculus. *Proc Indian Natn Sci Acad* 56: 269-276.

Srinivasan, M., Sudheer, A., Menon, V. P. 2007. Ferulic acid: Therapeutic potential through its antioxidant property. *J Clin Biochem Nutr* 40: 92-100.

Starvic, B., Lau, B. P. Y., Matula, T. I., Klassen, R., Lewis, D., Downie, R. H. 1997. Mutagenic heterocyclic aromatic amines (HAAs) in processed food flavour samples. *Food and Chemical Toxicology* 35: 185-197.

Suganuma, H., Inakuma, T. 1998. Protective effect of dietary tomato against endothelial dysfunction in hypercholesterolemic mice. *Bioscience, Biotechnology, and Biochemistry* 63: 78-82.

Sugimura, T., Wakabayashi, K. 1990. Mutagens and carcinogens in the diet. In: Mutagens and carcinogens in the diet. Pariza, M. W.; Felton, J. S.; Aeschbacher, H. U. and Sato, S. (eds). *Wiley-liss, New York,* pp.1-18.

Tada, A. M., Ochiai, K., Wakabayashi, H., Sugimura, T. N., Nagao, M. 1994. Identification of N–(deoxyguanosin-8-yl)-2-amino-3,4-dimethyl imidazo (4,5-f) quinoline (dG-C8-MeIQ) as amajor adduct formed by MeIQ with nucleotides

in vitro and with DNA in vivo. *Carcinogenesis* 15: 1275- 1278.

Turesky, R. J., Markovic, J., Aeschlimann, J. M. 1996. Formation and differential removal of C-8 and N2- guanine adducts of the food carcinogen 2-amino-3-methylimidazo (4,5-f) quinochem. *Res Toxicol* 9: 397- 402.

Twigg, J. P., Fulton, N., Gomez E. 1998. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: Lipid peroxidation, DNA fragmentation and effectiveness of antioxidant. *Hum Reprod* 13: 1429-1436.

Van Breda, S.G., Van Agen, E., Van Sanden, S., Burzykowski, T., Kleinjans, J. C., Van Delft, J. H., 2005. Vegetables affect the expression of genes involved in carcinogenic and anticarcinogenic processes in the lungs of female C57BI/6 mice. *J Nutr* 135: 2546-2552.

Wang, S., Konorev, E. A., kotamraju, S., Joseph, J., Kalivendi, S., Kalyanaraman, B. 2004. Doxorubicin induces apotosis in normal and tumor cells via distinctly different mechanisms. Intermediacy of H(2)O(2)- and p53- dependent pathways. *Journal of Biological Chemistry* 279: 25535- 25543.

Wyrobek, A. J., Gordon, L. A., Burhhart, J. G., Francis, M. W., Kapp, R. W.; Letz, G. Malling, H. V., Topham, J. C., Whorton, M. D. 1983. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. *Mutat Res* 115: 73-148.