Biological studies on the protective role of artichoke and green pepper against potential toxic effect of thermally oxidized oil in mice

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Wagdy K. B. Khalil;* Heba A. M. Abd El- Kader;* Mariam G. Eshak;* Ibrahim M. Farag* and Kadry Z. Ghanem**

Cell Biology Department, Food Science and Nutrition Department,** National Research Centre, Dokki, Giza, Egypt.

ABSTRACT

The present investigation was designed to evaluate oocyte competence including quality, meiotic progression and expression of ERK2 gene and activities of liver enzymes (ALT, AST and γ-GT) in female mice fed diets containing sunflower oil repeatedly used in frying processes (FO). The protective role of artichoke and green pepper against such potential dangers were also studied. Results revealed that mice fed FO diet had a significant decrease in number of good collected oocytes compared with those fed normal oil (NO) diet. Mice fed FO diet and artichoke extract at two levels, 0.5% (FO-A1) and 1% (FO-A2) as drinking water or FO diet plus dried ground green pepper at two levels, 5% (FO-G1) and 10% (FO-G2) had a significant increase in number of good collected oocytes compared with those fed FO diet. Animals fed FO-A1 or FO-G1 diets had the highest percentage of good oocytes. A statistically significant increase in the percentages of mature oocytes occurred in mice fed FO-A1, FO-A2, and FO-G1 diets as compared to those fed FO diet. The animals fed FO-G2 diet had a non-significant increase of percentages of mature oocytes than the animals fed FO diet. The expression of ERK2 gene was high in ovary tissues collected from female mice fed NO, FO, FO-A1, or FO-G1 diets. However, the ERK2 gene expression was low in mice fed FO-A2 or FO-G2 diets. Biochemical assay showed that mice fed FO diet had significant decrease in AST and ALT activities. A significant increase in enzyme activity of γ- GT compared with those fed NO diet was also observed. A statistically significant increase in activities of AST and ALT enzymes was observed in mice fed FO-A2, FO-G1 and FO-G2 diets. However, mice fed FO-A1, FO-A2, FO-G1 and FO-G2 diets had significant decrease in the activity of γ-GT enzyme compared with those fed FO diet. The present study demonstrated evidence that prolonged intake of diets containing sunflower oil repeatedly used in frying processes could be considered a cause of induced abnormalities in reproductive materials and liver enzymes activities of female mice. However, artichoke and green pepper proved to be suitable protectors against hazards of such toxic materials.

Key words: Sunflower oil, artichoke, green pepper, oocytes, RT-PCR, ERK2 gene, mice.

INTRODUCTION

Frying is one of the most commonly used methods of food preparation in the home and industry. However, the repeated use of oil in the frying processes may cause changes in its physical and/or chemical structure (Pavia and Gordon, 2005). Oxidation due to the frying process is one of the major causes of deterioration and decrease in the

nutritional quality of lipids (Frankel, 1996; Madhavi *et al.*, 1996 and Moure *et al.*, 2001). Lipid oxidation occurs through a chain reaction that involves three stages: initiation, propagation and termination (Gordon, 1990; Madhavi *et al.*, 1996; Hamilton *et al.*, 1997 and Coultate, 2002). During these stages, highly reactive free radicals are formed and consequently they react with oxygen or other fatty acids to form more free radicals and hydroperoxides. Through the termination stage, stable deterioration products, mainly carbonyl compounds are formed. These compounds may be responsible for the perception of rancidity in oxidized oil (Sikwese and Duodua, 2007). Also, a series of heterocyclic aromatic amines (HAAs) that have been found to be mutagens/carcinogens may arise in oil of fat during prolonged frying processes (Nagao *et al.*, 1997 and Raloff, 1999). The mutagenic and possibly carcinogenic products of HAAs are metabolized and activated by enzymes of cytochrome systems-mediated N-hydroxylation to a number of hydroxylated metabolites which react with the DNA to induce mutations (Davis and Snyderwine, 1995 and Schut *et al.*, 1997).

In animals or humans who ingest relatively a large amount of diet containing lipid peroxidation compounds (water soluble aldehydes), a significant higher risk of gene expression and cellular behavior is available (Viana *et al.*, 2005). Moreover, polyunsaturated fatty acids (PUFAs) which are found with abundance in vegetable oils have been observed to be more susceptible to peroxidation than other fatty acids. This is due to that PUFAs contain greater number of double bonds in their chemical structure (Guardiola *et al.*, 2008). The presence of oxidized PUFAs (due to frying processes) in the diets can alter cell membrane in mammalian cells and can affect growth and maturation of animal oocytes. This is because the main dietary n-6 PUFA is linoleic acid which was found to be implicated in oocyte growth and differentiation, in regulating meiotic arrest at the germinal vesicle stage and in preventing germinal vesicle breakdown (Moallem *et al.*, 1999 and Kim *et al.*, 2001).

Also, the oxidized PUFAs can act as competitive inhibitors for cyclooxigenation. Consequently, they could alter the function of transcription, and thus affect cellular concentrations of enzymes which regulate prostaglandin production and steroidogenic synthetic pathways (Abyasekara and Wathes, 1999 and Sikwese and Duodua, 2007).

Some medicinal plants such as artichoke and green pepper were found to have antioxidant effects (Pradhan *et al.*, 1999; Chatterjee *et al.*, 2007; Fratianni *et al.*, 2007 and Mehmetick, *et al.*, 2008). The artichoke is one of the world's oldest medicinal plants. The ancient Egyptians placed great value on the plant. It is clearly seen in drawings involving fertility and sacrifice. Moreover, this plant was used by the ancient Greek and Romans as a digestive aid. In 16^{th} century Europe, the artichoke was favored as a food by royalty (Brand, 1990). Artichoke leaves contain a wide number of active constituents, including cynarin, 1,3-dicaffeoylquinic acid, 3-caffeoylquinic acid, and scolymoside (Leung and Foster, 1996).Two phenolic compounds, namely 3,4-dihydroxyphenyl ethanol glucoside and 3,4 dihydroxy-6-(N- ethylamine) benzamide, are found in green pepper (Pradhan *et al.*, 1999). These natural phenolic antioxidants scavenge free radicals, and render them useful as radioprotective agents reducing DNA damage induced by UV and ionizing radiation (Kumar *et al.*, 2001). However, no reports exist on the role of pepper and artichoke in reducing DNA damage or alteration in the gene expression induced by thermally oxidized sunflower oil.

Therefore, the present investigation was designed to study the oocyte quality and maturation, *ERK2* gene expression and activities of liver enzymes (ALT, AST and γ-GT) in female mice fed diets containing thermally oxidized oil (through repeated use in frying processes). In addition, artichoke and green pepper were examined for their protecting ability against potential hazards caused by the oxidized oil rich diet.

MATERIALS AND METHODS

Experimental animals

Thirty adult female albino mice (8 wk old; 20-25 g) were used. These animals were obtained from the animal house laboratory, National Research Centre, Giza, Egypt. Apparently healthy acceptable animals were randomly assigned into six groups (5 mice each). The chosen mice were 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 hr and supplied with mice chew and fresh water *ad libitum*.

Preparation of frying oil

Sun flower oil was used for frying of potatoes. The frying processes were repeated for 5 times, 15 min for each time. The frying oil was then placed in a glass bottle and mixed with the basal diets, as shown in Table (1).

Preparation of artichoke

Artichoke flower petals were dried at 60°C for three days. Dried petals were ground using an electric mill.

Artichoke extraction

A weight of 0.5 gram or 1 gram of powdered dried artichoke petals were extracted with 100 ml tap water at 100°C for 5 min., and then filtered. The resulting crude extract was kept in the deep freezer until use.

Preparation of green pepper

The edible portion of the green pepper fruit were washed and dried at 60°C for three days. Dried green pepper was ground using an electric mill. Five or ten percent of this protector was added to the basal diet according to Phillip *et al.* (1993).

Preparation of basal diets

The composition of basal diets used are as follows; casein protein, sucrose, fat, salt mixtures, vitamin mixtures, fiber, corn starch (Phillip *et al.*, 1993) as shown in Table (1).

Experimental design

Mice were divided at random, into six groups (5 mice each). The first group, as a negative control, was fed the basal diet containing the natural sunflower oil NO, 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 the fourth groups were fed the basal diet containing FO plus artichoke leaves extract as drinking water at levels of 0.5g (W/V, FO-A1) and 1.0g (W/V, FO-A2). The fifth and the sixth groups were fed the basal diet containing FO plus dried ground green pepper at 5% (FO-G1) or 10% (FO-G2) of the diet. All mice groups were fed the corresponding previously mentioned diets for one month. Blood samples were collected from each mouse by orbital puncture and withdrawn on heparinized tubes for biochemical assays. The blood samples were centrifuged for ten minutes at 3000 rpm at 4°C. Plasma aliqurots were collected and stored at -20°C until use. Determinations of alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were performed according to the method of (Tiez, 1987). Also, γ-glutamyl- transferase (γ-GT) activity was determined by using the method of (Szasz,1976). Afterwards, the mice were sacrificed and tissue samples were collected

for further studies of oocyte quality, maturation and *ERK2* gene expression assays.

Ovarian collection

Ovaries were collected from each female mouse. The ovaries were dissected to release the oocytes. The collected oocytes were examined under microscope and classified according to their quality into: (a) good oocytes (surrounded with cumulus cells) and (b) denuded oocytes (without cumulus cells). Afterwards, the oocytes were fixed and stained with orcein stain to examine the meiotic progression (Polanski and Kubiak, 1999).

Semi-quantitative RT-PCR

RNA extraction

Immediately after animal scarification, ovary tissues were frozen in liquid nitrogen and stored at –80 °C prior to extraction. Total RNA was extracted from 50 to 100 µg of ovary tissues 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).

Reverse transcription

The complete Poly(A)+ RNA isolated from 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; Perkin-Elmer), 10 mM of each dNTP (Amersham, Brunswick, Germany), and 50 μM of oligo (dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 hr 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 DNA amplification through polymerase chain reaction (PCR).

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 family of mitogen-activated protein (MAP) kinases (42 and 44 kD), also termed extracellular signal-regulated kinases (*ERKs*), has been shown to have an important role during the M-phase of cell division (Kubelka *et al.*, 2002). Therefore, *ERK2* was the specific primer to detect the oocyte maturation, while, *globin* primer was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM $MgCl_2$, 1U/ μl taq polymerase,10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3; Gibco BRL, Eggenstein, Germany), and autoclaved water. The PCR cycling parameters were one cycle at 94 °C for 3 min, 33 cycles at 94 °C for 30 s, 59 °C to 60 °C for 30 s, 72 °C for 90 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 *globin* of the different mice samples.

Table (1): The composition of different diets.

Ingredient g/kg	Type of diets					
	1	2	3	4	5	6
	B+NO (Control)	B+FO	B+FO-A1*	B+FO-A2**	B+FO-G1	B+FO-G2
Casein	120	120	120	120	120	120
Sucrose	100	100	100	100	100	100
Salt mixture	50	50	50	50	50	50
Vitamin mix	10	10	10	10	10	10
Sunflower oil	60	0.0	0.0	0.0	0.0	0.0
Cellulose	40	40	40	40	40	40
Thermally oxidized sunflower oil	0.0	60	60	60	60	60
Dried green pepper (5%)	0.0	0.0	0.0	0.0	50	0.0
Dried green pepper (10%)	0.0	0.0	0.0	0.0	0.0	100
Starch	620	620	620	620	570	520

* Mice were given artichoke leaves extract (0.5g/ 100 ml) as drinking water
** Mice were given artichoke leaves extract (1g/ 100 ml) as drinking water
B = Basal diet
NO =Normal oil (not used in frying processes)
FO = Thermally oxidized oil (repeatedly used in frying processes)
FO-A1 = FO + artichoke extract at level 0.5%
FO-A2 = FO + artichoke extract at level 1%
FO-G1 = FO + dried ground green pepper at level 5%
FO-G2 = FO + dried ground green pepper at level 10 %

Table (2): Primer sequences used for PCR.

Genes	Primers sequences and positions	Annealing temp. (°C)	Cycles of PCR	Frag. size(bp)
ERK2	5′ primer (430-454) = upper primer 5′ -CACCGACCATCGAGCAGATGAAAGA- 3′ 3′ primer (873-897) = lower primer 3′ -TAAGACCCATAAGAACCTAGGGGCA- 5′	59	33	468
Globin	5′ primer (241-260) = upper primer 5′ -GCAGCCACGGTGGCGAGTAT- 3′ 3′ primer (555-657) = lower primer 3′ -GTGGGACAGGAGCTTGAAAT- 5′	60	27	257

RESULTS

Oocyte quality and maturation

Oocyte quality

The present results (Table 3) showed that the number of good oocytes was significantly lower in female mice fed FO diet than those of female mice fed NO diet. On the other hand, the number of good oocytes was significantly higher in female mice fed FO-A1 or FO-A2 diets than those fed FO diet. The highest number of good oocytes was observed in mice fed FO-A1 diet. Also, the number of good oocytes was significantly higher in female mice fed FO-G1 or FO-G2 diets than those fed FO diet. The highest number of good oocytes was observed in female mice fed FO-G1 diet. Moreover it was observed that the number of collected good oocytes was more than the

number of denuded oocytes in female mice fed FO -A1, FO-A2, FO-G1 or FO-G2 diets.

Oocyte maturation

Oocyte maturation results, summarized in Table (4), showed that the number of oocytes at GV stage was non-significantly increased in mice fed FO diet than those fed NO diet. However, the number of oocytes at GVBD stage was non - significantly decreased in FO group than those in NO group.

On the other hand, the number of oocytes at GV stage was higher in mice fed FO-A1, FO-A2, FO-G1 and FO-G2 diets than those fed FO diet (Table 4). These higher numbers were significant only in female group which were fed FO-A1, FO-G1, and FO-G2 diets. However, the number of oocytes at GVBD stage was significantly higher in female mice fed FO-A1, FO-A2 and FO-G1 diets than those fed FO diet (Table 4).

Semi-quantitative RT-PCR

A semi-quantitative RT-PCR assay was used to assess the relative abundance (RA) of mRNA of the *ERK2* gene, which is known to be involved in mammalian oocyte maturation. This was normalized with expression values of the *globin* gene.

Gene transcripts (mRNA) of the two genes were successfully detected in all ovary tissues within all treated groups (Fig. 1).

Figure (1) shows the relationship between the content of mRNA for the maturation gene and kind of diet. The results showed that the *ERK2* expression was highly up-regulated in the ovary tissues of the control group fed the basal diet mixed with NO. The same trend was observed in the ovary tissues collected from mice fed the basal diet mixed with frying oil FO alone, the FO-G1 group or the FO-A1 group. The results also showed that the expression level of the *ERK2* gene was low in the FO-G2 and FO-A2 groups. Whereas, the relative abundance of mRNA of the *ERK2* gene was lower in the ovary tissues collected from mice of the FO-G2 and FO-A2 groups.

Biochemical results

The present results showed that the AST and ALT activities were significantly lower in mice fed FO diet compared to that fed NO diet (Table 5). However, the γ-GT activity was significantly higher in the FO group than that of the NO group. On the other hand, the AST activity was higher in the group of mice fed FO-A1 diet than that of the FO group. However, this increase was not significant. Also, the ALT activity was significantly higher in the FO-A1 group than that of the FO group. However, γ-GT activity was significantly lower in FO-A1 or FO-A2 groups than those observed in the FO group.

The activities of AST and ALT were significantly higher ($P < 0.05$) in the FO-A2 group than that of the FO group. Moreover, the activities of AST and ALT were significantly higher in the FO-G1 and the FO-G2 groups compared to that of the FO group. The γ- GT activities were significantly lower in the FO-G1 and FO-G2 groups than that of the FO group.

Table (3): Oocyte quality of female mice fed different diets.

Types of diets	Oocyte quality	
	Good (M ± SEM)	Denuded (M ± SEM)
NO	15.25 ± 1.93 [c]	14.00 ± 1.35 [ab]
FO	13.75 ± 1.10 [d]	13.00 ± 3.67 [b]
FO-A1	23.75 ± 1.31 [a]	15.75 ± 1.65 [a]
FO-A2	20.00 ± 1.77 [a]	13.25 ± 1.43 [b]
FO-G1	22.75 ± 1.93 [a]	15.50 ± 0.64 [a]
FO-G2	18.50 ± 2.17 [ab]	9.75 ± 0.62 [c]

(a,b,c,d):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)
FO-A1 = FO + artichoke extract at level 0.5%
FO-A2 = FO + artichoke extract at level 1%
FO-G1 = FO + dried ground green pepper at level 5%
FO-G2 = FO + dried ground green pepper at level 10 %

Table (4): Nuclear stages in collected oocytes of female mice fed different diets.

Types of diets	Nuclear stages (M ± SEM)	
	GV	GVBD
NO	7.00 ± 1.66 [b]	6.50 ± 1.32 [b]
FO	7.75 ± 0.75 [b]	6.00 ± 1041 [b]
FO-A1	13.50 ± 0.28 [a]	10.25 ± 1.25 [a]
FO-A2	7.75 ± 1.31 [b]	12.25 ± 1.25 [a]
FO-G1	10.00 ± 1047 [a]	12.75 ± 0.75 [a]
FO-G2	11.75 ± 1.54 [a]	7.00 ± 0.91 [b]

(a,b):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)
FO-A1 = FO + artichoke extract at level 0.5%
FO-A2 = FO + artichoke extract at level 1%
FO-G1 = FO + dried ground green pepper at level 5%
FO-G2 = FO + dried ground green pepper at level 10 %

Table (5): Levels of ALT, AST and γ-GT (mean ± SEM) in female mice fed different diets.

Types of diets	AST	ALT	Y-GT
		µ/L	
NO	189 ± 7.20 [a]	109 ± 1.05 [a]	36 ± 5.11 [a]
FO	63 ± 5.21 [b]	65 ± 1.60 [b]	55 ± 5.22 [b]
FO-A1	64 ± 5.31 [ab]	92 ± 2.81 [a]	29 ± 6.12 [ac]
FO-A2	85 ± 3.31 [c]	98 ± 1.06 [a]	30 ± 4.35 [ac]
FO-G1	130 ± 1.50 [c]	92 ± 2.5 [ac]	29 ± 2.3 [ac]
FO-G2	110 ±5.20 [c]	89 ± 4.66 [ac]	39 ± 2.30 [ac]

(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)
FO-A1 = FO + artichoke extract at level 0.5%
FO-A2 = FO + artichoke extract at level 1%
FO-G1 = FO + dried green pepper at level 5%
FO-G2 = FO + dried green pepper at level 10 %

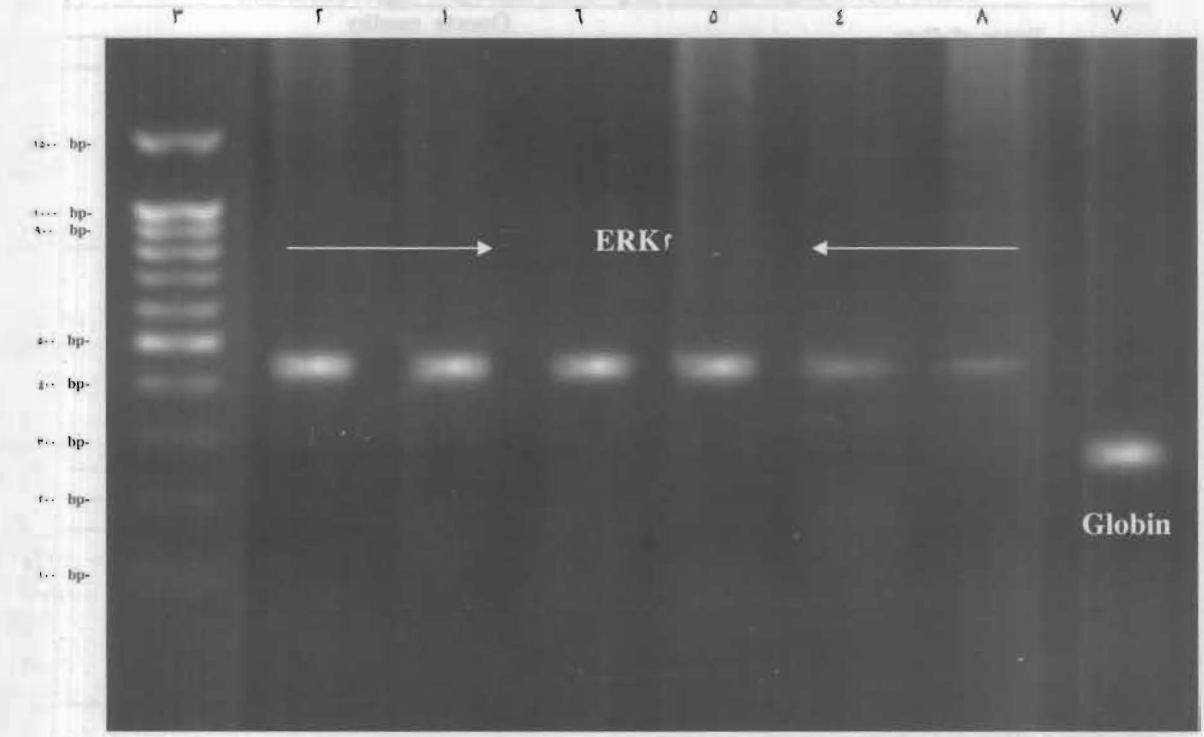

Fig. (١): Semi-quantitative RT-PCR analysis of ERK-٢ and Glob in-mRNAs in ovarian tissuescollected from female albino mice fed basal diet with frying oil (FO) and dried ground green pepper (GP) or artichoke extract (AE). Lane ١ represents DNA ladder. Lane ٢ represents ovarian samples from mice fed basal diet mixed with fresh oil (NO). Lane ٣ represents ovarian samples from mice fed basal diet mixed with FO. Lane ٤ represents ovarian samples from mice fed basal diet mixed with FO and ١٠٥ GP (FO-G١). Lane ٥ represents ovarian samples from mice fed basal diet mixed with FO and ١٠٥ AE as their drinking water (FO-A١). Lane ٦ represents ovarian samples from mic e fed basal diet mixed with FO and ١٠٥ GP (FO-G٢). Lane ٧ represents ovarian samples from mice fed basal diet mixed with FO and ١٠٥ AE as their drinking water (FO-A٢). Lane ٨ represents mRNA of the Globin gene. The RNA recovery rate was estimated as the ratio between the intensity of ERK٢ gene and the Globin gene.

DISCUSSION

In the present study, it was observed that the mean number of collected good oocytes and also of matured oocytes were lower in mice fed FO diet compared with those collected from mice fed NO diet. However, mice fed FO diet and had artichoke extract in their drinking water or dried ground green pepper had higher mean number of good collected oocytes. Those animals also had higher mean number of matured oocytes compared with those fed FO diet alone.

These results indicate that the use of FO, in the diets, has negative effects on the reproductive performance. This negative effect may be due to the formation of free radicals and hydroperoxides. Consequently, these forms can affect oocyte growth and differentiation (Moallem *et al.*, ١٩٩٩ and Kim *et al.*, ٢٠٠١).

To our knowledge, there were no data regarding reproductive effects of FO diets or

diets of FO plus natural products such as artichoke and green pepper. Furthermore, the overall contribution of environmental exposures to infertility is unknown. However, a growing scientific database suggests that exposure to various environmental factors could dramatically affect fertility. Studies of various contaminant-exposed wildlife populations suggest that multiple mechanisms contribute to changes in maturation of oocyte and fertilization (Guillette and Moore, 2006). In addition, Alm *et al.* (2006) reported that oocyte quality is significantly reduced by feeding of mycotoxin to gilts. Where, oocytes with compact cumuli collected from gilt supplemented with mycotoxin contaminated feed showed a reduced proportion having immature chromatin in comparison to gilts fed on normal feed. We have found that the oocytes collected from ovaries exposed to FO were not progressed in meiosis in comparison with those received the FO diet supplemented with artichoke or green pepper.

The family of mitogen-activated protein (MAP) kinases (42 and 44 kD), also termed extracellular signal-regulated kinases (*ERK*s), has been shown to have an important role during the M-phase (Kubelka *et al.*, 2002). The activation of MAP kinase is important for the transition of oocytes from GV to GVBD, and M I to M II (Verlhac *et al.*, 1994). In mouse oocytes, it is MAP kinase, not maturation promoting factor (MPF), that is responsible for the transition from GV to GVBD and M I to M II, because MPF activity declines between Anaphase I (A I) and Telophase I (T I) while MAP activity continues to be high during maturation.

In the present study, we have found that *ERK2* expression was highly up-regulated in the ovary tissues of control and FO alone, FO-G1 or FO-A1 which most of the oocytes of these groups were arrested in GV stage (not matured). However, the relative abundance of mRNA of the *ERK2* gene was lower in FO-G2 and in FO-A2 since their oocytes reached GVBD stage. In agreement with our results, Robert *et al.*(2000) showed that Cyclin B-mRNA in bovine oocytes decreased with the increase of meiotic progression. Similar results were proposed by Zaitsevskaya and Cooper, (1992) where, the MAP (*ERK*) kinase transcripts were found to be more abundant in Xenopus oocytes arrested in VG stage than in those of GVBD stage. These results suggest that large amounts of Cyclin B- and ERK-mRNA are synthesized and stored in immature follicles to allow synthesis of MPF and MAP kinase protein during maturation (Robert *et al.*, 2000). Also, Fair *et al.* (1997) reported that mRNA molecules were stored during the early stages of follicular development. This stored mRNA was later used for coding the proteins required for chromatin condensation and at germinal vesicle break down stage (GVBD), while the new transcription was silenced (Krischek and Meinecke, 2002).

As known the oils repeatedly used in frying without immersion undergo degradation by oxidative reactions (Gouveia De Souza *et al.*, 2004). Dietary PUFA supplementation can influence biosynthetic pathways involved in both prostaglandin synthesis and steroidogenesis that have multiple roles in the regulation of reproductive function (Sikwese and Duodua, 2007). Furthermore, lipid peroxidation compounds resulted from oil frying (water soluble aldehydes) can modulate gene expression and alter cellular behavior (Viana *et al.*, 2005). Therefore, we can suggest that oxidative reactions in the frying oil may affect the activation of *ERK2* expression during the oocyte maturation as a result of the influence of the steroidogenesis (Sekwese and Duodua, 2007). In addition, 0.5% artichoke extract or 5% dried ground green pepper (low doses), could not induce the oocyte to be progressed in meiosis. This means that these

products did not protect the oocytes against the oxidation process. However, using 5% artichoke extract or 10% dried ground green pepper (high doses), could induce the oocyte to be progressed in meiosis. This means that these higher levels protected the oocytes against the oxidative compounds.

The action mechanism of how the frying oil modulates the gene expression of *ERK2* and therefore the oocyte maturation are not investigated yet. It is known that induction of the oocyte maturation is done through several compounds: luteinizing hormone (LH) induces the elevation time of the intracellular calcium required for resumption of meiosis (Petr *et al.*, 2001 and Tanghe *et al.*, 2002). Calcium ions (Ca^{2+}) acts as a promoter to induce mRNA expression and the essential protein synthesis for the maturation processes. Therefore, oxidative compounds may modulate the gene expression through affecting the mechanism of action of LH and/or Ca^{2+}. However, the use of artichoke extract or dried ground green pepper at the higher dose level can prevent the role of oxidative compounds in this mechanism.

The present study showed that, the enzymatic activities of AST and ALT were decreased in mice fed FO diet than those observed in mice fed NO diet. However, the activity of γ-GT enzyme increased in FO group than NO group. These results indicate that FO has toxic effect on the liver function. This hazard effect may be due to the formation of free radicals and hydroperoxides (Coultate, 2002; Guardiola *et al.*, 2008; Viana *et al.*, 2005 and Sikwese and Duodua, 2007). Consequently, these dangerous compounds can affect the enzymatic activities in liver (Abyasekara and Wathes, 1999 and Guardiola *et al.*, 2008).

Also the biochemical assay in the present studies showed that the activities of AST and ALT were higher in mice supplemented with artichoke extract at the levels of 0.5% or 1% compared to those of FO group. However, the activities of γ- GT were lower in groups supplemented with artichoke extract at the levels of 0.5% or 1% than those of FO group. The increase in activities of AST and ALT enzymes and the decrease in activity of γ -GT enzyme may be due to the protective effect of some components of chemical structure of artichoke on the toxicity of thermally oxidized oil. Phenols and cyarine are major components of artichoke. These components were found to be significantly helpful in detoxification and as a support of liver functions. Also, these compounds stimulate the clearance of bile and prevent congestion in the liver (Mehmetick *et al.*, 2008). Consequently, such compounds have hepatoprotective effects (Fratianni *et al.*, 2007 and Mehmetick, *et al.*, 2008).

Moreover, it was also observed that the activities of AST and ALT were higher in mice fed diet containing dried ground green pepper at both levels of 5% and 10%. However, γ- GT activity was lower in these animals compared to those of FO group. These results may be due to the protective effect of phenolic compounds of the green pepper on the toxicity of thermally oxidized oil. The major phenolic compounds of green pepper namely,3,4-dihydroxyphenyl ethanol glucoside (A) and 3,4-dihydroxy-6-(N-ethyl-amino) benzamide (B) and phenolic acid glycosides were found to have the ability to scaverge1,1-diphenyl-2-picrylhydrazl (DPPH) radical and inhibit lipid peroxidation (Pradhan *et al.*, 1999 and Chatterjee *et al.*, 2007).

In conclusion, the present study demonstrates evidence that prolonged intake of diets containing sunflower oil repeatedly used in frying processes is considered a cause of inducing abnormalities in each of reproductive materials and enzyme activities of the liver in female mice. However, artichoke and green pepper proved to be

suitable protectors against the hazards of such toxic oil.

REFERENCES

Abayasekara, D. R. E. and Wathes, D. C.(1999). Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. Prostaglandins Leukot Essent Fatty Acids, 61: 275-287.

Alm, H.; Brussow, K. P.; Torner, H.; Vanselow, J.; Tomek, W.; Danicke, S. and Tiemann, U.(2006). Influence of fusarium–toxin contaminated feed on initial quality and meiotic competence of gilt oocytes. Reprod. Toxicol., 22(1): 44-50.

Brand, N. (1990). *Cynara scolymus L.*-The artichoke. Zeitschrift Phytother, 11: 169-175.

Chatterjee, S.; Niaz, Z.; Gautam, S.; Adhikari, S.; Variyar, P. S. and Sharma, A. (2007). Antioxidant activity of some phenolic constituents from green pepper (*Pipper nigrum* L.) and fresh mutmeg mace (*Myristica fragrans*). Food Chemistry, 101(2): 515-523.

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

Davis, C. D. and Snyderwine, E. G.(1995). Protective effect of N-acetylcysteine against heterocyclic amine- induced cardiotoxicity in cultured myocytes and in rats. Fd. Chem. Toxic., 33(8): 641-651.

Fair, T.; Hulshof, S. C.; Hyttel, P.; Greve, T. and Boland, M. (1997). Nucleus ultrastructure and transcriptional activity of bovine oocytes in preantral and early antral follicles. Mol. Reprod. Dev., 46: 208-215.

Frankel, E. N. (1996). Antioxidants in food and their impact on food quality. Food Chemistry, 57: 51-55.

Fratianni, F.; Tucci, M.; Palma, M.; Pepe, R. and Nazzaro, F. (2007). Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus*). Food Chemistry, 104 (3): 1282-1286.

Gordon, M. H. (1990). The mechanism of antioxidant action *in vitro*. In: Food Antioxidants (Hudson, B.J., ed): 42-48. Elsevier Science Publishers, London.

Gouveia De Souza, A.; Oliveira Santos, J. C.; Conceicao, M. M.; Dantas Silva, M. C. and 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. and 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.

Guillette L, J. and Moore, B. C. (2006). Environmental contaminants, fertility and multiooocytic follicles: a lesson from wildlife. Reprod. Med., 24(3):134-141.

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

Kim, J. Y.; Kinoshita, M.; Oshnishi, M. and Fukui, Y. (2001). Lipid and fatty acid analysis of fresh and frozen-thawed immature and *in vitro* matured bovine oocytes. Reproduction, 122: 131-138.

Krischek, C. and Meinecke, B. (2002). *In vitro* maturation of bovine oocytes requires polyadenylation of mRNAs coding proteins for chromatin condensation, spindle assembly, MPF and MAP kinase activation. Anim. Reprod. Sci., 73: 129-140.

Kubelka, M.; Anger, M.; Kalous, J.; Schultz, R. M. and Motlik, J. (2002). Chromosome condensation in pig oocytes: Lack of a requirement for either cdc2 kinase

or MAP kinase activity. Mol Reprod Dev; 63: 110-118.

Kumar, l. D.; Kathervil, M.; Rao, G. V. and Nagaraju, J. (2001). DNA profiling of disrributed Chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. Forensic Sci. Int.,116(1): 63-68.

Leung, A. Y. and Foster, S (1996). Encyclopedia of common natural ingredients used in food, drugs, and cosmetics. 2nd edn., John Wiley & Sons, pp: 42-43.

Madhavi, D. L.; Deshpande, S. S. and Salunkhe, D. K. (1996). Food antioxidants: Technological, toxicological and health perspectives: 2-7. Marcel Dekke, New York.

Mehmetcik, G.; Ozdemirler, G.; toker, N. K.; Cevikbas, U. and Usal, M. (2008). Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress. Experimental and toxicological Pathology, 60(6): 475-480.

Moallem U.; Folman, Y.; Bor, A.; Arva, A. and Sklan, D. (1999). Effect of calcium soaps of fatty acids and administration of somatotropin on milk production, preovulatory follicular development, and plasma and follicular fluid lipid composition in high yielding dairy cows. J. Dairy Sci., 82: 2358-2368.

Moure, A.; Cruz, J. M.; Franco, D.; Dominguez, J. M. and Dominguez, H. (2001). Natural antioxidants from residual sources. Food Chemistry, 72: 145-171.

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

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

Petr, J.; Rozinek, J.; Hruban, V.; Jilek, F.; Sedmikova, M.; Vanourkova, Z. and Nemecek, Z. (2001). Ultrastructural localization of calcium deposits during *in vitro* culture of pig oocytes. Mol Reprod Dev., 58: 196-204.

Phillip, G. R.; Forrest, H.; Nielsen, G. C. and 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.

Polanski, Z. and Kubiak, J. Z. (1999). Meiosis. In: Encyclopedia of Reproduction. Knobil, E.(ed.); 111:160-167. Academic Press, San Diego, California.

Pradhan, K. J.; Variyar, P. S. and Bandekar, J. R. (1999). Antimicrobial activity of Novel phenolic compounds from green pepper (*Piper nigrum L.*). Lebensmittel- Wissenschaft und-Technologie, 32 (2): 121-123.

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

Robert, C.; Barnes, F. l.; Hue, I. and Sirard, M. A. (2000). Subtractive hybridization used to identify mRNA associated with the maturation of bovine oocytes. Mol. Reprod. Dev., 57: 167- 175.

Schut, H. A. J.; Wang, C. L.; Twining, L. M. and 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.

Sikwese, F. E. and Duodua, K. G. (2007). Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. Department of Food Science, University of Pretoria, Pretoria 0002, South Africa.

Szasz, G. (1976). Reaction- rate method for γ-glutamyltransferase activity in serum. Clin. Chem., 22(12): 2051-2055.

Tanghe, S.; Van Soom, A.; Nauwynck, H.; Coryn, M. and de Kruif, A. (2002). Minireview: Functions of the cumulus oophorus during oocyte maturation, ovulation, and fertilization. Mol Reprod Dev; 61: 414-424.

Ties, N. W. (1987). Fundamentals of clinical chemistry, 3rd edn.W.B. Saunders, Philadelphia. pp. 372.

Verlhac, M. H.; Kubiak, J. Z.; Clarke, H. J. and Maro, B. (1994). Microtubule and chromatin behavior follow MAP kinase activity but not MPF activity during meiosis in mouse oocytes. Development, 120: 1017-1025.

Viana, M.; Villacorta, L.; Bonet, B.; Indart, A.; Munteanu, A.; Sanchez, I.; Azzi, A. and Zingg, J. M. (2005). Effects of aldehydes on CD36 expression. Free. Radic. Res., 39(9): 973-979.

Zaitsevskaya, T. and Cooper, J. A. (1992). Developmentally regulated expression of a mitogen-activated protein kinase in *Xenopus laevis*. Cell Growth Differ., 3: 773-782.

الملخص العربي

دراسات بيولوجية على الدور الواقى للخرشوف والفلفل الأخضر ضد التأثير السام المحتمل للزيت المؤكسد حرارياً فى الفئران

وجدى خليل بسالى خليل*، هبة أحمد محمد عبد القادر*، مريم جرجس إسحق*، إبراهيم محمد فرج* و قدرى ذكى غانم**
قسم بيولوجيا الخلية، ** قسم الصناعات الغذائية والتغذية، المركز القومى للبحوث، الدقى، الجيزة، مصر.

صُممت هذه الدراسة لتقييم جودة ونضج البويضات، وقياس التعبير الجينى لجين *ERK2* وكذلك قياس نشاط بعض إنزيمات الكبد مثل ALT ، AST و γ-GT فى إناث الفئران المعملية الصغيرة التى تم تغذيتها على زيت عباد الشمس الذى تكرر إستخدامه فى عمليه التحمير (FO). وكذلك تم دراسة التأثير الواقى للخرشوف والفلفل الأخضر على التأثير السام المحتمل حدوثه من هذا الزيت. أوضحت نتائج هذه الدراسة أن الفئران التى تم تغذيتها على الغذاء المحتوى على FO إحتوت على عدد قليل معنوياً من البويضات الجيدة مقارنة بالإناث التى تم تغذيتها على الزيت العادى (NO). كما أظهرت النتائج إرتفاعا معنويا فى متوسط عدد البويضات الجيدة فى الفئران التى تم تغذيتها على عليقة تحتوى على زيت FO وتم إضافة مستخلص الخرشوف بالتركيزين 0.05% (FO-A1) و1% (FO-A2) إلى مياه الشرب أو التى تغذت على عليقة تحتوى على زيت FO مضافاً إليه مستخلص الفلفل الأخضر بالتركيزين 5% (FO-G1) و10% (FO-G2) مقارنة بتلك التى تم تجميعها من مجموعة الـ FO. وأوضحت النتائج أن حيوانات مجموعتي، FO-A1 أو FO-G1 تميزت بأعلى نسبة تجميع من البويضات الجيدة. كما بينت النتائج ازدياد نسبة البويضات الناضجة بدرجة معنوية فى الفئران التى تغذت على زيت FO-A1، FO-A2 و FO-G1 بالمقارنة بتلك للفئران التى تغذت على زيت FO. وأظهرت أيضا النتائج أن نسبة البويضات الناضجة فى الفئران التى تغذت على زيت FO-G2 قد ازدادت بدرجة غير معنوية عن فئران مجموعة FO. وأوضحت نتائج تحليل التعبير الجينى للجين المسئول عن نضج البويضات وهو *ERK2* انه كان عاليا فى أنسجة المبيض التى تم تجميعها من إناث فئران مجموعات NO ، FO ، FO-A1 أو FO – G1. بينما قل التعبير الجينى لجين *ERK2* فى فئران مجموعتى FO- A2 أو FO- G2. وأوضحت نتائج التحاليل الكيميائية الحيوية إنخفاضاً معنوياً فى مستوى نشاط إنزيمات AST و الـ ALT وارتفاع نشاط أنزيم γ-GT بدرجة معنوية فى فئران مجموعة FO مقارنة بالمجموعة الضابطة. وقد لوحظ زيادة معنوية فى النشاط الإنزيمي لإنزيمات AST ، ALT فى فئران مجموعات FO-A2، FO-G1 و FO-G2 بينما إنخفض النشاط الأنزيمى لإنزيم γ-GT بدرجة معنوية فى فئران مجموعات FO-A1 ، FO-A2 ، FO-G1 أو FO-G2 مقارنة بنشاط الإنزيم فى مجموعة الـ FO.أوضحت الدراسة الحالية أن كثرة تناول الأغذية المحتوية على زيت عباد الشمس الذى تكرر إستخدامه فى عملية التحمير عدة مرات يمكن أن تؤدى إلى إحداث أضرار على الصفات التناسلية بالإضافة إلى تغير فى أنشطة إنزيمات الكبد فى إناث الفئران. ويمكن تقليل أو تجنب هذه الأضرار بإضافة الخرشوف وكذلك الفلفل الأخضر إلى مثل هذه الأغذية.