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Fennel (*Foeniculum vulgare)* essential oil ameliorates DNA and histopathological damage induced by cyclophosphamide in mice

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The possible protective role of fennel fruits essential oil (FEO) against cyclophosphamide (CP)-induced DNA damage and histopathological injury in liver and testis was investigated.FEO extraction was subjected to gas chromatography-mass spectrometry (GC-MS) to determine its active constituents. For chromosomal aberration and histopathological studies, FEO was administrated orally at concentrations of 0.2, 0.4 and 0.6mL/kg daily for 5 days and at the 5th day mice were i.p. injected with CP at 20 mg/kg. For sperm abnormalities mice were administrated FEO and CP for 5 consecutive days and samples were taken at 35th day of the first treatment. The results revealed non-significant effects in chromosomal aberrations in bone marrow and sperm shape abnormalities after repeated oral treatments with FEO alone at the highest tested dose (0.6mL/kg). The same dose showed normal hepatic architecture, as well as, it showed normal seminiferous tubules with different stages of spermatogenic cells and normal interstitial tubules in the testis. Also, FEO recorded minimum immunoreactions of caspase-3 expression in the cells of liver and testis. In addition, statistically-significant percentage of inhibition in chromosomal aberrations was recorded in bone marrow cells and morphological sperm abnormalities in CP groups pre-treated with different concentrations of FEO compared with CP groups. The histological alterations induced by CP in liver and testis tissues were restored to a good degree in those animals pre-treated with the two higher doses of FEO (0.4 and 0.6mL/kg).The hepatic and testis tissues also showed marked improvement in caspase-3 expression in the CP-treated groups with FEO. The results demonstrated the biosafety of the essential oil of fennel. It also has the ability to improve chromosome, sperm and histopathological alterations induced by CP.

Keywords: Fennel (*Foeniculum vulgare*), Cyclophosphamide, Chromosome aberrations, Sperm abnormalities, Bone marrow, Histopathological changes.

INTRODUCTION

Antioxidants play an important role in adsorbing and neutralizing free radicals such as lipid peroxyl, peroxide, or hydroperoxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Fahmy et al., 2015). Recently, there has been a growing interest for substitution of synthetic antioxidants with natural ones mainly from medicinal plants (Salami et al., 2016). Selection of plants with high antioxidant activity is very important for improving human health protection. In the food industries, the use of natural alternatives and resources has greatly increased and researchers found that antibacterial and antioxidant activities of natural alternatives are of special importance for improving the quality

of final product. Herbs and spices are the most important targets in which to find strong natural antioxidants and antibacterial (Prathapan et al., 2011).

Foeniculum vulgare (family: *Abiaceae*) is commonly known as fennel. It is a perennial herb native to the Mediterranean region. It is also cultivated in other parts of the world specifically in Asia, North America, and Europe. Fennel has a great economic importance in food and medicinal industries. Also seeds/fruits are largely used as flavouring for condiments, perfumes and liqueurs and the essential oils are used for anethole production and the formation of anise-flavoured compounded oils (Rapisarda 2004; Badgujar et al., 2014).

Fennel finds extensive use as spice in culinary purposes thus forming a part of dietary regime due to its valuable nutritional composition with respect to the presence of essential fatty acids (Barros et al. 2010) and also to its biologically active secondary metabolites (Badgujar et al., 2014). This herb is known worldwide and traditionally used as curative herbal therapy for treatment of a wide range of aliments related to digestive, respiratory, endocrine, renal and reproductive systems. Additionally, it is also used as a galactagogue agent for lactating mothers (Badgujar et al., 2014).

Fennel is a medicinal and aromatic plant with a diverse pharmacological spectrum (Sheweita et al., 2016). Several biological studies of fennel in many forms such as extracts, seeds and essential oil using a variety of methods concluding that fennel possesses a plethora of pharmacological activities from which: hepatoprotective (Sheweita et al., 2016), antioxidant (Mohamad et al., 2011), anti-inflammatory/analgesic (Aprotosoaie et al., 2016), chemopreventive, anti tumour/anti mutagenic (Mohamad et al. 2011; Sheweita et al., 2016; Elkady 2018) , antidiabetic (El-Soud et al., 2011) as well as antimicrobial and antiviral activities (Diao et al., 2014; Kwiatkowski et al., 2017). Fennel extract possesses memory-enhancing property (Koppula and Kumar 2013) and may be employed in treatment of cognitive disorders such as dementia and Alzheimer's disease (Joshi and Parle 2006).

Fennel is one of the common Egyptian foods. So, in a continuation of our efforts to evaluate the pharmaceutical properties of Egyptian flora, this work is designed to evaluate the safety use of fennel fruits essential oil (FEO) and its protective role. The mutagenic/antimutagenic and hisopathological protective activities of FEO were evaluated against toxicity induced by the antineoplastic drug CP.

MATERIALS AND METHODS

Plant Material*:*

Fennel fruits were collected from the Experimental Agricultural Station, Faculty of Agriculture, Cairo University, Giza Governorate during the season of 2016-2017.

Samples of this plant were subjected to botanical identification by Prof. Dr. Ahmed Shalaby, Prof. of Medicinal and Aromatic Plants in the National Research Centre, Dokki, Giza and the % essential oil was calculated.

Extraction Methods*:*

Plant samples were used for the determination of volatile oil content. The volatile oil of the studied fruit samples was extracted by hydro-distillation method (for 3 hrs.) in a Clevenger's apparatus (Guenther 1953). The sample was done in triplicate and the mean values of the oil content (%) were recorded.

Identification of the chemical composition of volatile oils:

Gas chromatography–mass spectrometry analysis (GC-MS):

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 *μ*m film thickness). Analysis were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:30, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 50-550. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Experimental animals:

Adult male white Swiss mice (*Mus Musculus*), were used in all experiments. The animals were obtained from a closed breeding and research colony of the National Research Centre (Egypt). The mice used for each experiment were selected from mice of similar age 9-12 weeks and weight 20-25gm. Animals were housed in polycarbonate boxes with steel-wire tops and bedded with wood shavings. Ambient temperature was controlled at 22± 3 ºC with a relative humidity of 50 ± 15% and a 12 h light/dark photoperiod. Food and water were provided *ad libitum*. All experiments were carried out in accordance with research protocols established by the Animal Care Committee of the National Research Centre.

Chemicals*:*

Cyclophosphamide (CP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals used in the extraction were purchased from ADWIC (Cairo, Egypt).

Experimental design and doses:

After one week of acclimatization, total of 90 mice were fasted overnight before treatment and were divided randomly into 12 groups, six groups (10/group) for chromosomal aberrations and histopathological investigation and six groups (5/group) for sperm abnormalities. Mice were distributed as follows:

For cytological and histopathological examinations:

Group 1:

Negative control group in which mice were orally administrated olive oil (vehicle) 4 mL/kg.

Group 2:

Mice i.p. administrated CP at a dose of 20 mg /kg.

Group 3:

For determining the safety use of FEO, mice were orally administrated with the highest tested dose 0.6 mL/kg for five consecutive days.

Groups (4-6):

For determining the protective effect of FEO**,** Mice were orally administrated different doses of FEO (0.2, 0.4 and 0.6 mL/kg) for five consecutive days and at the last day CP 20 mg/kg was i.p. injected.

For sperm abnormalities

Group 7:

Mice received oral administration of olive oil 4mL/kg (negative control group).

Group 8:

Mice received i.p. treatment with CP at 20 mg/kg.

Group 9:

Mice received oral administration of FEO at 0.6mL/kg.

Groups (10-12):

Mice received CP plus FEO (0.2, 0.4 & 0.6mL/kg). Animals were sacrificed 24h after the last treatment for chromosomal and histological analysis. While, for sperm preparation animals left for 35 days starting from the first day of treatment. For chromosome preparations, animals from the different groups were i.p.injected with colchicine (0.1mg/kg), 2-3h before sacrifice.

Procedures

Cytogenetic analysis:

Chromosome aberrations (CAs) in bone marrow cells:

Chromosome preparations from bone-marrow (somatic cells) carried out according to the method of Yosida and Amano (1965). 100 well spread metaphases were analyzed per mouse. Metaphases with different types of structural and numerical chromosomal aberrations were recorded under 2500× magnification with a light microscope.

Sperm-shape abnormalities:

Sperm were prepared according to Wyrobek and Bruce (1978)**.** 1000 sperm were examined/animal. Different head and tail sperm abnormalities were recorded under 1000× magnification with a light microscope.

Histopathological Examination:

After sacrificing, parts of the liver and testis tissues were collected for histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, embedded in paraffin, cut into 4-5 μm thick sections, and stained with haematoxylin-eosin (H&E) dye for histopathological investigation (Drury and

Wallington 1980)**.** Images were captured and processed using Adobe Photoshop Version 8.

Immunohistochemistry for Caspase-3:

Immunohistochemical staining of anti-caspase-3 antibodies was performed with streptavidin-biotin (Duan et al., 2003)**.** Sections of 4 µm thick were deparaffinized and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti caspase-3 antibodies as the primer antibody at a 1: 100 dilution. The reaction was visualized by adding DAB Substrate. The specimens were counter stained with hematoxylin. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

Statistical analysis:

Data were computerized and analyzed using Statistical Package of Social Science (SPSS Inc, version 20, Armonk, New York: IBM Corp). One way analysis of variance (ANOVA) followed by Duncan's multiple comparison test was used to determine the difference among the means. The level of statistical significance was set at $P < 0.05$**.** Evaluation the activity of the FEO to reduce abnormalities induced by CP was carried out according to the following formula:

Inhibitory index (II) = [1- (FEO plus CP – control) / (CP - control)] ×100 (Madrigal-Bujaidar et al., 1998).

RESULTS

The essential oil composition:

GC-MS of FEO fruits was represented in Table (1) which showed that the total concentration of the oil reached 97% of the total extract. The table also showed that Anethole represents the main constituent (82%), followed by D-Lemonene (9.68%) and L-Fenchone (5.52%).

Cytogenetic analysis

Chromosomal aberration (CAs) in bone marrow:

Table (2) showed the number and percentage of CAs in groups of: control, FEO, CP and CP with FEO. The percentage of aberrant cells in animals treated with oil extract at 0.6 mL/kg was nearly close to the control group. The results also showed the ability of different doses of FEO to reduce aberrations in mice treated with CP in a dose-dependent manner.

Sperm abnormalities:

No significant differences between the animals treated with FEO alone and the control group (Table 3).The mean percentage of sperm were 8.80 %, 7.32 % and 6.78 % in CP groups pre-treated with the three tested doses of FEO respectively compared with 13.14 % for CP alone. The reduction in sperm abnormalities was dose-dependent.

Histopathological findings:

Control group showed normal histological structure containing cords of hepatocytes with sinusoids between these cords and prominent round nuclei. Central and portal veins also appeared normal (Fig. 1A). CP-treated group showed loss of hepatocyte architecture and congested central vein. CP induced dense focal inflammatory cells or necrotic tissues with marked degeneration of hepatic cells and pyknotic nuclei (Fig. 1 B). The hepatic tissues in groups treated with FEO at 0.6 mL/kg showed normal cell structure compared to the negative control (Fig.1 C). Pre-administration of FEO at low dose (0.2mL/kg) with CP group induced a decrease in hepatocytes vacuolizations, sinusoidal dilatation and partially disruption in radial arrangement and moderate congestion in the central vein as compared to CP group (Fig.1 D). The two higher doses of FEO (0.4 and 0.6 mL/kg) induced marked improvements in the liver cell damage induced by CP and the tissues maintained a histological picture nearly similar to that of the control group (Figs. 1 E & F). However, proliferation of binucleated hepatocytes was noticed in all groups treated with FEO and CP.

Histological examination of the control mice testis revealed testicular parenchyma that consisted of seminiferous tubules lined by stratified germinal epithelium. Narrow interstitial in between the tubules contained of interstitial cells and blood vessels as shown in (Fig. 2 A). Cyclophosphamide induced varying histopathological changes in the testis: atrophied seminiferous tubules, inflammatory cells infiltration, and reduction in the number of germ cells that appeared with pyknotic nuclei. Degeneration, vacuolation, exfoliation of germ cells into the lumen of seminiferous epithelium, thickness in interstitial space and degenerated Leydig cells were also noticed (Fig. 2B). Treatment with FEO alone at 0.6mL/kg showed normal structure (Fig. 2 C). The histological

alterations induced by CP were restored to a good degree in those animals pre-treated with FEO. But the most pronounced restoration was seen in groups treated with the medium and highest tested doses respectively (Figs. 2 D, E & F).

Immunohistochemical findings:

Immunohistochemical stained sections of caspase-3 expression not observed in liver tissues of control and FEO groups (Figs. 3 A & C). While, elevation in the expression of caspase-3 in the hepatocytes of CP treated group was observed (Fig. 3 B).

Table (1): GC-MS of fennel (*Foeniculum vulgare*) essential oil:

"No.	Compound	Rt	Concentration %
1	α-Pinene	4.66	0.88
2	Camphene	5.10	0.03
3	Sabinene	5.68	0.28
4	β-Pinene	5.84	0.06
5	β-Myrcene	6.15	0.09
6	α-Phellandrene	6.69	0.06
7	D-Limonene	7.40	9.68
8	1,8-Cineole (Eucalyptol)	7.52	0.68
9	γ-Terpinene	8.41	0.12
10	L-Fenchone	9.65	5.52
11	trans-p-Menth-2,8-dienol	10.98	0.05
12	cis-Limonene oxide	11.34	0.08
13	trans-Limonene oxide	11.53	0.11
14	Camphor	12.01	0.11
15	Terpinen-4-ol	13.33	0.02
16	Methyl chavicol	14.20	0.11
17	Anethole	15.23	82.00
	Unknown		0.12"
	Total essential oil (%)		97%
Rt: retention time			

Table 2: Frequency of chromosomal aberrations induced in bone marrow cells after treatment with different doses of cyclophosphamide & fennel essential oil (FEO):

Treatment and doses	Total Abnormal Metaphases			No. of different types of metaphases with:						Inhibitory Index Excluding Gaps
	No.	Mean(%) ± SE		G.	Frag. and/or Br.	Del.	C.F.	M.A.	Poly p.	
		Including Gaps	Excluding Gaps							
I. Control (olive oil)	21	4.20±0.48^{a}	2.00±0.65^{a}	11	7	3	0	0	0	-
II.CP (20mg/kg)	151	30. 20±0.55^{d}	26.80±0.50^{d}	17	66	12	5	48	3	-
III. FEO (0.6 mL/kg)	22	4.40±0.50^{a}	2.40±0.60^{a}	10	10	2	0	0	0	-
IV. FEO (0.2 mL/kg) +CP	119	23.80±0.65^{cd}	21.60±0.45^{cd}	11	52	10	3	39	4	21
V. FEO (0.4mL/kg) + CP	110	22.00±0.55^{cd}	19.20±0.70^{bc}	14	48	8	4	33	3	31
VI. FEO (0.6 mL/kg)+CP	98	19.60±0.55^{bc}	17.00±0.48^{bc}	13	50	5	2	25	3	40

Number of examined metaphases=500 (100 metaphase/animal, 5 animals/group); G.: Gap; Frag: Fragment; Br.: Break; Del.: Deletion; C.F.: Centric Fusion; M.A.: Multiple Aberrations; Polyp: Polyploidy. The values having different superscript letters in each column are significantly different from one to another as calculated by ANOVA. The data were presented as mean ± SE (n=5).

Table 3: Sperm abnormalities induced after treatment with cyclophosphamide & fennel essential oil (FEO).

Treatment and doses	Abnormal sperm		No. of different types of sperm head abnormalities					Coiled tail	Inhibitory index
	No.	Mean % ± SE	Triangular	Banana shape	Amorphous	Without hook	Small head		
I. Control (olive oil)	178	3.56±0.45[a]	57	3	78	29	1	10	-
II. CP (20 mg/kg)	657	13. 14±0.50[d]	97	76	246	164	7	67	-
III. FEO (0.6 mL/kg)	165	3. 30±0.86[a]	41	7	83	19	0	15	-
IV.FEO (0.2mL/kg) + CP	440	8.80±0.55[bc]	85	51	129	118	5	52	46
V. FEO (0.4mL/kg) + CP	366	7.32±0.60[bc]	71	26	141	79	2	47	61
VI.FEO (0.6 mL/kg)+ CP	339	6.78±0.58[ab]	84	17	138	57	4	39	67

Total number of examined sperm 5000 (1000 sperm/animal, 5 animals/ group).
The values having different superscript letters in each column are significantly different from one another as calculated by ANOVA. The data were presented as mean ±SE (n=5).

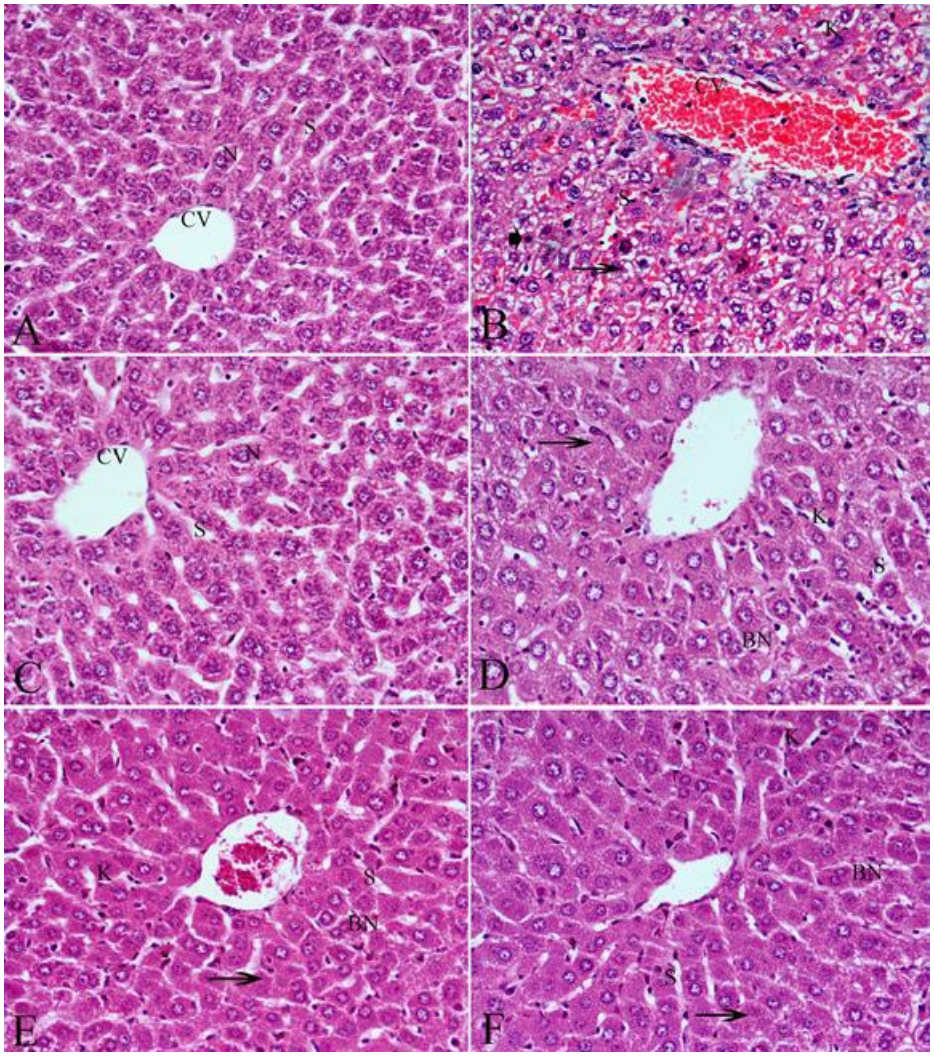

Figure. 1. (A) Normal liver section from control mice showing normal structure, central vein (CV), hepatic sinusoids(S) and prominent nucleus (N).
(B) Cyclophosphamide group showing loss of lobular architecture, necrosis of hepatocytes (arrow), accompanied by infiltration of inflammatory cells. Dilatation and congestion of central vein (CV) and blood sinusoids, activated Kuppfer cells (K), with pyknotic nucleus were observed (arrowhead).
(C) Plant group treated showing normal hepatic architecture central vein (CV), with hepatic sinusoids(S) and prominent nucleus (N).
(D) Low dose of plant and cyclophosphamide group showing moderate ameliorative effect, some hepatocytes appear necrosis (arrow), dilatation blood sinusoids(S), bineucleated hepatocytes (BN), and activated Kuppfer cells (K) were noticed.
(E) Medium dose of plant and cyclophosphamide group showing the liver architecture appeared nearly normal with a few of necrosis (arrow) and bineucleated of hepatocytes (BN), dilated blood sinusoids and activated Kuppfer cells (K).
(F) High dose of plant and cyclophosphamide group showing the liver architecture appeared nearly normal with bineucleated of hepatocytes (BN), dilated blood sinusoids and activated Kuppfer cells (K). (H & E. stain, X400).

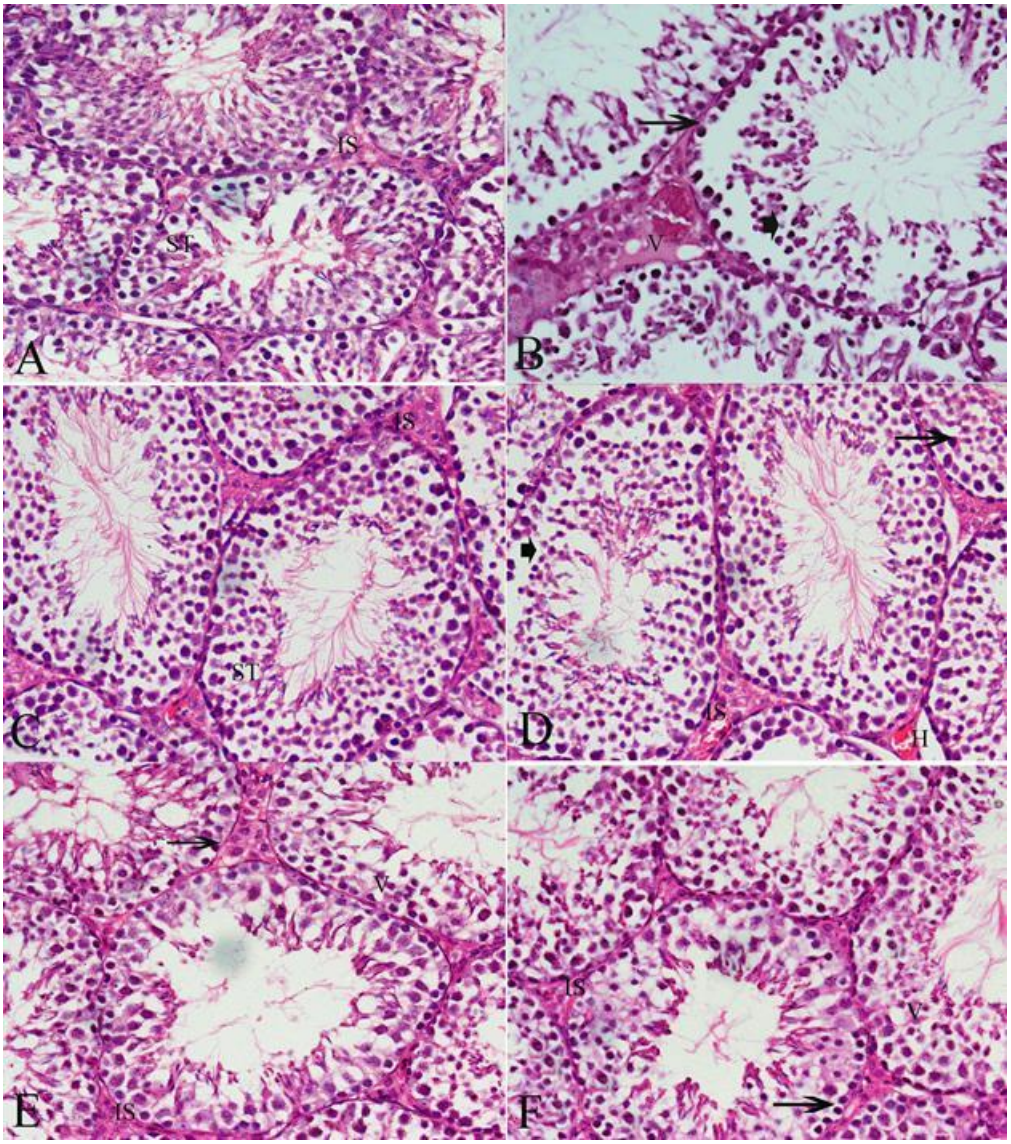

Figure. 2. (A) Photomicrograph of the testis of mice from control group is showing normal seminiferous tubule (ST) with different stages of spermatogenic cells and interstitial tubules (IS)
(B) Cyclophosphamide group showing degeneration and exfoliation of spermatogenic cells lining seminiferous tubules (arrow), interstitial spaces were widening with degeneration of Leydig cells; vacuolization of the interstitial cells (V) and pyknotic nuclei (arrowhead) were also observed
(C) Plants group showing normal seminiferous tubule with different stages of spermatogenic cells (ST) and interstitial tubules (IS)
(D) Low dose of plant and cyclophosphamide group showing moderate ameliorative effect, degeneration of some spermatogenic cells (arrow), few pyknotic nuclei of some spermatogenic cells (arrowhead) and the interstitial congestion were found (H)
(E) Medium dose of plant and cyclophosphamide group showing nearly normal structure of seminiferous tubule with few degeneration and vacuolation (V) of some spermatogenic cells (arrow) and interstitial tubules (IS)
(F) High dose of plant and cyclophosphamide group showing nearly normal structure of seminiferous tubule with few degeneration and vacuolation (V) of some spermatogenic cells (arrow) and interstitial tubules (IS) (H & E. stain, X400).

It was found that the caspase-3 positive existed in the cytoplasm and were dyed into brown yellow. Medium-increase reaction was noticed in CP-treated with FEO at 0.2 mL/kg (Fig. 3 D). The hepatic tissues showed a marked improvement in caspase-3 expression in the CP-treated groups with the two higher doses of FEO in a dose-dependent manner (Figs. 3 E & F).

In the testis, a few expressions of caspase-3 were observed in tissues of control and FEO groups (Figs. 4 A & C)**.** In cyclophosphamide group, immunoreactivity for caspase-3 was generally observed scattered in the cytoplasm and the perinuclear region (Fig. 4 B). The low dose of FEO showed a moderate improvement in expressions of caspase-3 in testis tissue of mice treated with CP (Fig. 4 D). However, pre-treatment of CP group with the medium and the high doses of FEO induced minimum immunoreactions of caspase-3 expressions (Figs. 4 E & F).

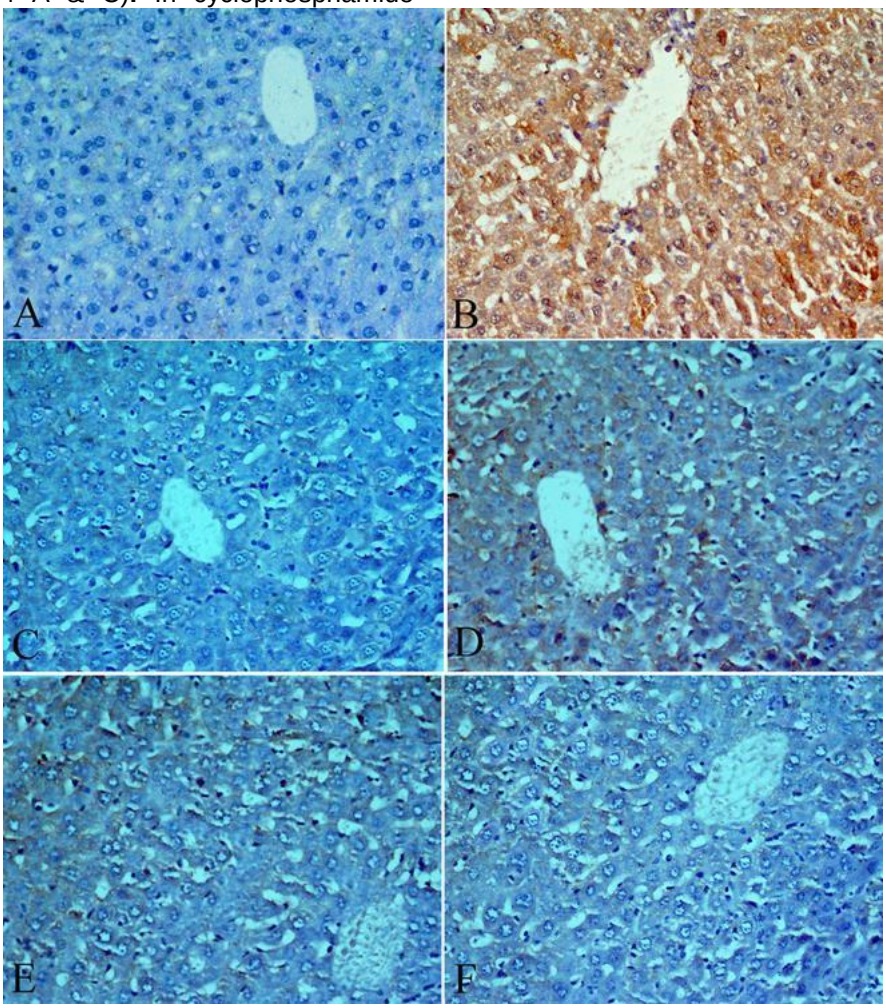

Figure. 3. (A) Normal liver from control mice showing no caspase-3 expression
(B) Cyclophosphamide group showing positive immunoreactions of caspase-3 expressions as brownish cytoplasmic stain
(C) Plants group showing minimum immunoreactions of caspase-3 expressions.
(D) Low dose of plant and cyclophosphamide showing moderate immunoreactions of caspase-3 expressions
(E) Medium dose of plant and cyclophosphamide showing mild immunoreactions of caspase-3 expressions
(F) High dose of plant and cyclophosphamide showing minimum immunoreactions of caspase-3 expressions. (Caspase-3 immunohitochemistry stain, X400).

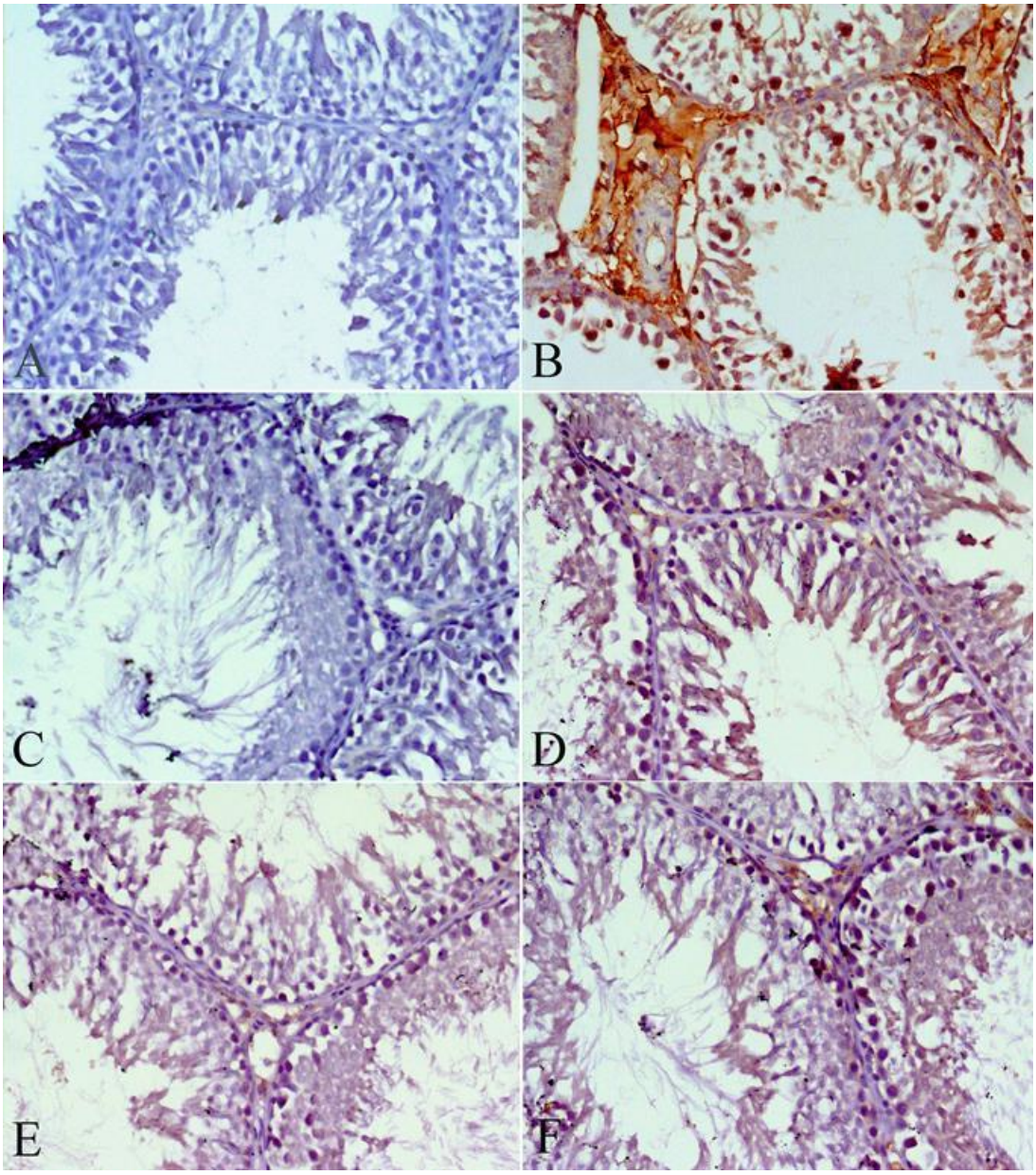

Figure. 4. (A) Photomicrograph of the testis of mice from control group showing minimum immunoreactions of caspase-3 expressions
(B) Cyclophosphamide group showing positive immunoreactivity for caspase-3 was observed scattered in the cytoplasm or in the perinuclear region of germ cells.
(C) Plants group showing minimum immunoreactions of caspase-3 expressions
(D) Low dose of plant and cyclophosphamide group showing moderate immunoreactions of caspase-3 expressions
(E) Medium dose of plant and cyclophosphamide showing mild immunoreactions of caspase-3 expressions
(F) High dose of plant and cyclophosphamide showing minimum immunoreactions of caspase-3 expressions.(Caspase-3 immunohitochemistry stain, X400)

DISCUSSION

Cyclophosphamide is a chemotherapeutic drug widely used in the field of cancer therapy. It is extensively used as a positive control in genotoxic/mutagenic experiments in both *in vivo* and *in vitro* studies (Fahmy et al., 2015).

The present results revealed that CP induced strong damage in mouse bone marrow chromosomes and affect sperm-shape. A statistically significant percentage of CAs in bone marrow cells was recorded after treatment with CP. These results are in agreement with the findings of other authors who reported that CP is a strong inducer for CAs, micronuclei, sister chromatid exchange and mutation in somatic cells (Jain and Jain 2012; El-Souda et al., 2014). On a consideration of sperm morphology, the results revealed significant abnormalities in sperm head and tail which respectively reflect DNA damage and reduced fertility. Effect of CP on sperm morphology, sperm counts, germinal cell degeneration and apoptosis in specific stages of germinal cells were detected after CP treatment **(**Tripathi and Jena 2008; Jalali et al., 2012; Fahmy et al., 2015). Moreover, it was found that adult male patients treated with CP suffered from infertility, decreased sperm counts and an absence of spermatogenic cycle in their testicular tissues (Harel et al., 2011; Green et al., 2014). Oligozoospermia/azoospermia was also detected in experimental models (Shen et al., 2005).

For histopathological studies, our results proved that CP induced liver damage evidenced by loss of normal hepatic architecture, marked degeneration of hepatic cells, congested veins, and appearance of dense focal inflammatory cells, pyknotic nuclei and necrotic tissues. These findings are coinciding well with the results of other authors. Ince et al., (2014) demonstrated sever damage in liver tissue of rats exposed to i.p. injection with CP 75 mg/kg body weight. Liver DNA fragmentation and sever damaging effect on liver tissue, in the form of congestion in the main blood vessels, dilatation and massive infiltration of inflammatory cells with irregular pattern were demonstrated in mice i.p treated with CP at 20 mg/kg (Fahmy et al., 2015). CP was also reported to induce liver dysfunction in mice through the elevation in the activities of liver enzymes (AST, ALT, and ALP) and massive histological changes (Sheweita et al., 2016).

In the present work CP induced varying histopathological changes in the testis: atrophied seminiferous tubules, inflammatory cells infiltration, and degeneration in germ cells that appeared with pyknotic nuclei. Also degeneration of Leydig cells was noticed. These pathological changes are compatible with the results obtained by other authors (Subramanian et al., 2006; Østensen et al., 2006). Immunohistochemical stained sections of caspase-3 expression in both liver and testis showed massive elevations after CP treatment.

In spite of the therapeutic importance of CP, a wide range of deleterious side effects especially hepatotoxicity, genotoxicity and reproductive toxicity etc have been demonstrated following to its use (El-Souda et al. 2014; Fahmy et al., 2015 ; Sheweita et al., 2016).

CP is metabolized mainly in the liver by cytochrome P450 system into the two active metabolites acrolein and phosphoramide mustard and acrolein represents the proximate toxic metabolite. It is a highly reactive α, β- unsaturated aldehyde, and was identified as the initiator of lipid peroxidation. This reactivity is the main reason of the cytotoxicity in all cells exposed to acrolein (Kehrer and Biswal 2000). Cytotoxicity to normal cells is the major limitation of using CP in clinical practice. Acrolein can interact with the big molecules of the cells such as proteins, membrane lipids and nucleic acids (Lata et al., 2014). Oxidative stress which lead to induction of free radicals is another pathway for initiating CP toxicity (Wahlang et al., 2015).

The genotoxicity of CP which detected in the present work can be related to the alkylating properties of its metabolites that can alkylate DNA and protein, producing cross links and breaks (Liu et al., 2014) and interfering with normal cell division in all rapidly proliferating tissues (Li et al., 2006). Several researchers showed that CP induced DNA damage and apoptosis by a free radical mediated mechanism. It was also reported to induce DNA damage and apoptosis in non-tumor cells which eventually lead to mutation and secondary tumors in the cells of patients under therapy (Zhang et al., 2009; Yadav et al., 2014).

Recent studies suggested that dietary antioxidants supplementation can influence the response to chemotherapy as well as improve their adverse side effects (Fahmy et al., 2015). The bioactive compounds of plant origin are nowadays of special interest in protecting DNA and different tissues from deleterious effects of ROS and other free radicals generated during chemotherapy (Arash et al., 2009; Melek et al., 2015; Sharma et al., 2017). Fennel (*Foeniculum*

vulgare) is an herbaceous, aromatic plant widely used in traditional medicine for treating a wide range of ailments. It possesses many pharmaceutical properties and its essential oil proved to be a prolific source of bioactive natural compounds (Badgujar et al., 2014; El-Sheikha and Galal 2015). The present study aimed to evaluate the safety use of fennel fruits essential oil (FEO) and its ameliorative role against mutagenicity, histopathological deteriorations in liver and testis induced by cyclophosphamide.

In the present study, FEO did not exhibit mutagenic/genotoxic effect by chromosomal aberration analysis or sperm abnormalities science the higher dose do not reached significant effect compared to the negative control. Histopathological studies also showed normal effect on liver and testis after FEO treatment. The safe bioactivity of FEO was previously documented (El-Sheikha and Galal 2015; Sheweita et al., 2016). The antigenotoxic evaluation showed that pre-treatment with FEO was able to reduce chromosomal aberrations and sperm abnormalities induced by CP with a dose-dependent manner. The reduction values reached 21, 31 & 40% in bone marrow and 46, 61 & 67% in sperm abnormalities after treatment with the three tested doses of FEO respectively. The number of metaphases with multiple aberrations that pronounced in bone marrow of CP group was nearly reduced to the half after co-administration with the highest tested dose of FEO. The antimutagenic effect of fennel against CP was also proved by Tripathi et al., (2013) who reported that oral administration of fennel seeds essential oil (1 and 2 mL/kg) was significantly inhibited the frequencies of chromosomal aberrations, micronuclei formation, and cytotoxicity in mouse bone marrow cells induced by CP treatment and was also produced a significant reduction of abnormal sperm. Chemopreventive potential of fennel seeds was proved by Singh and Kale (2008) who reported that diet containing different concentrations of fennel seeds exhibiting a significant reduction in the skin and forestomach tumor incidence and multiplicity induced by 7,12 - Dimethylbenz (a) anthracene (DMBA) and benzo (a) pyrene (B(a)P), respectively, in Swiss albino mice as compared with the control group. In this study significant enhancement in the activities of antioxidant enzymes was recorded especially at 4% and 6% tested diets of fennel. Also it was reported that the methanolic extract and the volatile oil of fennel seeds have a remarkable anti-inflammatory properties (Badgujar et al., 2014) and anticancer potential against liver cancer cell line (HepG-2) and breast cancer cell line (MCF-7) (Mohamad et al., 2011).

Histopathological investigation of liver and testis in the present study revealed good improvement in groups co-administrated with FEO plus CP compared with CP groups. Different concentrations of FEO attenuate the destructive alterations induced by CP in liver and testis tissues in a dose-dependent manner and the tissues tend to the normalcy with the highest tested dose of FEO (0.6 mL/kg). Also FEO attenuates the elevations in the expression of caspase-3 which observed in hepatocytes and testis of CP treated mice. Such effect decreased to a good extends with the two higher doses of FEO. Hepatoprotective effect of fennel was previously proved using different estimating parameters. Özbek et al., (2003) reported that oral administration of FEO decreases the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin induced by carbon tetrachloride. FEO was also reported to improve liver infiltration, coagulative necrosis in liver and spleen tissues and alleviated liver enzymes induced by sub-chronic treatment with the insecticide emamectin benzoate in male rats (El-Sheikha and Galal 2015).The same effect was observed by using the methanol extract of fennel seeds against hepatotoxicity induced by tienilic acid. This appeared from the improvement of hepatic functions, atherogenic markers, Na+/K+ATPase activity, endogenous antioxidants and hepatic lipid peroxidation level (Abdel-Wahhab et al., 2016). The possible effect of fennel oil against hepato-nephro toxicity of sodium-valproate (SVP) in albino rats was investigated (Al –Amoudi 2016). The results have confirmed that fennel oil has positive effects on the histological structure of the liver and kidney and the biochemical levels of AST, ALT, ALP, bilirubin, total proteins, creatinine and urea. The same protective effect was obtained by fennel seeds essential oil against biochemical and histological liver alterations induced in Swiss male albino mice after repeated oral treatment with CP 2.5 mg /kg for 28 days (Sheweita et al., 2016).

According to the aforementioned results of the current study and the literature reviewed, it can be concluded that the mechanism by which fennel (oil, seeds or extracts) offers its protective effect against toxicity induced by xenobiotics based on:

the strong antioxidant activity of fennel which prevent oxidative damage such as lipid peroxidation, the ability to increase the endogenous defense capacity, the reduction of pro-inflammatory cytokines, protection of nucleophilic sites of DNA and prevention /or scavenging of free radicals which may result in inhibition of the endogenous formation of mutagens (Badgujar et al., 2014; Abdel-Wahhab et al., 2016; Al –Amoudi 2016)**.**

Fennel is an effective antioxidant and different *in vitro* antioxidant assays support this fact. Kontogiorgis et al., (2016) demonstrated that fennel (beverage) interaction with DPPH (2, 2-diphenyl-picrylhydrazyl) has shown antioxidant capacity rate > 80% as well as lipid peroxidation inhibition compared with the standard antioxidants Trolox and NDGA (nordihydroguaiaretic acid). When fennel is compared to rosemary, fennel showed higher measured antioxidant activity by the carotene/ linoleic acid assay (Mata et al., 2007).

Essential oils are volatile and liquid aroma compounds derived from plants. In the present work GC/MS analysis of fennel fruits essential oil revealed the presence of 17 compounds with a total concentration of 97% where anethole represents the major constituent with a concentration of 82% followed by D-limonene, L-fenchone, α-pinene and eucalyptol with concentrations of 9.68% , 5.52%, 0.88%, 0.68% respectively. γ-terpinene, β-myrcene, β–pinene, trans and cis limonene oxide, camphene and camphor were also exist with low concentrations as active secondary metabolites. Anethole is the main fragrance and bioactive compound of fennel, and more than other 20 plant species. It is widely used as flavor agent in food industry and other industries, in cosmetics, perfumery, and pharmaceuticals. In the last few years, various studies have revealed multiple beneficial effects of anethole for human health, such as anti-inflammatory, anticarcinogenic and chemopreventive, antidiabetic, immunomodulatory, neuroprotective, or antithrombotic, that are mediated by the modulation of several cell signaling pathways, mainly NF-kB and TNF-α signaling, and various ion channels (Ritter et al., 2013; Galicka et al., 2014; Al-Amoudi 2016; Aprotosoaie et al., 2016). With respect to the anticancer effect anethole, it was previously reported to exhibit anticancer activity against Ehrlich ascites carcinoma induced in a tumor model in Swiss albino mice (Al Harbi et al. 1995). Moreover, Alkady (2018) demonstrated that anethole inhibits the proliferation of human prostate cancer *via* induction of cell cycle arrest and apoptosis. Very potent anticancer activity of fennel seeds essential oil against human breast cancer (MDA-Mb) and cervical epithelioid carcinoma (Hela) cell line was also reported by Akhbari et al., (2018). In this study the authors found that anethole (80.63%), L-Fenchone (11.57%), Estragole (3.67%) and Limonine (2.68%) represented the major components of the oil. Anethole is confirmed as “GRAS” (Generally Recognized as Safe) by the FDA (Food and Drug Administration) and FEMA (Flavor Extract Manufactures Association) in the U.S.A. (Zahid et al., 2015).

In the present work the monoterpene D-Limonene represents the second major compound in FEO (9.68%). The therapeutic effects of limonene have been extensively studied, proving anti-inflammatory, antioxidant, anticancer, antidiabetic, and antiviral properties (Miller et al., 2015; Suh et al., 2017; Vieira et al., 2018). Ozbek et al., (2003) suggested that the constituents (d-limonene and *β*-myrcene) of essential oil may have played a key role in the protection of hepato-toxicity. Moreover, it was reported that the antioxidant activities of essential oils are dependent on the presence of double bonds in the chemical structures of terpene hydrocarbons e.g limonene (Saleh et al., 2010). The monoterpene eucalyptol (1,8-cineole), a terpenoid oxide (represents 0.68%) is a promising compound as it has been shown to have anti-inflammatory and antioxidant effects in various diseases. It reduces the oxidative stress through the regulation of signaling pathways and radical scavenging activities (Juergens et al. 2003; Huang et al. 2015; Seol and Kim 2016). Strong antioxidant, antiproliferative and anti-inflammatory properties of the terpene EO components (L-fenchone, α-pinene, β-myrcene β-pinene, camphene and γ-terpinene) were reported (Meguel 2010; Sobral et al., 2014). Finally, it is worthy to mention that fennel essential oil is a complex of several secondary metabolites which in a combination play a significant antioxidant role protecting big molecules of the cells such as proteins, membrane lipids and nucleic acids produced by the xenobiotics and their metabolites.

CONCLUSION

Accumulating evidence suggests that free radical reactions play a key part in the development of degenerative diseases and that

an antioxidant-rich diet is a major defense against these free radical reactions. The scientific studies in this work showed that FEO has safe bioactivity and antioxidant potential that ameliorate the destructive effects of the anticancer drug cyclophosphamide. It attenuates both mutagenic and histophathological alterations of CP-treatment an effect that can be attributed to the presence of majorital compounds. It can be concluded that, FEO is a promising candidate in the field of drug construction and can be used concomitantly as a supplement to protect people undergoing chemotherapy.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

M A. F. and A. A. F. designed the work, shared in the experiments and also wrote and revised the manuscript. E.E.H. Share in designing the experiments, performed animal treatments, tissue collections, data analysis and reviewed the manuscript. E.M.H. Share in designing the work, identification of the chemical composition of essential oil. N.S.A-S and E.M.A.-G. Share in practical wok, statistical analysis of the results and reviewed the manuscript .E.A.O. Share in practical work, collect the tissues, share in writing the manuscript.

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