Antimutagenic and Chemoprevention Potentialities of Sweet Fennel (*Foeniculum vulgare* Mill.) Hot Water Crude Extract

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Abstrac: The use of medicinal plants by the general population is an old and still widespread practice that makes studies of their mutagenicity and/or antimutagenic/anticarcinogenic very essential. Fennel, *Foeniculum vulgare*, one of the most common use in Egyptian kitchen as a spice and beverage as well as in traditional medicine for its estrogenic, lactagouge, diuretic, antioxidant, immune booster and its usefulness in dyspepsia. It contains many phytochemicals such as thymol, carvacrol, terpinenes, P-thymene and thymol methyl ether, phenolic glycosides, flavonoids, phytosterols-triterp and saponins. Therefore, the potential antimutagenic and cancer chemoprevention effects of the hot water crude extract of sweet fennel (*Foeniculum vulgare* Mill.) seeds were evaluated in well known genetic model organisms mice and Drosophila: using mutagenicity, molecular and biochemical assays. In mice, Mitomycin C (MMC) was administered to mice as a positive control alone before and after treatment with 5 or 0.5 mg/Kg body weight or in combination with Fennel crude extract as acute (24h) and sub acute (5 consecutive days) doses, respectively. Chromosomal aberration assay in mice bone marrow cells revealed slight insignificant effect of fennel extract on aberrant mitosis rate, while it gave remarkable significant reduction of the MMC induced chromosomal aberrations. This effect was found to be dose-dependent. However, random amplified polymorphism of DNA (RAPD) showed clear variation between different classes of treated and non treated animals against MMC treatment, which reflected DNA protective effect of fennel extract. Biochemical studies showed slight effects on liver and kidney functions. Nucleic acids system (RNA, DNA, RNAase, DNAase and total soluble protein of liver), also the serum uric acid, urea and creatinine (kidneys function) and liver function (GOT and GPT activities) were slightly affected by MMC, which were improved by the ingestion of Fennel extract, whereas fennel extract alleviated MMC toxic effects. In Drosophila, Fennel extract significantly decreased the frequency of cholchicine induced aneuploidy and chromosomal aberrations in post and pre-treatments. [Journal of American Science 2010; 6(9):831-842]. (ISSN: 1545-1003).

Keywords: Sweet Fennel, Aneuploidy test, Chromosomal aberration, RAPD, Genotoxicity

1. Introduction

Use of antimutagens and anticarcinogens in everyday life has been suggested to be the most effective procedure for preventing human cancer and genetic diseases **(Ferguson, 1994)**. Medicinal plants have components as bioactive compounds which act as strategy to block or reverse carcinogenesis at early stages **(Lippman *et al*., 1994)**. Moreover, they are considered to be an inexpensive, effective and easily applicable approach to control cancer **(Wattemberg, 1985)**. The wide spread medicinal, edible and herbal plants have been tested for their antimutagenic activity and proved to inhibit the mutagenic and/or carcinogenic effects of some chemical mutagens **(Sarkar *et al*., 1997; Sripanidkulchai *et al*., 2002)**. In recent years, there has been a global surge in the popularity of herbal/traditional medicine, and currently there is enormous interest in developing new pharmaceutical products from such resources **(Karekar *et al*., 2000; Fahmy *et al*., 2007).**

Sweet fennel (*Foeniculum vulgare* Mill.) contains volatile oils (trans-anethole, thymol, fenchone, carvacrol, terpinene, P-thymene and thymol methyl ether), phenolic glycosides, flavonoids, phytosterols, triterpines, saponins **(Ph.Eur. 2005)**. Sweet fennel is an estrogenic (**Albert-Puleo, 1980; Malini *et al.*, 1985; Annusuya *et al*., 1988**), lactagouge, diuretic, antioxidant; immune booster is useful in dyspepsia. It has also bronchodilatory effects (**Boskabady *et al.*, 2004**), and a depressive action on arterial blood pressure **(Abdul-Ghani and Amin 1988)** that may be due to fennel extract which acts mainly as a diuretic and a natriuretic (**El Bardai *et al.*, 2001).** Fennel and its herbal drug preparations are widely used for dyspeptic complaints such as mild, spasmodic gastric-intestinal complaints, bloating and flatulence **(Chakurski *et al.*, 1981)**. Different studies had shown that the extract of *Foeniculum vulgare* is effective in the treatment of colic in breastfed infant **(Alexandrovich *et al*., 2003; Savino *et al.*, 2005)**.

Aqueous extracts of fennel (*Foeniculum vulgare*) proved to have antioxidant activity higher than some well known antioxidant such as ascorbic acid **(Satyanarayana *et al.*, 2004)**. The fennel extract showed also antioxidant effects, reducing malondialdehyde (MDA) level and increasing plasma

superoxide dismutase (SOD) as well as catalase activities (**Roberto *et al.*, 2000**). In addition, *F. vulgare* is claimed to possess an analgesic and is concerned in relieving inflammation (**Choi and Hwang 2004**).

Essential oil of *F. vulgare* showed hepatoprotective activity against carbon tetrachloride (CCl4) which induces liver injury in rat's model as it decreases levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin (**Ozbek *et al*., 2003**). *F. vulgare* M. revealed immunomodulatory effect of NFkappaB activities that plays critical roles in apoptosis and immunomodulation **(Kaileh *et al.,* 2007).** Fennel contains minor amounts of polyacetylenes in nonpolar extracts, which shows cytotoxicity against five different lymphoblastic cell lines **(Zidorn *et al.,* 2005).**

The mutagenicity, antimutagenicity and anticarcinogenicity of fennel and some of its components were subjected to some investigations. **Gorelick (1995)** indicated that trans-anethole, the main component of fennel oil, does not increase the mutant frequency in the Salmonella/microsome test as well as it did not induce chromosome aberrations in Chinese hamster ovary cells. In addition, pretreatment with trans-anethole and eugenol led to significant antigenotoxic effects against ethyl methane sulfonate (EMS), cyclophosphamide (CPH), procarbazine (PCB), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and urethane (URE). Both trans-anethole and eugenol exerted dose-related to antigenotoxic effects against PCB and URE. There was no significant increase in genotoxicity of trans-anethole and eugenol even when administered at high doses **(Abraham 2001).**

The anticarcinogenic, cytotoxicity and non-clastogenic activities of anethole was reported where, it increased the survival time and reduced the tumor weight and volume and body weight of the Ehrlich ascites tumors (EAT) bearing mice. The results also indicated the nature of anethole **(Al-Harbi *et al*., 1995)**.

The chemopreventive effect of different doses of test diet of Foeniculum vulgare Mill (Fennel) seeds was tested on DMBA-induced skin and B(a)Pyrene-induced forestomach papillomagenesis in Swiss albino mice. Fennel seeds exhibited a significant reduction in the skin and the forestomach tumor incidence and tumor multiplicity compared to the control group. Biochemical assays revealed a significant increase in the content/activities of phase I and phase II enzymes with a significant enhancement in the activities of antioxidant enzymes. These findings were indicative of the chemopreventive potential of Fennel against carcinogenesis **(Singh and Kale 2008)**.

The short term mutagenicity assays were proved to be good tools to assess the mutagenicity and predicted carcinogenicity of single compounds as well as mixed compounds either in direct applications or under environmental exposure. Mice chromosomal aberration assay was used for long time as a well valid and sensitive assay to evaluate the clastogenic effect of mutagenic agents (**Tice *et al*., 1994**). Aneuploidy plays a significant role in adverse human health conditions including birth defects, pregnancy wastage and cancer **(Aardema *et al.*, 1998)**. Aneuploidy and chromosomal aberration test in germ-line cells of Drosophila is considered one of the most sensitive and more efficient to detect chemically induced inherited aneuploidy as well as chromosome damage in germ-line cells (**Awad 1992; Fahmy *et al.*, 1997**). Recently, RAPD-PCR technique has been used to assess the mutagenic potentiality of genotoxic agents in which changes in DNA fragments amplification patterns due to mutagenic effect could be used as an indication of genotoxicity **(Savva 1998)**. RAPD is a sensitive assay for the detection of genotoxin-induced changes at the DNA level. Indeed, alterations in RAPD profiles were obtained after exposure of diverse aquatic systems to low concentrations of physical and chemical genotoxic agents under in vivo conditions (e.g. mussel embryo larvae exposed to 0.37–370 Bq, algae irradiated to 1.3 W/m2 UV-A **(Atienzar *et al*., 2002),** larvae barnacles exposed to 0.1–10 mg/L 4-n-nonyl phenol or 10 mg/L 17b-estradiol **(Atienzar *et al*., 2002),** Daphnia exposed to 12.5 mg/L B(a)P **(Atienzar *et al.*, (1999)** or 15 mg/L copper **(Atienzar *et al*., 2001)**. Nevertheless, studies comparing the RAPD method with routinely used genotoxicity/mutagenicity assays are necessary to further evaluate the potential of the RAPD assay for the detection of DNA damage and mutations.

In eco-genotoxicity studies, to elucidate the potential genotoxic effects of environmental contaminants, a powerful strategy could be firstly to use the RAPD assay as a screening method and secondly to use more specific methods measuring for instance DNA adducts gene mutations or cytogenetic effects **(Atienzar and Jha, 2006).**

Many studies indicated the usefulness of randomly amplified polymorphic DNA (RAPD) analysis for assessing the genotoxic effects of many tested substances and/or environments, in zebra fish **(Rong and Yin 2004)**, mice **(Noel and Rath 2006)**, rats **(El-Rahim *et al*., 2008)** as well as in plants **(Enan 2006; Cenkci *et al*., 2009).**The aim of this study was to evaluate the potential mutagenic and antimutagenic of the hot water crude extract of sweet fennel seeds as well as its chemoprotective against cytotoxic agents such as Mitomycin C and colchicine on mice and

Drosophila using a battery of mutagenicity test systems as well as molecular and biochemical assays.

2. Materials and Methods

2. A. Materials

1- Sweet fennel crude extract was prepared by hot water extraction method (**Tayel *et al.*, 2003).**

2- Mytomycin C (MMC: $C_{15}H_{18}N_4O_5$) was used as positive mutagens and carcinogens. Manufactured by Bristol-Myers Squibb Caribbean Company Mayaguez, puetro UAS.

3- Colchicine (Col: $C_{22}H_{25}NO_6$) was used as positive mutagens and carcinogens, manufactured by El Naser Pharmaceutical Chemicals Company "ADWIC" Abu-Zaabal-A.R.E.

2. B. Methods

2. B. 1- Mice assays: Animals (Male swiss mice) two doses of fennel extract were orally given (0.5 and 5.0 mg/kg) acutely (24 h) and sub acutely (5 consecutive days) successively. Mitomycin C was used as a positive. Untreated animals were used as negative control.

a. **Chromosomal aberration assay**: Standard Flame-drying technique was followed for metaphase preparations from the bone marrow cells according to **Preston *et al*., (1987).**

b. **Biochemical analysis**: Liver and kidney nucleic acid **(Dische 1955 and Schneider 1957)**. DNAse and RNAse activities for liver and kidneys tissue were assessed **(Bergmeyer 1974)**. Liver function plasma GOT and GPT activities **(Reitman and Frankel 1957)** and plasma bilirubin **(Peters, 1968).** For kidneys function, uric acid and urea in plasma and creatinine (**Schirmeister 1964**) were used to asses the biochemical and toxicological effects of MMC and fennel extract.

c. **RAPD profiles and data analysis**: DNA was extracted from the tested animals according to the method described by **Sharma *et al.* (2000)**. The concentration of DNA and its relative purity were determined using a spectrophotometer based on absorbance at 260 and 280 nm, respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gels using DNA molecular weight marker (Eurobio, Paris, France). Random amplified polymorphic DNA (RAPD) was carried out using the random primers (Operon Technology, USA) presented in Table 1. RAPD–PCR was performed on DNA for all liver samples using the previously described methods of **Williams *et al.* (1990)** and **Plotsky *et al.* (1995)**. All mutagen that presented an RAPD profile was defined by loss or addition of bands compared with the control. All amplifications were repeated twice in order to confirm the reproducible amplification of scored fragments. Only reproducible and clear amplification bands were scored. The marked changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control treatments) were evaluated.

Table (1): List of primer names and their nucleotide sequences.

Primer names	Sequences
A01	5` CAGGCCCTTC 3`
A02	5` TGCCGAGCTG 3`
A03	5` AGTCAGCCAC 3`
A04	5` ATTCGGGCTG 3`
A05	5` AGGGGTCTTG 3`
A06	5` GGTCCCTGAC 3
A07	5` GAAACGGGTG 3`
A08	5` GTGATCGCAG 3`
A09	5` GGGTAACGCC 3`

2. B.2. Drosophila assay: Chromosomal aberrations and aneuploidy test in Drosophila germ-line cells were carried out **(Awad 1992),** using the same previous substances. Different broods of larvae were given colchicines as positive control.

a. ***Drosophila Stocks***: ***ATE Strain;*** ♂w/Y^{w+}; se/se , ♀w/w; se/se, this strain carries white-eye mutation (*w*) on X chromosome and sepia eyes (*se*) on the third chromosome. Moreover, the Y chromosome carries the segments of X chromosome that carries the w^+ locus. So that, the male of this strain has sepia eyes, while the female has white eyes phenotype **(Awad, 1992).**

b. ***Drosophila Crosses and Treatments***: To evaluate the mutagenic potentiality of fennel crude extract, virgin *white* females were crossed to sepia males. After 2 days the parental flies were removed and 56-68 hours old larvae were collected and immersed in solution containing 1mM of Colchicine as positive control for 2h, after treatment, larvae were washed with tap water and dried on filter paper, and then they were reared on standard Drosophila media until emergency. in pre-treatment, the early second instar larvae (48-hs old) of F_1 were collected and treated with fennel in standard Drosophilae media (50mg/lml) which was chosen after a preliminary study. Then larvae were collected and they were immersed in 1mM of Colchicine for 2h. Meanwhile, in post treatment, the 56hs old larvae were immersed in colchicine solution for 2h then washed and dried. As described before, the treated larvae were transferred to standard Drosophilae media contains fennel for (6hs) after colchicine treatments in all experiments. The emerged virgin females were collected and mated to 3 days old males of ATE strains (30 Females: 15 males/ bottles) in two

brooding systems with 6 days interval. The progeny of each experiment was screened and scored for aneuploidy males. All Drosophila stocks and crosses were maintained at 25°C.

2. B.3. Statistical analysis: All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (**SAS, 1982**). χ^2 test was used to evaluate the significant of differences between negative control and other treatments (**DeGroot, 1988**). All statements of significance were based on probability of $P \leq 0.05$ and $P \leq 0.01$. However data for RAPD–PCR analysis were carried out using SPSS statistical program for windows (Version 11).

3. Results:

3.1. Biochemical analysis:

The chemo-protective effects of fennel extract were evaluated on mice that were treated with Mitomycin C as strong cytotoxic agent by measuring standard biochemical markers. MMC induced strong damage to liver and kidney cells as indicated by highly increase of liver GOT, GPT and bilirubin biomarkers as well as kidney biomarkers, i.e., Uric acid, Urea and Creatinine (Table 2) in addition to decreasing the DNA and RNA nucleic acids contents in both organs (Table 3). Fennel extract itself showed little effect on both organs. The chemo-protective effects of fennel extract is very pronounced in against the severe toxicity of MMC in liver and kidney of treated mice, MMC induced abnormalities and toxic effects either in pre or post treatments (Table 4).

3.2. Bone-marrow chromosomal analysis:

The capabilities of sweet fennel tested extract to induce chromosomal aberrations (CA) and its anticlastogenic activity against the clastogenic effects of MMC were evaluated using *in vivo* clastogenicity assay of mouse bone marrow cells. Two doses: low (L, 0.5 mg), and high (H, 5.0 mg) doses of hot water fennel extract were tested alone or in combination with Mitomycin C as pre and post-treatments compared with the negative and positive (MMC) controls. Results are summarized and presents in Table (4). Fennel treatments showed slight non-significant increase in chromosomal aberration frequency, but it was in the spontaneous range of aberrations. MMC induced strong clastogenic effects, where it reduced mitotic index. Highly significant increases of all types of chromosomal aberrations (27.6 +1.0) were recorded after 24h administering of MMC (Table 5).

In pre treatments with acute dose, administration of 0.5 mg/kg fennel with MMC showed no significant differences in the frequency of CA caused by MMC. While the high dose 5 mg/kg fennel decreased the percentage of CA from (27.6 +1.0) to (19.6 +2.4). This showed a dose dependent effect of fennel versus the positive control at 24h of the treatment.

Meanwhile, significant differences in the percentage of aberrant mitosis of the studied samples and time dependent effects after treatment with fennel sub-acutely were observed. The fennel water extract decrease the clastogenic effect of MMC to 21.6 +1.3 (0.5mg/kg) and 16.0 +1.4 (5mg/kg). Fennel produces low percentage of aberrations, but still significantly higher that of the negative control.

In post treatments, administration of fennel for long time before treatment with MMC caused a marked decrease in the aberrant mitosis. The obtained data showed a significant decrease in CA from 27.6 +1.0 to 11.0 + 0.5 in the group receiving the high dose of 5mg/kg. While in the group of low dose (0.5mg/kg) a significant decrease from 27.6 +1.0 to 16.2 + 1.1 in CA was observed. Fennel extract decrease significantly the number of cells with numerical aberrations and increase the mitotic activity (MI) of the cells

In conclusion, all samples showed lower percentage of aberrations versus MMC in acute and sub acute treatments. This effect was found to be dose- and time dependent, and showed that Fennel exhibited antioxidant activities. This suggests that the plant does not pose a hazard when used as herbal medicine. In general, both pre and post- treatments of fennel extracts were capable to reduce the frequency of MMC induced chromosomal aberrations in acute and sub-acute treatments. These results indicated that fennel extract exerted dose and time dependent anticlastogenic effects against MMC.

3.3. RAPD profiles

Aimed at verifying genetic effect of Sweet fennel on genetic material, RAPD analysis was performed on DNA extracted from liver of animals after acute and sub-acute treatments with, Mitomycin C, and fennel extract as well as in pre and post treatments comparing with the negative control. In total, ten 10-mer oligonucleotide primers of 60–70% GC content were used for screening Mice genome for alterations, however only five primers generated specific and stable results with the total number of 30 bands. The molecular size of amplified bands ranged from 100 (primer OP-A08) to 740 pb (primer OP-A07) in control. Each primer generated between 6 (primer OP-A04) and 12 (primer OP-A06) bands with an average of 8 bands per primer. RAPD profiles showed substantial differences between control and fennel-treated mice with apparent changes in the number and size of amplified DNA fragments for different primers.

The disappearing of a normal band and/or appearing of a new band are the obvious changes in

the RAPD patterns generated by fennel treatments. Table 7 and Fig. 2 present a summary of all RAPD profile modifications and RAPD products of selected 5 primers, respectively. In total, 30 normal RAPD bands of control [in the range of 650,800bp (primer OP-A06) were disappeared in all treatments. On the other hand, 23 extra bands (in the range of 160 and 1300 bp) appeared in all treatments and majority of these new PCR products (77%) were smaller fragments (<1 kb). the number of new bands was obviously higher than the number of lost bands in the treated mice in all treatments using primer OP-A06 (Table 6) and Fig. (1). Nevertheless, there is a positive correlation between pre or post treatments with fennel and total number of disappearing and appearing RAPD bands.

In conclusion, Recorded results of RAPD-PCR assay as a mutagenicity test system using extracted DNA of control and treated mice and arbitrary random primers, MMC, fennel, post and pre treatments increased the number of amplified fragments of DNA over the control ones. However, there was an ascending increment of band numbers in fennel extract treatments at low and high doses, while post-treatment with low dose exhibited the lowest band numbers for all tested primers compared with positive and negative controls. Obtained results affected DNA banding pattern in all treatments either with fennel hot water extract in high and low doses or after MMC treatments alone or in post and pre treatments. It's worthy to notice that each line represented the total extracted DNA from each treated mice group to reduce the intra specific variation between animals in each group. This result showed also the presence of genetic heterogenicity between treated animals.

3.4. Male Germ line Aneuploidy test

The Antimutagenic potential of Sweet Fennel Crude Extract was evaluated through induction of aneuploidy in drosophila male Germ line cells using colchicine as strong aneuogenic agent against Fennel extract on drosophila ATE strain. The des-mutagenic and bio-antimutagenic activities were studied through pre and post treatments.

In control experiments, the frequency of spontaneous mutation (white eyed male mutants) in male-germ line cells varied between (0.11%) and (0.19%) through the two broods. While, the over all average was (0.15%). On the other hand, the frequencies of spontaneous chromosome gain (sepia eyed females) were (0.24%) and (0.20%) in the two broods with an average of (0.22%). The total frequencies of aneuploidy were (0.18%) and (0.20%) in the two broods. While, the over all average of aneuploidy was (0.19%), respectively.

In colchicine experiment, the frequency of chromosome loss (white eyed male mutants) was significantly increased (0.85%) and (1.00%) in the two broods. With regard to chromosome gain, colchicine treatment significantly increased the frequency of chromosome gain in the two broods. The frequency of chromosome gain (sepia female) was significant in the first brood (0.93%) and highly significant in the second brood (0.1.08%) comparing with the negative control. The over all average of chromosome loss frequency was (0.93%) and the over all average of chromosome gain was highly significant (0.1.02%).

After fennel treatment, the frequency of aneuploidy was increased over the control level for the chromosome loss which was slightly increased but it was in-significant when compared with the control (0.16%) as shown in Table (7).

Fennel extract treatment gave only insignificant effect on aneuploidy and chromosomal aberration frequency in male germ-line cells in contrast to the well known aneuogenic agent, colchicine that induced high significant increase of chromosomal mutations compared to the negative control (Table 7). In general, little reduction could be seen in aneuploidy frequency after fennel treatment comparing with negative control and this may be due to antimutagenic activity of fennel, which was capable to reduce the frequency of aneuploidy via des-mutagenic or bio-antimutagenic activity.

In pre treatment, it could be observed that fennel was capable to reduce the frequency of aneuploidy that was induced after colchicine treatment. Where the total frequency of aneuploidy at the first brood was non significant (0.38%) and it was highly significant at the second brood with a total average of aneuploidy of (43 %), respectively. The reduction in chromosome loss rate in these experiments varied from 55% at the first brood to 57% at the second brood, while, the total reduction in chromosome loss was 56%. With regard to the reduction in chromosome gain rate in this experiment, it was 53% in the first brood and 55% at the second brood with an average of 54%. The highest reduction rate in pre treatment was observed at the second brood as shown in Table (6) and Fig. (1).

In post treatment, the frequency of aneuploidy was also decreased rather than colchicine treatment, where the frequencies of chromosome loss or gain mutants were lower than the level of the same induced mutants after colchicine treatment. They were insignificant compared with the negative control. The over all average of chromosome loss was significant and the over all average of chromosome gain was highly significant. On the other hand, the total frequency of aneuploidy was significant in the first brood (0.44 %) and highly significant in the second brood as well as over all average of aneuploidy in this

experiments (0.46% and 0.53%, respectively).The reduction rate of chromosome loss was about 48% at the first and second broods. The total reduction in Post treatment was about 53% as indicated in Table (7) and Fig. (1).

Fennel extract was to decrease the frequencies of colchicine induced mutations in pre and post treatments. In general, fennel extract showed more effectiveness in pretreatment (Table 7, Figure 1). Recorded results revealed that fennel has an antimutagenic potentiality and may have a protective effect against aneuogenic agents. The obtained results indicated that fennel reduced the aneugenic effect of colchicine when used as pre treatment which reflects that it may activate some enzymes, or mechanisms winch detoxificate the colchicine compound or activate the metabolite system of larvae to reduce the biological hazard of colchicine.

Table (2): Chemical profiles of liver and kidney functions of animals after acute and sub- acute treatments with, Mitomycin C, and fennel extract as well as in pre and post treatments comparing with the negative control.

Treatment	Liver function								Kidney function			
	GOT (U/1)	%	GPT (U/1)	%	Bilirubin mg/d1	%	Uric acid mg/d1	%	Urea mg/d1	%	Creatinine mg/d1	%
Normal control	26.11 ± 1.66 a	100	20.00 ± 1.11 a	100	00.91 ±0.07 a	100	3.89 ± 0.25 a	100	17.21 ± 1.23 a	100	0.78 ± 8.05 a	100
MMC	55.12 ± 3.33 b	211	64.23 ± 4.26 b	321	02.42 ± 0.18 b	266	10.00 ± 0.98 b	251	51.21 ± 3.26 b	298	1.69 ± 0.08 b	217
Fennel + MMC 0.5 mg	41.11 ± 2.83 c	157	50.12 ± 3.92 c	251	20.00 ± 0.13 c	220	7.23 ± 0.48 c	183	40.23 ± 3.33 c	234	1.31 ± 0.10 c	168
Fennel + MMC 5.0 mg	38.61 ± 2.91 c	148	40.01 ± 1.27 d	200	1.50 ± 0.10 d	165	6.11 ± 0.32 d	157	32.11 ± 0.17 d	187	1.11 ± 0.09 d	142
Pre-treatment 0.5 mg	35.12 ± 2.17 d	135	36.00 ± 2.34 e	180	1.36 ± 0.08 e	149	6.00 ± 0.27 d	154	30.00 ± 0.19 e	174	0.99 ± 0.06 e	127
Pre-treatment 5.0 mg	33.78 ± 2.17 d	129	34.11 ± 3.00 e	171	1.30 ± 0.09 e	144	6.01 ± 0.27 d	154	32.00 ± 2.71 d	186	1.09 ± 0.08 d	140
Post-treatment 0.5 mg	32.60 ± 2.00 d	125	30.72 ± 1.66 f	154	1.24 ± 0.07 f	136	5.31 ± 0.31 e	137	27.72 ± 0.17 f	161	1.03 ± 0.07 e	132
Post-treatment 0.5 mg	31.00 ± 2.22 f	119	30.21 ± 1.78 f	152	1.19 ± 0.07 f	131	4.92 ± 0.30 f	126	26.41 ± 2.00	153	1.07 ± 0.06 e	137

Different letters (a,b,c,d,e and f) in the same column are significantly different ($p \leq 0.05$) using student's t test.

Table (3): Quantification of nucleic acids (DNA and RNA) content in liver and kidney of animals after acute and sub- acute treatments with, Mitomycin C, and fennel extract as well as in pre and post treatments comparing with the negative control.

Treatment	Liver				Kidney			
	DNA mg/100g	%	RNA mg/100g	%	DNA mg/100g	%	RNA mg/100g	%
Normal control	30.00 ± 2.11 a	100	120.01 ± 10.21 a	100	40.01 ± 3.12 a	100	45.11 ± 3.74 a	100
MMC	25.67 ± 1.79 b	86	72.12 ± 6.27 b	60	34.44 ± 2.23 a	86	30.01 ± 2.00 b	67
Fennel + MMC 0.5 mg	26.10 ± 1.89 b	87	88.12 ± 7.12 c	73	35.74 ± 2.22 b	89	35.21 ± 1.99 c	78
Fennel + MMC 5.0 mg	27.89 ± 1.92 b-a	93	110.11 ± 9.99 a	92	37.01 ± 2.74 b-a	93	39.98 ± 2.14 a	89
Pre-treatment 0.5 mg	28.11 ± 1.87 b-a	94	111.12 ± 10.21 a	93	37.17 ± 3.00 b-a	93	41.27 ± 3.11 a	91
Pre-treatment 5.0 mg	28.12 ± 1.99 b-a	94	110.00 ± 10.00 a	92	37.51 ± 2.76b-a	94	41.11 ± 3.01 a	91
Post-treatment 0.5 mg	28.07 ± 1.92 b-a	94	109.00 ± 9.87 a	91	37.72 ±2.41 b-a	94	40.00 ± 2.94 a	89
Post-treatment 0.5 mg	29.27 ± 2.00 a	98	107.27 ± 9.88 a	89	38.98 ±3.01 a	97	41.21 ± 2.74 a	91

Different letters (a, b and c) in the same column are significantly different ($P \leq 0.05$) using student's t test

Table (4): DNA and RNA nuclease activities in liver and kidney of experimental animals after acute and sub- acute treatments with, Mitomycin C, and fennel extract as well as in pre and post treatments comparing with the negative control.

Treatment	DNAase activity (IU/g tissue)				RNAase activity (IU/g tissue)			
	Liver mg/100g	%	Kidney mg/100g	%	DNA mg/100g	%	RNA mg/100g	%
Normal control	61-11 ± 4.44 a	100	51.23 ± 3.47 a	100	39.21 ± 2.12 a	100	30.23 ± 1.76 a	100
MMC	55.61 ± 3.72 a	91	47.24 ± 2.81 a	92	36.12 ± 3.00 a	92	28.82 ± 1.86 a	95
Fennel + MMC 0.5 mg	56.34 ± 3.61 a	92	48.11 ± 3.12 a	94	37.00 ± 2.78 a	94	28.89 ± 1.87 a	96
Fennel + MMC 5.0 mg	58.75 ± 4.71 a	96	47.87 ± 2.92 a	93	37.84 ± 2.64 a	97	29.12 ± 1.91 a	96
Pre-treatment 0.5 mg	59.89 ± 4.10 a	98	48.90 ± 3.11 a	95	38.24 ± 2.74 a	89	29.47 ± 1.72 a	97
Pre-treatment 5.0 mg	58.02 ± 4.09 a	95	49.00 ± 3.11 a	96	38.01 ± 2.94 a	97	29.52 ± 1.81 a	98
Post-treatment 0.5 mg	59.72 ± 3.79 a	99	48.77 ± 3.00 a	95	38.47 ± 2.93 a	98	30.11 ± 2.00 a	100
Post-treatment 0.5 mg	60.00 ±4.61 a	98	49.01± 2.81 a	96	39.00 ± 2.91 a	99	30.01 ± 1.98 a	99

Different letters (a, b and c) in the same column are significantly different ($P \leq 0.05$) using student's t test

Table (5): Frequencies of chromosomal aberrations in affected mouse bone marrow cells after acute and sub- acute treatments with, Mitomycin C, fennel extract as pre and post treatments comparing with the negative control.

Dose mg/kg	Route	Time	Total cell/n	MI %	Total chromosomal aberrations						Total numerical aberrations (Mean ± S.D.)
					g	b	f	d	Without gaps	Mean ± S.E	
Control	P.O.	24h	500/5	3.98	3	-	1	-	1	0.2 ± 0.2 E	1.4 ± 0.2 C
MMC	P.O.	24h	500/5	2.2	32	98	16	24	138	27.6 ± 1.0 A	17.8 ± 2.2 A
Fennel + MMC (0.5 mg)	P.O.	24h	500/5	2.38	35	83	21	27	131	26.2 ± 1.5 A	16.2 ± 1.7 A
MMC + Fennel (5.0 mg)	P.O.	24h	500/5	3.28	23	53	18	27	98	19.6 ± 2.4 B	9.2 ± 1.3 B
Pre-treatment (0.5 mg)	P.O.	5 days	500/5	2.74	29	62	24	22	108	21.6 ± 1.3 B	10.2 ± 1.3 B
Pre-treatment (5.0 mg)	P.O.	5 days	500/5	2.94	16	43	18	19	80	16.0 ± 1.4 C	8.6 ± 0.8 B
Post-treatment. (0.5 mg)	P.O.	5 days	500/5	3.16	20	39	15	27	81	16.2 ± 1.1 C	9.2 ± 1.3 B
Post-treatment (5.0 mg)	P.O.	5 days	500/5	3.4	13	22	14	19	55	11.0 ± 0.5 D	7.8 ± 1.0 B

MI = mitotic index, S.E. = standard error, g = gap, b = breaks, f = fragments and d = deletion.
Different letters (A, B,C, and D) in the some column are significantly different ($P \leq 0.05$) using student's t test.

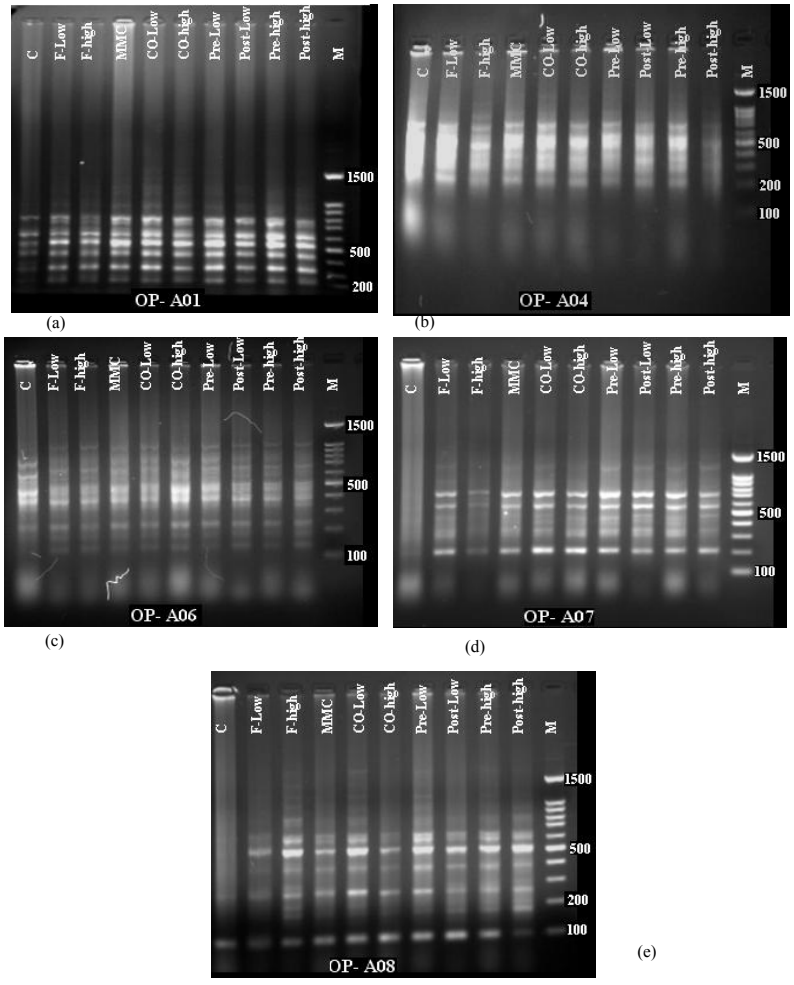
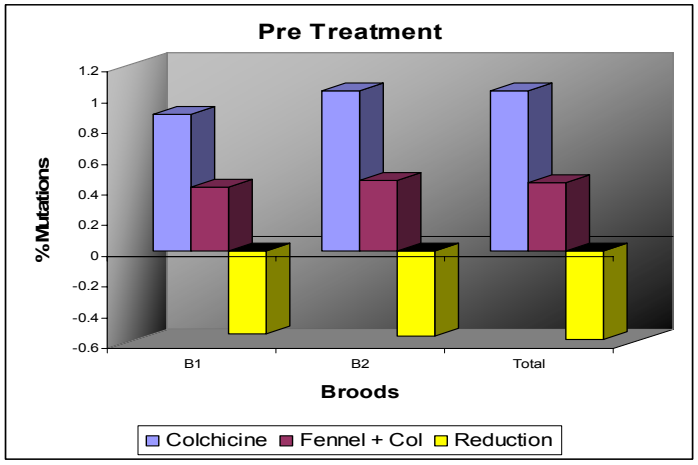


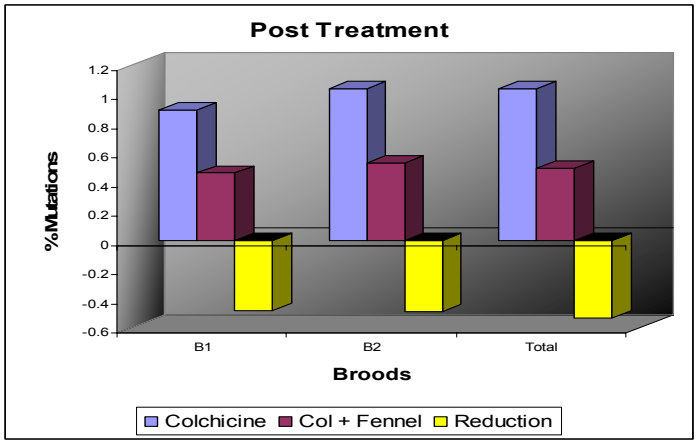
Figure (1): Comparison of RAPD fingerprinting profiles of different mice genomic DNA treated with two doses of low (L, 0.5 mg), and high (H, 5.0 mg) doses of hot water fennel extract alone or in combination with Mitomycin C as pre and post-treatments compared with the negative and positive (MMC) controls. (a), (b), (c), (d), and (e), represent PCR products with primers OP-A01, A04, A06, A047 and A08 respectively. C represents negative control. M represents DNA marker.

Table (6):The number of bands in control treatment (C) and molecular sizes (base pair, bp) of appearance (+) and disappearance (−) of bands for primers OP-A01, A04, A06, A07, A08 in affected mouse bone marrow cells after acute and sub- acute treatments with, Mitomycin C, fennel extract as pre and post treatments compared to control using Software UVIsoft image analyzer system. ND (none detection) indicates that there is no change in fennel-treated RAPD profiles compared to control.

Primer	c		Fennel-low	Fennel high	MMC	Co-low	Co-high	Pre-low	Post-low	Pre-high	Post-high
OP-A01	5	+	700, 240,	700, 240	700, 240, 900, 1300	700, 240, 900, 1300	240, 700, 900, 1300	240, 700, 900, 1300	240, 700, 900, 1300	240, 700, 900, 1300	240, 700, 900, 1300
		-	ND	ND	ND	ND	ND	ND	ND	ND	ND
OP-A04	6	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
		-	ND	ND	ND	ND	ND	ND	ND	ND	ND
OP-A06	5	+	1000,680, 600,500, 190,150	1000,680, 600,500, 190,150	1000,680, 600,500,190,150	1000,680, 600,500,320, 190,150	1000,680, 600,500,190, 150	1000,680 ,600,500, 190,150	1000,680, 600,500,320, 190,150	1000,680, 600,500,3201 90,150	1000,680,600 ,500,320, 190,150
		-	800,650	800,650	800,650	800,650	800,650	800,650	800,650	800,650	800,650
OP-A07	4	+	1300,320,300	1300,320 ,300	1300,320, 300	1300,320, 300	1300,320, 300	1300,320, 300	1300,320, 300	1300,320, 300	1300,320 300
		-	ND	500,400	ND	ND	ND	ND	ND	ND	400
OP-A08	5	+	600,500	850, 800, 650, 600, 500,400,290,180,160	650,600, 500, 400	850, 800 650, 600, 500,400, 180,160	650, 600, 500,400,180, 160	850, 800, 650, 600, 500,400, 180,160	650, 600, 500,400,180, 160	650,600, 500,400, 180,160	850,800,650, 600,400,400, 290, 180,160
		-	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	30		2 (-); 13 (+)	4 (-); 20 (+)	2(-); 17(+)	2(-);22 (+)	2(-); 19(+)	2(-); 21(+)	2(-); 20(+)	2(-); 13(+)	3(-); 23(+)



(a)



(b)

Figure (2): Diagram represents the frequencies and reduction rate of colchicine induced chromosomal aberrations and aneuploidy mutations in male germ-line cells of Drosophila after treatment with colchicine and fennel extract: a) pre treatment (fennel + colchicine) and b) post treatment (colchicine + fennel).

Table (7): Frequencies of chromosomal aberrations and aneuploidy mutations in male germ-line cells of Drosophila; after larvae treatment with colchicine, water crude extract of fennel, pre treatment (fennel + colchicine), and post treatment (colchicine + fennel) comparing with negative control.

Treatment	Brood	Males				Females				Total tested flies			
		N	White eyed male mutants	%	Reduction	N	Sepia eyed female mutants	%	Reduction rate	N	Total Mutations	%	Reduction rate
Control	B1	3690	4	0.11		4158	10	0.24		7848	14	0.178	
	B2	3077	6	0.19		3524	7	0.20		6601	13	0.197	
	Total	6767	10	0.15		7682	17	0.22		14449	32	0.187	
Colchicine 1mM	B1	2115	18	0.85**		2246	21	0.93**		4361	39	0.89*	
	B2	2725	27	1.00**		2864	31	1.08**		5589	58	1.04*	
	Total	4840	45	0.93**		5110	52	1.02**		9950	97	1.04*	
Fennel		3105	5	0.16		3265	6	0.19		6370	11	0.17	
50 mg/ml		3635	9	0.25		3276	7	0.21		6911	16	0.23	
	T	6740	14	0.21		6541	13	0.20		13281	27	0.20	
Fennel + col		3684	14	0.38	0.55	3374	15	0.44*	0.53	7058	29	0.41*	0.54
Pre-treatment		3922	17	0.43*	0.57	3489	17	0.49*	0.55	7411	34	0.46*	0.56
	T	7606	31	0.41*	0.56	6863	32	0.47*	0.54	14469	63	0.44*	0.58
Col + fennel		3376	15	0.44*	0.48	3215	15	0.47*	0.49	6591	30	0.46*	0.48
Post- treatment		3338	19	0.57*	0.43	3510	17	0.48*	0.56	6848	36	0.53*	0.49
	T	6714	34	0.51**	0.45	6725	32	0.475*	0.53	13439	66	0.49*	0.53

N = number of tested flies

* and ** = significantly different from the control, p> 0.05 and 0.01 , respectively, using χ^2 test.
The relative frequency was calculated as frequency of colchicine induced mutation in pre- or post-treatments with Fennel extract/ frequency of induced mutations in colchicine treatment. Reduction rate = 1- relative frequency.

4. Discussion and Conclusion:

The protection of the adjoining normal tissue as well as non-target organs is one of the major concerns in cancer therapy. Both Mitomycine C and colchicines, well known as cytotoxic agents are used today in some medicinal treatments. Mitomycin C (MMC) is a natural antitumor, antibiotic, and cytotoxic agent used against solid tumors, gastric, pancreatic, oesophageal carcinomas and bladder cancer. The cytotoxicity of MMC acts primarily through the formation of DNA adducts and interstrand cross-links **(Iyer and Szybalski 1964; Paz *et al*., 2004; Lee *et al.*, 2006).** Although MMC is an effective anticancer drug, its clinical use is restricted owing to its toxicity.

MMC induces chromosomal damage during the S phase (Adler 1976). The DNA damage, as well as drug-associated adverse events such as cardiovascular and skin toxicity, may be related to the formation of reactive oxygen species **(Gutiérrez 2000)**. The obtained results showed liver and kidney damage due to exposure to MMC as well as the chemoprotection effect of fennel extract against this damage. This result is in agreement with the results of **Ozbek *et al*., (2003)** who indicated the hepatoprotective activity of *Foeniculum vulgare* essential oil against carbon tetrachloride (CCl4) induced liver injury model in rats with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. Although hot water crude extract of fennel contains little amounts of essential oils, but it contains high amounts of many antioxidants **(Satyanarayana, *et al.*, 2004)** which can give chemo-protections against many chemically induced damage in liver and kidney cells. The our results also indicated that, hot water extract of sweet fennel is an effective hepatoprotective agent, improving the liver and kidney functions and decreasing the damage caused by MMC.

Little is known about antimutagenic activity of fennel extracts, but there were some experimental evidences about the anti carcinogenic activity of fennel extracts.

Clastogenic effect of Mitomycin C is due to its alkylating activity. Accordingly, MMC requires a bioreductive transformation to form active species that crosslink DNA **(Wang *et al*., 2007).** Depending on the biotransformation pathway, metabolism of MMC may generate ROS **(Gustafson and Pritsos, 1992)**. When ROS interact with cells and exceed endogenous antioxidant systems, there is indiscriminate damage to biological macromolecules such as nucleic acids, proteins, and lipids (**Offord *et al*., 2000**). In presence of fennel extract in pre treatment, the clastogenic effect of MMC was effectively reduced, which reflected the capability of this extract to interfere with the mutagenic mechanism of MMC and that may be due to the antioxidant potentiality of fennel as reported by **Roberto *et al.*, (2000)** and **Satyanarayana *et al.*, (2004).** However, fennel extract showed also antimutagenic effect in post treatments which reflected its activity to enhance DNA repair system(s).

The aneuploidy and chromosomal aberrations test in male germ line cells of Drosophila revealed that fennel extract has anti-aneuogenic properties showing the presence of substance able to protect spindle fibers from colchicine action. However this point needs more investigations.

The overall analysis of obtained results

indicated that fennel extract may have slight genotoxic effects on mice rather than Drosophila. In addition, the biochemical, chromosomal aberrations in mice bone marrow as well as aneuploidy and chromosomal aberration test in Drosophila male germ-lines confirmed the antimutagenic effects of fennel extract against MMC and colchicine induced mutations. However, the pre and post treatment analysis revealed that hot water crude extract of fennel may contain some compounds that can act as dis-antimutagen and some compounds can act as bio-antimutagen. The molecular studies using RAPD indicated the effect of fennel extract to induce DNA changes as confirmed by biochemical assays. These results suggest that fennel does not possess a hazard on chromosomal integrity when used as herbal medicine, but still need more investigations on the molecular level to assay its effects on DNA. Further studies should be made to establish the mechanism of action of these compounds in fennel extract with the possibility to formulate potent antimutagenic activity prescriptions.

Acknowledgment

Gratefulness and thanks to ***Dr.Mary Therase Ibrahim Fahmy*** Associate Professor of Genetics,Cell Biology Department, National Research Center for precious advices and valuable help while revising this paper.

References:

1. Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM, Sarrif AM, Stringer DA and Taalman RD. (1998). Aneuploidy: a report of an ECETOC task force. Mutat Res.; 410(1):3-79.
2. Abdul-Ghani, A.S. and Amin, R. (1988). The vascular action of aqueous extracts of Foeniculum vulgare leaves. J Ethnopharmacol. 24(2-3), 213-8.
3. Abraham SK. (2001). Anti-genotoxicity of trans-anethole and eugenol in mice. Food Chem Toxicol.; 39 (5):493-8.
4. Adler ID. (1976). Aberration induction by mitomycin C in early primary spermatocytes of mice. Mutation Res; 35:247-56.
5. Albert-Puleo, M. (1980). Fennel and anise as estrogenic agents. J Ethnopharmacol. 2(4): 337-44.
6. Alexandrovich, I., Rakovitskaya, O., Kolmo, E., Sidorova, T. and Shushunov, S. (2003). The effect of fennel (Foeniculum Vulgare) seed oil emulsion in infantile colic: a randomized, placebocontrolled study. Altern Ther Health Med. 9 (4), 58-61.
7. Al-Harbi MM, Qureshi S, Raza M, Ahmed MM, Giangreco AB and Shah AH. (1995). Influence of anethole treatment on the tumour induced by Ehrlich ascites carcinoma cells in paw of Swiss albino mice. Eur J Cancer Prev.; 4(4):307-18.
8. Annusuya, S., Vanithakumari, G., Megala, N., Devi, K., Malini, T. and Elango, V. (1988). Effect of Foeniculum vulgare seed extracts on cervix, vagina of ovariectomised rats. Indian J Med Res. 87, 364-7.
9. Atienzar F.A., B. Cordi, M.E. Donkin, A.J. Evenden, A.N. Jha, M.H. Depledge, (2000). Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, Palmaria palmata, Aquat. Toxicol. 50 1–12.
10. Atienzar F.A., M. Conradi, A.J. Evenden, A.N. Jha, M.H. Depledge (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a]pyrene, Environ. Toxicol. Chem. 18 2275–2282.
11. Atienzar F.A., V.V. Cheung, A.N. Jha, M.H. Depledge (2001). Fitness parameters and DNA effects are sensitive indicators of copper induced toxicity in Daphnia magna, Toxicol. Sci. 59 241–250.
12. Atienzar F.A., Z. Billinghurst, M.H. Depledge (2002). 4-n-Nonylphenol and 17-beta estradiol may induce common DNA effects in developing barnacle larvae,Environ. Pollut. 120 735–738.
13. Atienzar Franck A. and Awadhesh N. Jha (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: Mutation Research 613: 76–102
14. Awad, A.A.M. (1992). Genetic assays for screening chromosomal aberrations in Drosophila after treatment with medical drugs. Pro. 1st Internat. Conference of Enviromental Mutagcnsis in Human Populations at Risk, Cairo, Egypt, pp. 1480-1490.
15. Bergmeyer HU, Gawehn K, Grassl M (1974) Enzymatic assay of fumarase. In HU Bergmeyer, ed, Methods of Enzymatic Analysis, Vol 1. Academic Press, New York, pp 543–545.
16. Boskabady, M.H., Khatami, A. and Nazari, A. (2004). Possible mechanism(s) for relaxant effects of Foeniculum vulgare on guinea pig tracheal chains. Pharmazie. 59(7): 561-4.
17. Cenkci S, Yildiz M, Ciğerci IH, Konuk M, Bozdağ A. (2009) Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (Phaseolus vulgaris L.) seedlings. Chemosphere, 76(7): 900-906
18. Chakurski, I., Matev, M., Koichev, A., Angelova, I. and Stefanov, G. (1981) Treatment of chronic colitis with an herbal combination of Taraxacum officinale, Hipericum perforatum, Melissa officinaliss, Calendula officinalis and Foeniculum vulgare. Vutr Boles.; 20(6): 51-4.
19. Choi, E.M. and Hwang, J.K. (2004). Antiinflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. Fitoterapia. 75(6), 557-65.
20. Dische, Z. 1955. New color reactions for determination of sugars in polysaccharides. In D. Glick, ed. Methods of biochemical analysis. Vol. 2, pp. 313–358. New York, USA, Interscience Publishers.
21. El Bardai, S., Lyoussi, B., Wibo, M. and Morel, N. (2001). Pharmacological evidence of hypotensive activity of Marrubium vulgare and Foeniculum vulgare in spontaneously hypertensive rat. Clin Exp Hypertens. 23(4), 329-43.

22. El-Rahim WM, Khalil WK, Eshak MG. (2008) Genotoxicity studies on the removal of a direct textile dye by a fungal strain, in vivo, using micronucleus and RAPD-PCR techniques on male rats. J Appl Toxicol.; 28(4):484-90.
23. Enan MR. (2006) Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol Appl Biochem.; 43(Pt 3):147-54.
24. Fahmy KH , A. B. Abd El-Razek and S. Baumgartner (2007). Effect of Spirulina on Chemoprevention of Cancer and Somatic Mutations in Drosophila melanogaster. Egypt J. Genet. Cytol., 36:163-180
25. Fahmy, KH., T. A. Tantawi, and A. A. M. Awad (1997). A genetic assay for detecting of aneuploidy in the female germ- line cells of Drosophilae. Annals Agri. Sci., 42 (2), 513-523.
26. Ferguson LR (1994) Review antimutagens as cancer chemopreventive agents in the diet. Mutat Res 307:395-410
27. Gorelick NJ (1995). Genotoxicity of trans-anethole in vitro. Mutat Res.; 326(2):199-209.
28. Gustafson DL, Pritsos CA (1992). Oxygen radical generation and alkylating ability of mitomycin C bioactivated by xanthine dehydrogenase. Proceedings of the Western Pharmacology Society.;35:147–151.
29. Gutiérrez PL. (2000). The metabolism of quinine-containing alkylating agents: free radical production and measurement. Frontiers in Biosciences.; 5:629–638.
30. Iyer VN and Szybalski W. (1964). Mitomycins and porfiromycin: Chemical mechanisms of activation and cross-linking of DNA. Science; 145:55-8.
31. Kaileh M, Berghe WV, Boone E, Essawi T and Haegeman G. (2007). Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J Ethnopharmacol.; 113(3):510-6
32. Karekar V, Joshi S and Shinde SL (2000). Antimutagenic profile of three antioxidants in the Ames assay and the Drosophila wing spot test. Mutation Research 468:183-184.
33. Keyes SR, Heimbrook DC, Fracasso PM, Rockwell S, Sligar SG and Sartorelli AC. (1985). Chemotherapeutic attack of hypoxic tumor cells by the bioreductive alkylating agent mitomycin C. Adv Enzyme Regul; 23:291-307.
34. Lee Y-J, Park S-J, Ciccone SLM, Kim C-R and Lee S-H. (2006). An in vivo analysis of MMC-induced DNA damage and its repair. Carcinogenesis.; 27(3):446–453.
35. Lim SN, Cheung PCK, Ooi VEC and Ang PO (2002). Evaluation of antioxidative activity of extracts from a brown seaweed, Sargassum siliquastrum. J Agric Food Chem 50:3862-3866.
36. Lippman SM, Benner SE, Hong WK (1994). Cancer Chemoprevention. J Clin Oncol 12, 851-873.
37. Malini, T., Vanithakumari, G., Megala, N., Anusya, S., Devi, K. and Elango, V. (1985). Effect of Foeniculum vulgare Mill. seed extract on the genital organs of male and female rats. Indian J Physiol Pharmacol. 29(1), 21-6.
38. Meis, J.E. and F. Chen (2003). EPICENTRE Forum 10 (2), 8.
39. Mohapatra R, Ramesh A, Jayaraman G, Santhiya ST, Gopinath PM. (2009). Modulatory action of 2-deoxy-D-glucose on mitomycin C-and 4-nitroquinoline-1-oxide-induced genotoxicity in Swiss albino mice In vivo . J Can Res Ther; 5:53-6
40. Noel S and Rath SK (2006). Randomly amplified polymorphic DNA as a tool for genotoxicity: an assessment. Toxicol Ind Health; 22(6):267-75.
41. Offord E, van Poppel G and Tyrrell R (2000). Markers of oxidative damage and antioxidant protection: current status and relevance to disease. Free Radical Research.;33(supplement):S5–S19.
42. Ozbek, H., Ugras, S., Dulger, H., Bayram, I., Tuncer, I., Ozturk, G. and Ozturk, A. (2003). Hepatoprotective effect of Foeniculum vulgare essential oil. Fitoterapia. 74(3), 317-9.
43. Paz MM, Kumar GS, Glover M, Waring MJ and Tomasz M. (2004). Mitomycin dimers: polyfunctional cross-linkers of DNA. Journal of Medicinal Chemistry.; 47(12):3308–3319.
44. Peters,T., (1968). Protein colorimetric method. Clin. chem. 14: 1147-1159.
45. Ph.Eur. 2005: Pharmacopoea Europaea, 5th ed., www.pheur.org Strasbourg
46. Plotsky, Y., Kaiser,M.G., Lamont, S.J., (1995). Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Anim. Genet. 26, 163–170.
47. Pors K and Patterson LH. (2005). DNA mismatch repair deficiency, resistance to cancer chemotherapy and the development of hypersensitive agents. Current Topics in Medicinal Chemistry.; 5(12):1133–1149.
48. Preston J., B.J. Dean, S. Galloway, H. Holden, A. Mc-fee and M. Shelby (1987). Mammalian in vivo cytogenetic assays-Analysis of chromosomal aberrations in bone marrow cells. Mutat. Res. 189), pp. 157–165.
49. Reitman S, Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol;28:568.
50. Roberto, G., Baratta, M.T., Deans, S.G. and Dorman, H.J. (2000). Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Med. 66(8), 687-93.
51. Rogers, B.J., (1998). Environ. Mol. Mutagen. 31 (Suppl. 29): 12.
52. Rong Z and Yin H. (2004). A method for genotoxicity detection using random amplified polymorphism DNA with Danio rerio. Ecotoxicol Environ Saf.; 58(1):96-103.
53. Sarkar A, Basak R, Bishayee A, Basak J, Chatterjee M (1997). Beta-carotene inhibits rat liver chromosomal aberrations and DNA chain break after a single injection of diethylnitrosamine. Br J Cancer, 76 855-861
54. SAS, (1982). SAS User's Guide: Statistics. SAS Institute, Cary, NC.

55. Satyanarayana, S., Sushruta, K., Sarma, G.S., Srinivas, N., Subba Raju, G.V. (2004). Antioxidant activity of the aqueous extracts of spicy food additives:-evaluation and comparison with ascorbic acid in in-vitro systems. J Herb Pharmacother. 4(2): 1-10.
56. Savino, F., Cresi, F., Castagno, E., Silvestro, L. and Oggero, R. (2005). A randomized double-blind placebo-controlled trial of a standardized extract of Matricariae recutita, Foeniculum vulgare and Melissa officinalis (ColiMil) in the treatment of breastfed colicky infants. Phytother Res.; 19(4): 335-40.
57. Savva, D. (1998). Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicol. Environ. Saf. 41, 103–106
58. Schirmeister J. et al.(1964).Dtsch Med.Wsehr 89 :1018 .
59. Schneider W. C. (1957). Determination of nucleic acid in tissues by pentose analysis. In: Methods in Enzymology, Vol. 3 (S. P. Colowick and N. O. Kaplan, ed.). Academic Press, New York, pp. 680-684.
60. Sharma, D., Appa Rao, K.B.C., Totey, S.M., (2000). Measurement of within and between population genetic variability in quails. Br. Poult. Sci. 41, 29–32.
61. Singh B and Kale RK. (2008). Chemomodulatory action of Foeniculum vulgare (Fennel) on skin and forestomach papillomagenesis, enzymes associated with xenobiotic metabolism and antioxidant status in murine model system. Food Chem Toxicol.; 46(12):3842-50.
62. Sripanidkulchai B, Tattawasart U, Laupatarakasem P, Vinitketkumneur U, Sripanidkulchai K, Furihata C, Matsushima T (2002). Antimutagenic and anticarcinogenic effects of Phyllanthus amarus. Phytomed. 9: 26-32.
63. Stashenko, E.E., Puertas, M.A. and Martinez, J.R. (2002). SPME determination of volatile aldehydes for evaluation of in-vitro antioxidant activity. Anal Bioanal Chem. 373(1-2), 70-4.
64. Szybalski W and Iyer VN. (1964). Cross linking of DNA by enzymatically or chemically activated mitomycins and porfiromycin, bifunctionally "alkylating" antibiotics. Federation Proc; 23:946-57.
65. Tayel A. A, Wafaa, A. M. Abdel-Naby, Kh. I. Fahmy and Rehab M. M. Salim (2003). Antimutagenic Activities of Some Medicinal Plant Extracts Using Two Biological Systems Proc 1st Egypt & Syr. Conf. For Agric. & Food. El Minin:Dec. 8-11.2003, Vol 1, No. 1. (301-322)
66. Tice RR, Hayashi M, MacGregor JT, Anderson D, Blakey DH, Holden HE, Kirsch-Volders M, Oleson FB Jr, Pacchierotti F, Preston RJ, Romagna F, Shimada H, Sutou S and Vannier B (1994). Report from the working group on the in vivo mammalian bone marrow chromosomal aberration test. Mutat Res.; 312(3):305-12.
67. Wang S-L, Han J-F, He X-Y, Wang X-R and Hong J-Y (2007). Genetic variation of human cytochrome P450 reductase as a potential biomarker for mitomycin C-induced cytotoxicity. Drug Metabolism and Disposition; 35(1):176–179.
68. Wattemberg LW (1985). Chemoprevention of cancer. Cancer Res 45:1-8.
69. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.
70. Workman P and Stratford IJ. (1993). The experimental development of bioreductive drugs and their role in cancer therapy. Cancer and Metastasis Reviews.; 12(2):73–82
71. Zidorn C, Jöhrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, Greil R, Ellmerer EP and Stuppner H. (2005). Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J Agric Food Chem.; 53(7):2518-23.
72. DeGroot. H. (1988). "A Conversation with George A. Barnard". Statistical Science 3 (2): 196–212.

8/1/2010