

PROTECTIVE EFFECTS OF *MENTHA SPICATA* AQUEOUS EXTRACT AGAINST IFOSFAMIDE INDUCED CHROMOSOMAL ABERRATIONS AND SPERM ABNORMALITIES IN MALE ALBINO MICE

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ABSTRACT

The present study was performed to investigate the protective effects of aqueous extract of *Mentha spicata* against the mutagenic effects of Ifosfamide (IFO) in male albino mice *Mus musculus* of BALB/c strain. In the present study, (20) male mice were divided into (5) groups (each group contained 4 animals), one group was treated daily and orally with phosphate buffer saline (PBS) as a negative control and one group was treated weekly (one time only) and intraperitoneally with IFO (50 mg/kg body weight) as positive control. The other three groups were treated with IFO (50 mg/kg b.w.) intraperitoneally at the beginning of the week for one time and also were treated with three different doses of the plant extract (40, 100, 400 mg/kg b.w.) for one week for testing the protective effect of *Mentha spicata* extract. The results of the study revealed that the *Mentha spicata* extract especially, at dose (400 mg/kg b.w.) exhibited a strong antimutagenic effect against ifosfamide clastogenic action in bone marrow cells and sperm abnormalities.

KEY WORDS: chromosomal aberrations, Ifosfamide, *Mentha spicata*, sperm abnormalities.

INTRODUCTION

The natural products, especially plants, have been used for the treatment of various diseases for thousands of years. Medicinal plants are now becoming more widely used by people all over the world (Shoeb, 2006). The antimutagens have been first reported almost four decades ago, and since then numerous studies have been carried out in order to identify compounds, which might protect humans against DNA-damage and its consequences. Antimutagenic and anticarcinogenic properties of a wide variety of dietary constituents and plant secondary metabolites have been reported (Sangwan, *et al.*, 1998; Shon, *et al.*, 2004).

Considerable emphasis has been laid down on the use of dietary constituents to prevent the mutagen induced mutation and/or chromosomal damage due to their relative non-toxic effects (Wongpa, *et al.*, 2007). The antimutagenic or protective effect has been attributed to many classes of phytochemicals mainly flavonoids and phenolic compounds present in food. However, such compounds have also been reported to exhibit a wide range of other biological activities such as antimicrobial, anti-inflammatory, antiallergic, antioxidant and free radical scavenging (Steinmetz and Potter, 1991; Negi, *et al.*, 2003; Shon, *et al.*, 2004). Antigenotoxic agents especially those present in natural substances act through different cellular pathways involving endogenous sequestration of mutagens by various enzymes (Flora, 1998; Heddle, *et al.*, 1999). Preventing the formation of carcinogens from precursors, blocking the metabolic activation of carcinogens by increasing the activation of detoxification enzymes might inhibit initiation of cancer (Dhuley, *et al.*, 1993).

Mentha spicata L. (spearmint) is commonly produced as a crop for their essential oil for food products, cosmetics and pharmaceuticals. Spearmint also produces rosmarinic acid (RA), a naturally occurring and potent polyphenolic antioxidant, which plays a role in modulating inflammatory diseases including allergies, asthma and atherosclerosis (Fletcher, *et al.*, 2010). Ifosfamide (IFO) is an oxazaphosphorine alkylating agent with a broad spectrum of antineoplastic activity. It is used alone or in combination regimens for the treatment of a variety of haematological malignancies such as lymphomas and multiple myeloma, and solid tumors including sarcoma, ovarian, testicular, cervical, breast, lung cancer and bone tumors (Dechant, *et al.*, 1991; Zhang, *et al.*, 2005; Goto, *et al.*, 2007) and is also used as systemic anticancer therapy in gynecological cancer patients with renal dysfunction (Li, *et al.*, 2007). IFO destroys tumor cells through apoptosis initiated by DNA damage, modulation of cell cycle and other antiproliferative effects. IFO is used concurrently with the uroprotective mesna to avoid hemorrhagic cystitis (Siu, and Moore, 1998). The main aims of the present work are to study the protective effects of *Mentha spicata* aqueous extract against ifosfamide induced mutagenicity in males of laboratory albino mice through studying: (a) chromosomal aberrations in bone marrow cell and (b) sperm abnormalities.

MATERIALS AND METHODS

Laboratory mice

Adult male laboratory mice *Mus musculus* strain BALB/c (8-10 weeks) in age, weighing (30-35) gm, were used.

Ifosfamide

The mutagenic material been used in this study was the ifosfamide (Baxter Oncology GmbH Company).

Antimutagenic herb

The herb which is used in this study was *Mentha spicata* L. (spearmint) that collected freshly from the green house of Biology department / College of Science / University of Salahaddin and identified in Herbarium of College of Science.

Aqueous extraction of plant contents

The plant components (fresh leaves and other edible parts) were extracted using (Ito *et al.*, 1986) method. The gummy extract of the plant was weighted and mixed with known size of phosphate buffer saline (PBS) preparing stock solution that preserved in refrigerator at 4°C and this solution used for preparation of the doses.

Animal treatment

In the present study, (20) male mice were divided into (5) groups (each group contained 4 animals), one group was treated daily and orally with phosphate buffer saline (PBS) as a negative control and one group was treated weekly (one time only) and intraperitoneally with IFO (50 mg/kg b.w.) as positive control. The other three groups were treated with IFO (50 mg/kg b.w.) intraperitoneally at the beginning of the week for one time and also were treated with three different doses of the plant extract (40, 100, 400 mg/kg b.w.) for one week.

Chromosome preparation

Chromosomal preparations from bone marrow cells were done by standard method of (Evans *et al.*, 1964).

Sperm preparation

Sperm was taken from epididymis using method of (Karanowska, 1976) and (Wyrubek and Bruce, 1978).

Statistical analysis

All data are expressed as mean \pm standard error (M \pm SE) and statistical analysis was carried out using statistically available software (SPSS version 17). Comparisons between groups were made using one-way analysis of variance (ANOVA) in combination with Duncan t-test post hoc analysis. Duncan t-test treats one group as a control, and compares all other groups against it. P values <0.01 were considered significant.

RESULTS AND DISCUSSION

1. Protective effect of *Mentha spicata* extract on ifosfamide induced chromosomal aberration in bone marrow cells of male albino mice

Table (1) summarizes the treatment effect of the three doses of *Mentha spicata* extract on ifosfamide induced chromosomal aberrations.

As revealed in the Table (1), a significant increase ($P<0.01$) was found in ifosfamide treated group (positive control) on total abnormal chromosome and most aberrations were studied like (centromeric gap, centromeric break, chromatid gap, chromatid break, ring chromosome, dicentric chromosome and acentric fragment) when compared with negative control (PBS), but there were non-significant effect ($P<0.01$) observed on (pulverization and polyploidy) (Figure 1).

The centromeric gap (5.75 ± 0.250) and ring chromosome (5.5 ± 0.957) have the highest value of aberration type, while the lowest value was polyploidy (0.5 ± 0.288). Treatment with *Mentha spicata* extract showed significant decrease ($P<0.01$) of all three doses of the plant extract on total abnormal metaphase and most other aberrations were studied compared with positive control and there was no significant difference between these groups and negative control in most analyzed parameters. It was clear from the Table (1) that all three doses of plant extract were minimized the effect of ifosfamide with respect to chromosome structure, but the highest protective dose was the third dose (400 mg/kg b.w.) and the lowest effective dose was the first dose (40 mg/kg b.w.).

2. Protective effect of *Mentha spicata* extract on sperm abnormalities induced by ifosfamide in male albino mice

Table (2) showed the treatment effect of the three doses of *Mentha spicata* extract on ifosfamide induced sperm abnormalities. Highly significant increase at ($P<0.01$) was found in ifosfamide treated group (positive control) on total abnormal sperms and all other sperm abnormalities (sperm without head, sperm without tail, sperm without hook, swollen head sperm, defective head sperm, and blunt hook sperm) when compared with negative control (PBS), but there were non-significant effect ($P<0.01$) observed on (double head sperm, double tail sperm and finger head sperm) (Figure 2).

Table 1. Protective effects of *Mentha spicata* extract against ifosfamide induced chromosomal aberrations in male albino mice. (Mean \pm SE) (P<0.01).

Treatments	Total normal Chromo.	Total abnormal Chromo.	Centro gap	Centro break	Chroma. gap	Chroma. Break	Ring Chro.	Di. Chromo.	Pulv.	Acentric Frag.	Pol.
Control (PBS)	95 \pm 0.577 ^d	5 \pm 0.577 ^a	0.75 \pm 0.250 ^a	0.25 \pm 0.250 ^a	1.5 \pm 0.288 ^a	0.25 \pm 0.250 ^a	0 \pm 0.000 ^a	0 \pm 0.000 ^a	0.75 \pm 0.250 ^a	1.25 \pm 0.250 ^a	0.25 \pm 0.250 ^a
IFO 50 mg/kg b.w.	65.75 \pm 1.314 ^a	34.25 \pm 1.314 ^d	5.75 \pm 0.250 ^d	5 \pm 0.408 ^d	5 \pm 0.707 ^c	3.25 \pm 0.478 ^b	5.5 \pm 0.957 ^c	4 \pm 0.912 ^b	2 \pm 0.912 ^a	3.25 \pm 0.629 ^b	0.5 \pm 0.288 ^a
D1 40 mg/kg b.w. 50 mg/kg b.w.	78.5 \pm 1.652 ^b	21.5 \pm 1.652 ^c	3.5 \pm 0.288 ^c	2.75 \pm 0.250 ^c	3.5 \pm 0.288 ^{bc}	2.75 \pm 0.478 ^b	2.25 \pm 0.250 ^b	2.25 \pm 0.478 ^{ab}	1.5 \pm 0.478 ^a	3 \pm 0.408 ^{ab}	0.25 \pm 0.250 ^a
D2 100 mg/kg b.w. 50 mg/kg b.w.	85.25 \pm 0.408 ^c	14.75 \pm 0.408 ^b	2.25 \pm 0.250 ^{bc}	2 \pm 0.000 ^{bc}	2 \pm 0.000 ^{ab}	1 \pm 0.288 ^{ab}	2.5 \pm 0.288 ^b	1.25 \pm 0.478 ^a	1.5 \pm 0.250 ^a	2 \pm 0.288 ^{ab}	0.25 \pm 0.250 ^a
D3 400 mg/kg b.w. 50 mg/kg b.w.	91 \pm 0.250 ^c	9 \pm 0.250 ^b	1.5 \pm 0.000 ^b	1.5 \pm 0.288 ^b	1.75 \pm 0.250 ^a	0.5 \pm 0.288 ^a	0.5 \pm 0.250 ^a	0.25 \pm 0.25 ^a	1.25 \pm 0.250 ^a	1.5 \pm 0.250 ^a	0.25 \pm 0.000 ^a

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

Table 2. Protective effects of *Mentha spicata* extract against ifosfamide induced sperm abnormalities in male albino mice. (Mean \pm SE) (P<0.01).

Treatments	Total normal sperm	Total abnormal sperm	Sperm without head	Sperm without tail	Sperm without hook	Double head sperm	Double tail sperm	Swollen head sperm	Defective head sperm	Finger head sperm	Blunt hook sperm
Control (PBS)	92.25 \pm 0.629 ^d	7.75 \pm 0.629 ^a	2 \pm 0.250 ^a	2.5 \pm 0.288 ^a	0.5 \pm 0.288 ^a	0.25 \pm 0.250 ^a	0.25 \pm 0.250 ^a	0.75 \pm 0.478 ^a	0.25 \pm 0.250 ^a	0.25 \pm 0.250 ^a	0.25 \pm 0.250 ^a
IFO 50 mg/kg b.w.	66.5 \pm 1.554 ^a	33.5 \pm 1.554 ^d	4.75 \pm 0.629 ^b	5 \pm 0.288 ^b	5.25 \pm 0.853 ^c	2 \pm 0.577 ^a	0.75 \pm 0.250 ^a	5.25 \pm 0.853 ^c	5.25 \pm 0.853 ^b	1 \pm 0.408 ^a	3.75 \pm 1.030 ^b
D1 40 mg/kg b.w. 50 mg/kg b.w.	75.5 \pm 2.362 ^b	24.5 \pm 2.362 ^c	4.25 \pm 0.478 ^b	3 \pm 1.000 ^a	4 \pm 1.154 ^{bc}	1.75 \pm 0.853 ^a	0.25 \pm 0.250 ^a	5.25 \pm 0.629 ^c	4 \pm 0.707 ^b	0.75 \pm 0.250 ^a	1.25 \pm 0.750 ^{ab}
D2 100 mg/kg b.w. 50 mg/kg b.w.	82 \pm 1.658 ^c	18 \pm 1.658 ^b	2.25 \pm 0.750 ^a	2.5 \pm 0.957 ^a	3.5 \pm 0.000 ^{bc}	1 \pm 0.408 ^a	0 \pm 0.000 ^a	3.75 \pm 0.250 ^{bc}	3.5 \pm 0.288 ^b	0.5 \pm 0.288 ^a	1 \pm 0.408 ^a
D3 400 mg/kg b.w. 50 mg/kg b.w.	87.75 \pm 0.912 ^d	12.25 \pm 0.912 ^a	2 \pm 0.288 ^a	2.5 \pm 0.816 ^a	1.75 \pm 0.478 ^{ab}	1 \pm 0.408 ^a	0 \pm 0.000 ^a	2.25 \pm 0.478 ^{ab}	2 \pm 0.500 ^{ab}	0.25 \pm 0.000 ^a	0.5 \pm 0.000 ^a

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

The highest value of abnormality type was sperm without hook (5.25 \pm 0.853), swollen head sperm (5.25 \pm 0.853) and defective head sperm (5.25 \pm 0.853), while the lowest value was double tail sperm (0.75 \pm 0.250). Treatment with *Mentha spicata* extract showed significant decrease (P<0.01) of all three doses of the plant extract on total abnormal sperm and all other sperm abnormalities were studied compare with positive control and there was non-significant difference between these groups and negative control in most analyzed parameters. From the Table (2) it is clear that all three doses of the plant extract decreased the effect of ifosfamide on sperm morphology, but the most effective dose was third dose (400 mg/kg b.w.) because there were non-significant difference between this group and negative control in most parameters analyzed, while the lowest effective dose was the first dose (40 mg/kg b.w.).

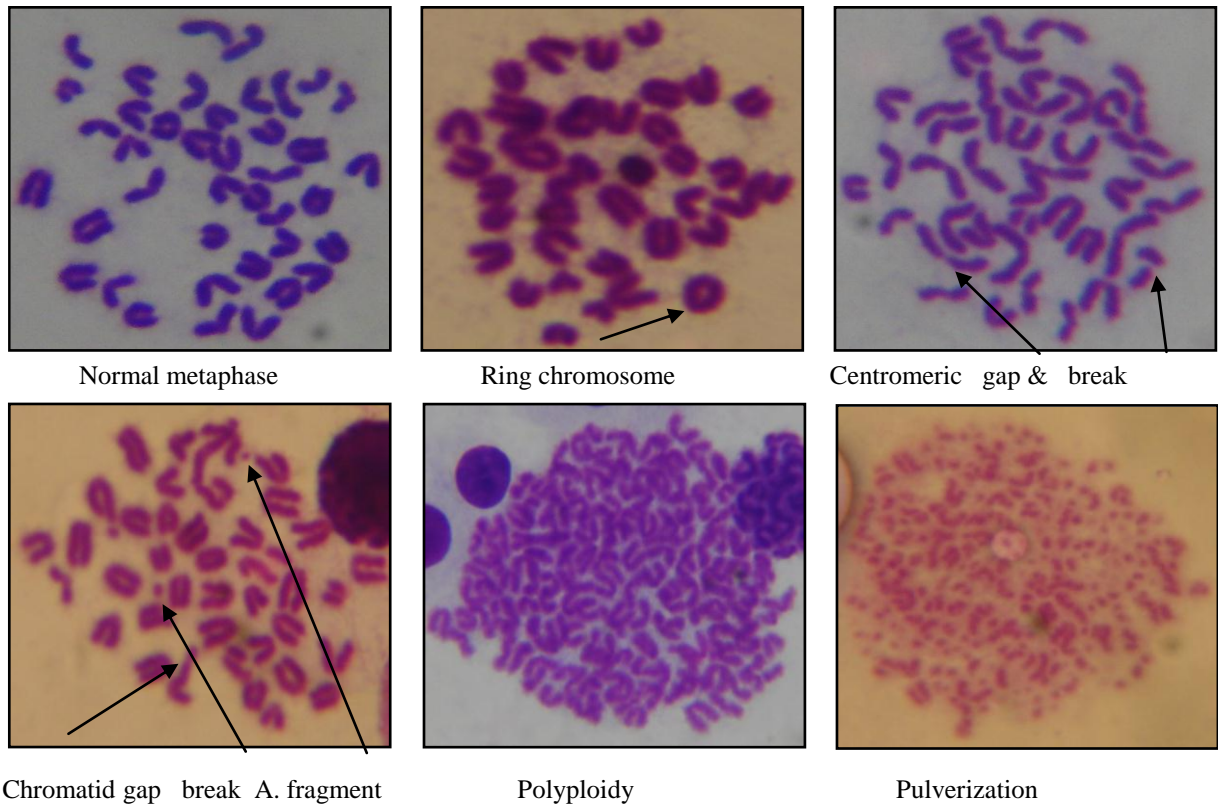


Figure (1): Types of structural and numerical chromosomal aberrations induced by ifosfamide (1000 X).

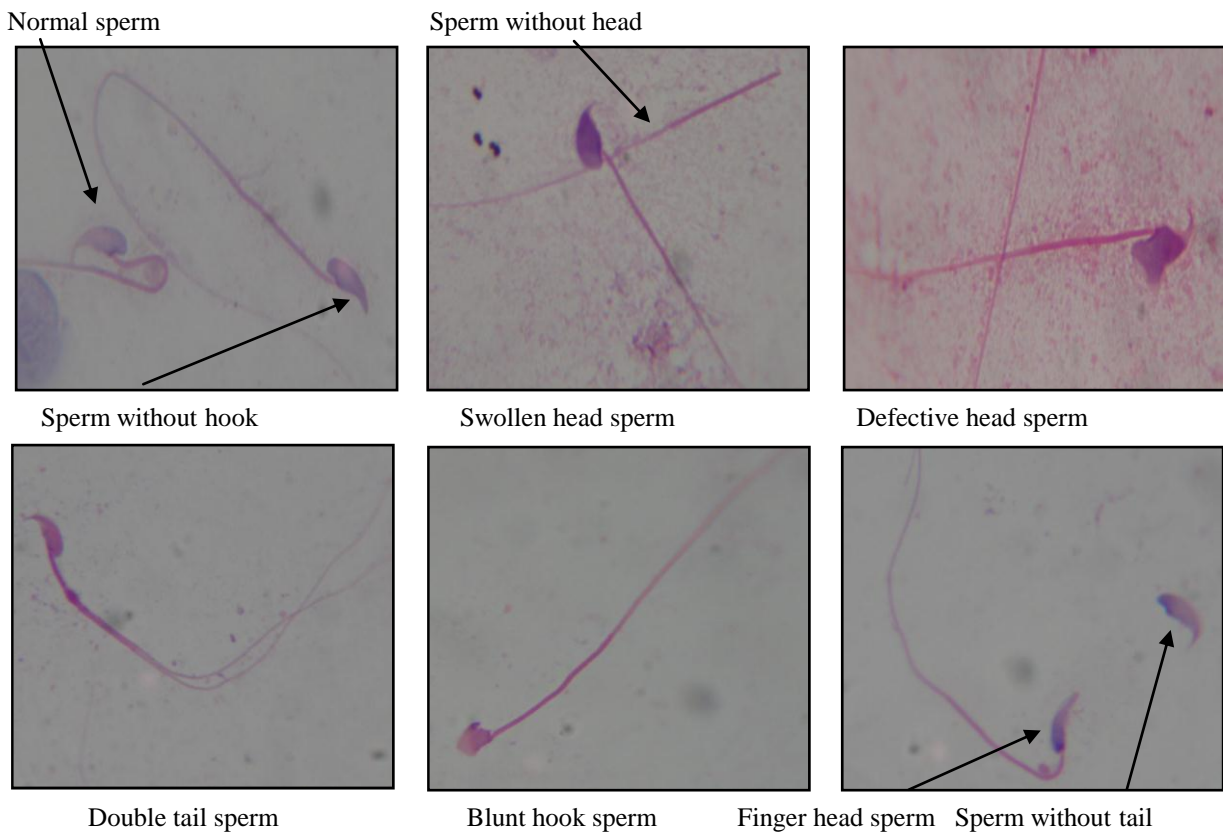


Figure 2. Types of sperm abnormalities of male albino mice induced by ifosfamide (1000 X).

DISCUSSION

1. Protective effect of *Mentha spicata* on chromosomal aberration induced by ifosfamide

Ifosfamide, as all other alkylating agents, destroy tumor cells through apoptosis initiated by DNA damage, modulation of cell cycle and other antiproliferative effects. Thus it can damage DNA during any phase of cell cycle, and therefore, it is not phase specific. The main mechanism is inhibition of DNA replication, as the interlinked strands cannot separate (Zhang, *et al.*, 2005). IFO induced highly significant percentage of structural chromosomal aberrations in mouse bone marrow cells and diakinesis metaphase I cells (spermatocytes) which increased with dose increasing (Donya, *et al.*, 2010).

Although the genotoxicity of ifosfamide has been investigated extensively, it is still valuable to search for agents than can combat its genotoxicity. In the present work ifosfamide used as a positive control to study the clastogenic effect *in vivo* in BALB/c albino male mice bone marrow. The present study showed that ifosfamide significantly increased chromosomal aberration compared with negative control, particularly total aberrant metaphase, centromeric gap, ring chromosome, centromeric break, chromatid gap, dicentric chromosome, acentric fragment and chromatid break, and there was no significant difference in pulverization and polyploidy. The results were similar to findings reported by (Donya, *et al.*, 2010). Frequencies of chromosomal aberrations in animals treated with *Mentha spicata* extract and ifosfamide were decreased significantly compare with positive control. These results may be due to the antagonistic effect of *Mentha spicata* against ifosfamide. Spearmint belongs to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*) (Reverchon, 2006). This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties (Palmer, and Ting, 1995; Bimakr, *et al.*, 2010). Compounds with antioxidant activities could presumably modulate genotoxicity induced by toxic materials (Povirk, 1996).

In the present study, Table (1) represented that the animals treated with *Mentha spicata* extract and ifosfamide at the same time in all three doses (40, 100, 400) mg/kg b.w. of the plant extract formed significant decrease in the rate of total abnormal chromosome and most other aberrations like (centromeric gap, centromeric break, chromatid gap, chromatid break, ring chromosome, dicentric chromosome and acentric fragment) when compared with positive control (IFO), only in the first dose (40 mg/kg b.w.) there was non-significant decrease on chromatid gap, chromatid break, dicentric chromosome and acentric fragment. These results may be due to the bioantimutagenic activity of *Mentha spicata* extract. The inhibitors which become active with or beyond administration of toxic materials are known as biotic inhibitors. These inhibitors use to repair the injuries through three main ways which are: (1) increasing the accuracy in DNA replication and (2) increasing the efficiency of repairing systems by minimizing repairing errors in addition to (3) enforcing the immune system. The most protective dose of the plant extract was the third dose (400 mg/kg b.w.) that showed higher reduction of total abnormal chromosome. The possible explanation might be due to the presence of chemicals in higher concentrations than other doses that neutralize the genotoxic effect of ifosfamide. Generally, results of the present study indicated that antimutagenic activity of *Mentha spicata* extract is dose dependent. Our findings are in agreements of other workers who have reported concentration dependent antimutagenic activity in other plants (Kaur, *et al.*, 2000; Kaur, *et al.*, 2002).

2. Protective effect of *Mentha spicata* extract on sperm abnormalities induced by ifosfamide

A sperm is a package of streamlined genetic information; intuitively one might expect that a change in genetic content be reflected by a change in the size or shape of sperm (Sun, *et al.*, 2006). The results of positive control as shown in Table (2), ifosfamide was induced sperm abnormalities significantly compared with untreated group (negative control), particularly total abnormal sperm, defective head sperm, sperm without hook, sperm without head, sperm without tail, swollen head sperm and blunt hook sperm. The results were similar to findings reported by (Donya, *et al.*, 2010). Wyrobek and Bruce, (1975) suggested that the abnormalities in sperm morphology are a consequences of chromosomal aberrations or may be due to genetic changes in the genes responsible for spermatogenesis.

Treatment with *Mentha spicata* extract showed significant effects of all three doses of the plant extract on the total numbers of abnormal sperm and all other sperm abnormalities were studied compare with positive control and it was non significance between these groups and negative control in most analyzed parameters. The mechanism of protection action of the *Mentha spicata* extract is not completely known, however, it may scavenge free radicals or inhibiting DNA strand breaks or may enhance DNA repair mechanism, there is obvious that ifosfamide produce DNA strand breaks (Siu and Moore, 1998). According to the study conducted by (Al-Rubaie, 2000), *Mentha spicata* extract contains flavonoids, glycosides, phenols, tannins, saponins and resins that enables the plant to act as a strong antioxidant activity and free radical scavenger. Among the natural compounds, phenolic compounds constitute one of the major groups of herbal compounds acting as radical scavengers and antioxidants (Lee, *et al.*, 2003a; Capecka, *et al.*, 2005). Two factors protect the sperm DNA from oxidative insult: the characteristic tight packaging of the DNA; and the antioxidants present in seminal plasma (Twigg, *et al.*, 1998b). However, oxidative stress (OS) may develop as a

result of an imbalance between ROS generation and antioxidant scavenging activities (Sikka, 2001). In general, DNA bases and phosphodiester backbones are very susceptible to peroxidation. In addition, spermatozoa are particularly susceptible to OS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of scavenging enzymes (Sharma and Agarwal, 1996). Furthermore, studies in which the sperm was exposed to artificially produced ROS resulted in a significant increase in DNA damage in the form of modification of all bases, production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal rearrangements (Twigg, *et al.*, 1998b; Duru, *et al.*, 2000).

The present work revealed a clear significant reduction against ifosfamide induced sperm abnormalities including total abnormal sperm and all other kinds of sperm abnormalities when *Mentha spicata* extract given in combination with ifosfamide compared with positive control. The best protective dose of the plant extract was the highest dose (400 mg/kg b.w.) which showed higher reduction of total abnormal sperm. The possible explanation might be due to the presence of chemicals in higher concentrations than other doses that neutralize the genotoxic effect of ifosfamide. Generally, information on herb drug interactions is still limited; from the results, we conclude that the *Mentha spicata* extract has antimutagenic action against mutagens. This phenomenon may explain the medical properties of *Mentha spicata*, well recognized already by traditional medicine.

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