The Egyptian Journal of Hospital Medicine Vol., 22 : 60 - 72 March 2006
I.S.S.N: 12084
1687 - 2002

Antioxidant effect of parsley and *panax ginseng* extract standardized with ginsenosides Rg3 against alteration induced in reproductive functions in male mice

Aziza M. Hassan[1] and Mosaad A. Abdel-Wahhab[2]
[1]Cell Biology Dept. and [2]Food Toxicology & Contaminants Dept.
National Research Center, Dokki, Cairo, Egypt

Abstract

In the present study, the antigcidant effects of parsley oil and *panax ginseng* have been evaluated against the clastogenecity of ZEN. One hundred and eight mature male mice were distributed into nine treatment groups, including the control group and the groups treated with parsley oil (0.6 ml/kg b.w), *panax ginseng* extract (40 mg/kg b.w) or parsley oil plus *panax ginseng* extract with or without ZEN (10 µg/kg b.w). Animals within different treatment groups were divided into two subgroups (A and B). Subgroup A were used for the determination of serum testosterone levels and chromosomal aberrations and received their respective doses for two weeks whereas, subgroup B were used for sperm abnormality and received their respective doses twice a day for one week and sacrificed after 30 days. The results indicated that ZEN treatment resulted in a significant decrease in testosterone concentration, sperm count and sperm motility. Whereas it caused a significant increase in abnormal sperms counts and total chromosomal aberrations in germ cells. Animals treated with parsley oil or *panax ginseng* extract alone or in combination were comparable to the controls regarding all the tested parameters. The combined treatment with ZEN and parsley oil, *panax ginseng* or parsley oil plus *panax ginseng* extract resulted in a significant improvement in all tested parameters. Moreover, parsley oil was found to be effective than *panax ginseng* extract and the combined treatment was more effective than the single treatment. It could be concluded that both parsley oil and *panax ginseng* extract induced a protective action against ZEN-induced alteration in the reproductive performance and the combined treatment may be useful than the single treatment.

Key words: Zearalenone, parsley oil, panax ginseng, cytogenetic, germ cells, testis, testosterone and chromosomal aberration.

Introduction

Herbal remedies are used world wide to alleviate symptoms, to treat illnesses, and to promote overall wellness. An estimated 60% of the world's population and 40% of Americans use herbal remedies (Astin, 1998). However, use of medicinal herbs may be unsupervised and unproved efficacy (Boniel and Dannon 2001; Ernst and Cassileth 1999).

Parsley (*Petroselinum crispum)* is an important culinary herb native to the Mediterranean area. It has been cultivated for more than 2,000 years. Parsley is a member of the Umbelliferae family that has been employed in the food, pharmaceutical, perfume, and cosmetics industries (Lopez *et al.*, 1999). It is widely distributed in Egypt and grown in gardens and fields. Parsley has been reported to have a number of possible medicinal attributes including, antimicrobial, (Wong and Kitts 2006) antianemic, menorrhagic, (Baytop, 1984) anticoagulant, antihyperlipidemic, antihep-atotoxic, (Ozturk *et al.,* 1991) antioxidant,

(Nielsen *et al.,* 1999) and laxative (Kreydiyyeh *et al.,* 2001). It has been used to treat lumbago, as a blood pressure regulator, to treat eczema, knee, ache, impotence and nose bleed (Manderfeld *et al.,* 1997). Parsley seed are also used as a diuretic and the hypoglycemic activity of parsley has been shown by Ozsoy *et al.,* (2006). The constituents of parsley which include ascorbic acid, carotenoids, flavonoids, coumarins, apiole, various terpenoic compounds, phenyl propanoids, phthalides, furano coumarins, and tocopherol, have been chemically investigated (Tunali *et al.,* 1999). Components of fresh parsley leaf scavenge superoxide anion in vitro (Campanella *et al.,* 2003), and the methanol extracts of parsley scavenge hydroxyl radical in addition to protecting against ascorbic acid induced membrane oxidation (Fejes *et al.,* 2000). Supplementation of diets with fresh parsley leaf can increase antioxidant capacity of rat plasma (Hempel *et al.,* 1999).

Ginseng is one of the most popular medicinal plants, the roots of which have long been traditionally used for strengthening immunity, providing nutrition and recovering health from fatigue (Blumenthal, 2000). The pharmaceutical activities of ginseng roots have been proven recently by many investigators, and ginseng has become the famous medicinal plant all over the world. Ginseng roots contain various pharmaceutical components ginsenosides (saponins), polyacetylenes, polyphenolic compounds and acidic polysaccharides, and among the components, ginsenosides are the most pharmaceutically active (Kim *et al.,* 2005). Until now, 38 ginsenosides have been isolated from ginseng roots, with five major ginsenosides (ginsenosides Rb1, Rb2, Rc, Re and Rg1) constituting more than 80% of the total ginsenosides (Kim *et al*, 1999). Moreover, ginseng intake was associated with a decreased risk for most cancers including carcinomas of the esophagus, stomach, colon, pancreas, lung and liver (Matsuda, 1986; Jeong *et al,.* 1997; Dayan and Paine 2001). In the recent years, many studies have aimed to convert major ginsenosides to the more active minor ginsenosides using heating, acid treatment, enzymatic and microbial conversion (Kim *et al.,* 2005). The minor ginsenoside, Rd, has been known to enhance the differentiation of neural stem cells, while other ginsenosides induce no differentiation of neurons (Shi *et al.,* 2005) and are known to protect neural systems against neurotoxicity by attenuating NO overproduction (Choi *et al.,* 2003, Mannaa *et al.,* 2006). The pharmaceutical property of ginseng roots in protecting neurons from neurotoxic chemicals, such as kainic acid, is attributed mostly to ginsenoside Rd (Lee *et al.,* 2003). Ginsenoside Rd has been known to protect kidney from apoptosis and DNA fragmentation caused by chemical drugs and cancer drugs (Yokozawa and Owada, 1999; Yokozawa and Dong, 2001, Mannaa *et al.,* 2006).

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin present in corn, as well as food mixture for farm animals (Hagler *et al.,* 1984; Molto *et al.,* 1997; Placinta *et al.,* 1999). Studies in various species (rodents, pigs and monkeys) have shown that ZEN and its metabolites have estrogenic and anabolic activities (Etienne and Dourmad 1994) and it has cytotoxic effects to mammalian cell cultures (Cetin and Bullerman, 2005). ZEN was associated with hyperestrogenism and several physiological alterations of the reproductive tract in several laboratory animals (mice, rat, guinea-pig, hamster, rabbits) (Creppy, 2002). ZEN ingestion through contaminated feed is associated with decreased reproductive capacity and other hyperestrogenic conditions, such as vaginal swelling, enlargement of mammary glands, and testicular atrophy in farm animals (Wannemacher *et al.,* 2000). The strong estrogenic effect of ZEN is due to its competition with 17 B-estradiol in the binding to cytosolic estrogen receptors present in the uterus, mammary gland, hypothalamus and pituitary gland (Kuiper-Goodman *et al.,* 1987; Creppy, 2002). Several toxic effects of ZEN were demonstrated, it induces apoptosis, DNA fragmentation (Kim *et al.,* 2003; Abid-Essafi *et al.,* 2003), micronuclei production (Ouanes *et al.,* 2003), chromos-ome aberration (IARC, 1993; Ouanes *et al.,* 2005) and DNA-adduct formation (Pfohl-

Leszkowicz *et al.,* 1995). These toxic effects are unlikely to be due to the estrogenic activity of ZEN. Several processes are known to play a role in the molecular events leading to cell damage. The carcinogenicity of ZEN is still questionable since it is classified by IARC in group 3, i.e. not classifiable as to its carcinogenicity to humans (IARC, 1993; Yosida and Amano, 1965).

The objectives of the current study were to evaluate the potential of panax ginseng extract and parsley oil against the cytotoxic effects and the reproductive disorders induced by ZEN in male mice.

Materials And Methods

Chemicals: ZEN standards was purchased form Sigma Chemical Co. (St. Louis, MO). Testosterone Kit was purchased from ELISA (Enzyme Linked Immuno System Assay)., Italy. All other chemical were of the highest purity commercial available.

Parsley oil were purchased from El-Captain Company (CAP PHARM), 6th October, Egypt.

Panax ginseng: The standardized *Panax ginseng* extract EFLA400 (Phoenix ginseng) (Batch No. 303298) of Panax ginseng C. A. Mayer was prepared according to the published procedure (Korean patent 0425022, PCT/KR2003/000003) and was supplied from Lotte Group R & D Centre (Seoul, Korea). The content of ginsenoside Rg3, a pharmacologically active ingredient of Phoenix ginseng, was 3.6% (w/w) as determined by HPLC.

Animals: A total of 108 adult male Swiss albino mice (eight-week old, weighing 25 ± 2 gm) purchased from Animal House Colony, NRC Dokki, Giza, Egypt. Animals were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum.* After an acclimation period of 1 week, animals were divided into nine groups (12 mice/ group) and housed in filter-top polycarbonate cages (six animals/cage) the animals were maintained in an environmentally standard controlled.

Experimental design: Animals within different treatment groups were treated as follow: group 1 untreated control; group 2, treated orally with corn oil; group 3, treated orally with Korean panax ginseng extract (40 mg/kg b.w) suspended in water; group 4, treated orally with parsley oil (0.6 ml/kg b.w); group 5, treated orally with panax ginseng extract plus parsley oil; group 6, treated orally with ZEN (10 µg/kg b.w) in corn oil; group 7, treated orally with ZEN plus panax ginseng extract; group 8, treated orally with ZEN plus parsley oil; and group 9, treated orally with ZEN plus panax ginseng extract and parsley oil.

All groups were divided into two subgroups A and B. Animals in subgroup A (within all treatment groups) were used for the determination of blood serum testosterone level and chromosomes analysis of germ cells and treated with the respective treatment for 15 days. On day 16 blood samples were collected for testosterone determination then animals were sacrificed by cervical dislocation and testes were removed for chromosomes analysis of germ cells. Whereas animals in subgroup B were used for the determination of sperm abnormality and treated twice/day with their respective doses for one week and sacrificed after 30 days and epididymis were removed for the sperm abnormality study.

Chromosome analysis of germ cells was carried out according to the method described by Evans *et al.,* (1964) and modified by Russo (2000). For testosterone determination, blood samples were collected from all animals in subgroup A from retro-orbital venous plexus (Schermer, 1967) then left to clot, centrifuged at 5000 rpm under cooling for 10 min and serum was separated for testosterone determination by enzyme linked immunoassay procedure as described by Parker (1981).

The technique of Wyrobek *et al.,* (1984) was adopted for sperm abnormality study with minor modifications. Epididymis (free of fats, vas deferens and other tissues) from each side of testis of either control or treated mice was dissected out and the inner content squeezed out into 10 ml of 0.87 % normal wormed saline separately. The content was thoroughly shaken, filtered

through a silken cloth and dropped on grease-free clean slides to determine the movement and swimming ability of sperm (motility) using microscope. Spermatozoa were counted using heamocytometer and a drop of a heamogenate smeared on a cleaned slide, allowed to air dry and stained by Eosin Yellow to determine the head and tail abnormality of sperms.

Statistical Analysis: Students't-test was conducted as per standard procedure for determining the significance level of the differences between treated and control data according to Parker, (1979).

Results

The results of the cytogenetic study (Table 1) revealed that treatment with ZEN resulted in a significant increase in frequency of aberrations in spermatocytes (19.0 ± 1.0) whereas, animals treated with either parsley or ginseng extract were nearly as the control groups. Cotreatment with parsley or ginseng extract plus ZEN showed a significant decrease ($p<0.01$) in the aberrations resulted from ZEN (11.1 ± 1.2 and 9.8 ± 0.1 respectively). Although a marked inhibition was observed in the group received ZEN and treated with ginseng plus parsley (8.2 ± 0.7), the total aberrations was still significantly higher than the control groups (Fig. 1).

The present study showed that serum testosterone levels were significantly decrease in male mice treated with ZEN compared to the control groups (Fig.2). Treatment with parsley alone or parsley plus ginseng extract caused a significant increase in the testosterone concentration compared to those of the control groups. Whereas, insignificant increase was found in animals with Phoenix ginseng alone compared to the control groups. On the other hand, the combined treatment of ZEN plus phoenix ginseng, parsley or phoenix ginseng plus parsley resulted in an amelioration of the testosterone level. It is of interest to mention that the recovery was obvious in the group treated with phoenix ginseng plus parsley than in the ZEN plus either phoenix ginseng or parsley oil.

The motility of cauda epididymal sperm of group treated with ZEN showed a significant decrease ($P<0.001$) compared to those of the control groups. The combined treatment with phoenix ginseng plus ZEN resulted in a significant improvement in the sperm motility compared with the ZEN alone group although the values were still lower than the control groups. On the other hand, animals treated with ZEN plus parsley oil alone or ZEN plus parsley oil and phoenix ginseng showed a complete recovery in the sperm motility and were comparable to the control groups (Table 2).

The sperm count in ZEN-treated group showed a significant decline compared to those of the control groups. Whereas, in animals treated with ZEN plus phoenix ginseng, ZEN plus parsley oil or ZEN plus phoenix ginseng and parsley showed a significant recovery ($p<0.01$) compared to the ZEN alone treated group and the improvement was pronounced in the later two groups.

The current results clearly indicated that treatment with either parsley or phoenix ginseng alone or in combination had no influence to induced sperm abnormalities (Fig.3). Animals treated with ZEN alone showed a significantly increase (29.5%) in the frequency of abnormal sperm compared to the control groups. Treatment with both parsley oil and phoenix ginseng alone or in combination to ZEN-treated mice considerably reduced sperm abnormality frequencies. The maximum efficacy being found in the animals received the combined treatment plus ZEN (8.2 %). In spite of this reduction, the percentage of sperm abnormality was still significantly higher than the control group. Various morphological sperm abnormalities in head and tail were recorded in both control and treated mice (Table3).

Table (1): The main structure aberration in spermatocytes of mice in different treatments (mean ± SE)

Treatment	X-Y univalent	Autosomal break	Chain	Total aberration
Control	0.4 ± 0.3	1.6 ± 0.5	0	2.0 ± 0.8
Corn oil	0.3 ± 0.1	0.9 ± 0.4	0	1.2 ± 0.6
Ginseng	0.9 ± 0.5	2.0 ± 0.5	0.3 ± 0.1	3.2 ± 0.48
Parsley	0.5 ± 0.1	1.8 ± 0.3	1.1 ± 0.25	3.4 ± 0.15
Ginseng+ Parsley	1.4 ± 0.5	1.4 ± 0.6	1.0 ± 0.38	3.8 ± 1.1
ZEN	5.6 ± 1.5	7.6 ± 0.5	5.8 ± 1.5	19.0 ± 1.0**
ZEN + Ginseng	3.2 ± 0.8	0.8 ± 0.3	3.1 ± 0.53	11.1 ± 1.2*
ZEN + Parsley	3.3 ± 1.5	4.0 ± 0.45	2.5 ± 0.6	9.8 ± 0.1*
ZEN + Ginseng+parsley	2.5 ± 0.45	3.6 ± 0.71	2.1 ± 0.54	8.2 ± 0.7*

Table (2). Cauda epididymal sperm count, motility and abnormality percentage.

Groups	Sperm Count X10^6	Sperm Motility (%)	Sperm Abnormality (%)
Control	35.63 ± 0.45	70.28 ± 0.58	1.4
Corn oil	33.2 ± 0.53	75.19 ± 0.43	2.0
Ginseng	38.0 ± 0.59	80.11 ± 0.48	1.9
Parsley	42.3 ± 0.45*	86.88 ± 0.6	2.3
Ginseng + Parsley	43.0 ± 0.81*	88.64 ± 0.42*	2.9
ZEN	20.52 ± 0.45*	42.46 ± 0.91**	29.5**
ZEN + Ginseng	30.45 ± 0.48*	60.94 ± 0.73*	15.3*
ZEN + Parsley	38.25 ± 0.81*	68.63 ± 0.54*	12.0*
ZEN + Ginseng + parsley	43.34 ± 0.74**	75.74 ± 0.75**	8.2**

*P<0.01, ** P<0.001

Table (3). Effect of different treatments on types of sperm abnormality in mice (means ± SE)

Groups	Tail abnormality	Head abnormality			Total abnormality
		Without hock	Banana	Amorphous	
Control	1.0 ± 0.55	2.3 ± 0.38	2.0 ± 0.6	3.1 ± 0.35	8.4 ± 0.45
Corn oil	2.3 ± 0.38	3.0 ± 0.2	1.3 ± 0.54	5.4 ± 0.45	12.0 ± 0.3
Ginseng	1.3 ± 0.45	3.0 ± 0.15	2.5 ± 0.38	4.6 ± 0.50	11.4 ± 0.72
Parsley	2.1 ± 0.5	2.4 ± 0.45	3.4 ± 0.45	5.9 ± 0.38	13.8 ± 0.95
Ginseng + Parsley	3.0 ± 0.48	5.1 ± 0.63	4.3 ± 0.82	5.0 ± 0.61	17.4 ± 0.37
ZEN	29.2 ± 1.5	52.4 ± 1.75	40.5 ± 2.5	54.9 ± 1.48	177.0 ± 3.8**
ZEN + Ginseng	16.0 ± 0.9	21.5 ± 0.92	24.3 ± 1.8	30.0 ± 0.85	91.8 ± 1.5*
ZEN + Parsley	12.0 ± 0.75	17.0 ± 0.78	19.5 ± 1.0	23.5 ± 0.75	72.0 ± 0.57**
ZEN + Ginseng + parsley	10.5 ± 0.45	10.0 ± 1.1	9.5 ± 0.75	19.2 ± 0.54	49.2 ± 0.45**

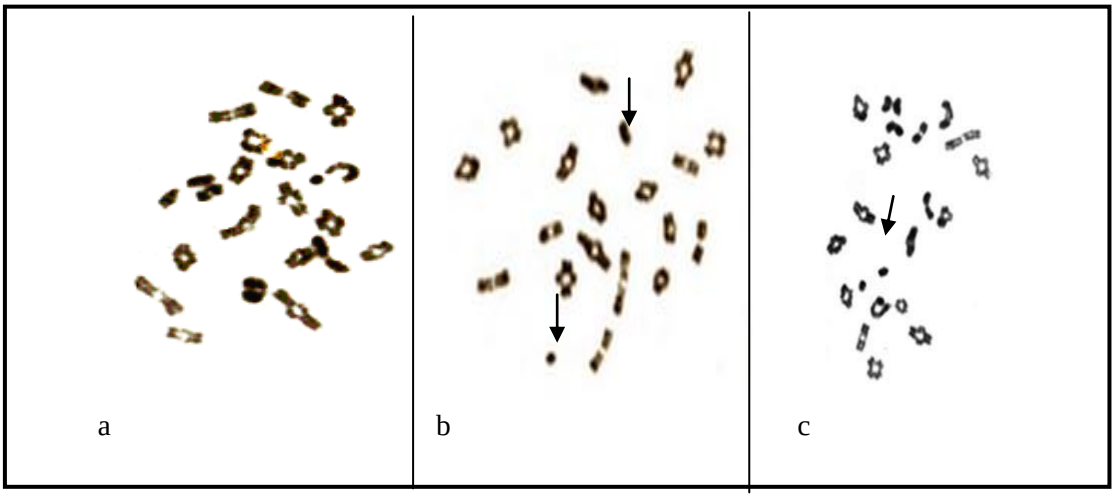

Fig. (1): Spread spermatocytes metaphases showing a- Normal metaphase, b- X-Y univalent, and c- autosomal break

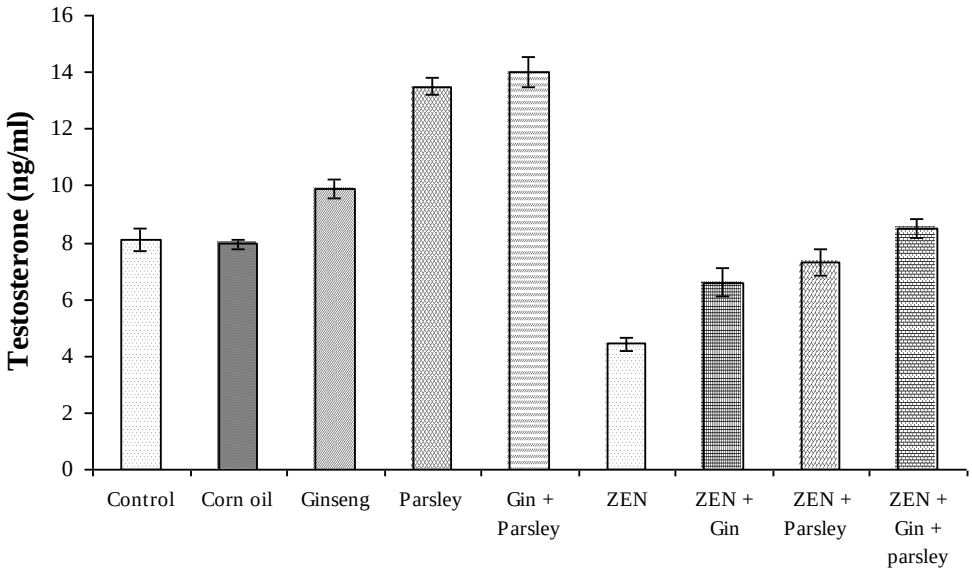

Fig. (2). Concentration of testosterone in serum of mice treated with ZEN with or without phoenix ginseng, parsley oil or phoenix ginseng plus parsley

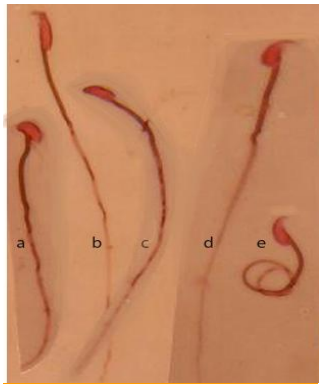

Fig. (3): Showing different sperm shapes abnormality: (a) amorphous head, (b) banana head, (c) head without hock, (d) normal sperm, and (e) sperm with coiled tail.

Discussion

The present investigation was carried out to explore the possible ameliorative role of phoenix ginseng and parsley herbs on ZEN-induced genotoxic effects of germ cells chromosomes, sperm abnormality as well as testosterone level in male mice. The selection of dose of ZEN and parsley oil was literature based (Ouanes *et al.,* 2005 and Nielsen *et al.*, 1999 respectively), and the selection of dose of phoenix ginseng was based on our previous work (Mannaa *et al.,* 2006). Different herbal plants were potentially offer distinct qualities of materials used for cuisine, food preservation and herbal medicine. Numerous studies have suggested that phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to their antioxidant and antibacterial activities (Kaur and Kapoor, 2002). Flavonoids, the major group of total phenolic compounds, are found in greater concentration in parsley and ginseng (Justesen and Knuthsen 2001).

ZEN is a fusariotoxin occurring worldwide in cereals, animal food and forages (Placinta *et al.,* 1999) that adversely affects reproduction (Creppy, 2002) thus, ZEN was first known to be a potent disrupter of the reproductive system. It is also associated with several human diseases of unknown etiology (Placinta *et al.*, 1999). Many studies have been carried out in order to show whether or not ZEN induced clastogenic effects. The induction of chromosome aberrations by ZEN was demonstrated for the first time by Tennant *et al.* (1987). Recently, Lioi *et a*l., (2004) demonstrated that ZEN induced a significant increase in the aberration frequency in bovine lymphocyte cultures. In addition, very convincing data do exist that show a capability of ZEN to induce DNA adducts in mice (Pfohl-Leszkowicz *et al.*, 1995), DNA fragmentation in cell cultures (Abid-Essefi *et al.,* 2003), micronucleus formation and apoptosis both in vivo and in vitro (Ouanes *et al.,* 2003). According to Ouanes *et al.,* (2005) the chromosome aberrations induced by ZEN could be attributed to the parent compound itself as well as to its metabolities, especially α-zearalenol and α-ZENalanol, which both have demonstrated mutagenic potency (Scheutwinkel *et al.,* 1986; Metzler and Pffeifer, 2001). These effects can be also attributed to active oxygen species generated by ZEN (Abid-Essefi *et al.*, 2004).

The findings of the current study revealed that treatment with ZEN resulted in a significant decrease in testosterone concentration. This effect suggests that ZEN had harmful effects on the endocrine system as well as the stressful of testicular cells. In this regards, Whitehead and Lacey (2003) reported that ZEN increased the enzymes that convert androgens to estrogens. Moreover, ZEN was found to decrease the size and weight of testis and resulted in the decrease of testosterone secretion by the cells (Gajecki, 2002; Kim *et al.,* 2003) and the inhibition of other endocrine glands mainly the pituitary gland (Thomas *et al.,* 2000). In the current study, treatment with ZEN resulted in a significant increase in the frequency of abnormal sperm and meiotic germ cells. The positive correlation between cytogenetic damage in germ cells and sperm abnormalities was previously reported in man and in mice (Kim *et al.,* 2003). Precocious separation of X-Y chromosome has been discussed as a mechanism of male sterility (Ford, 1969) however; the effect of ZEN on germ cells may resulted in the reduction of fertility. El-Makawy *et al.* (2001) reported that ZEN was shown to increase chromosome breaks in mouse spermatocytes at a dose of 10µg/kg and this results confirmed our present data. Moreover, ZEN has been reported to disturb spermatogenesis and decrease fertility in male rats fed naturally contaminated corn (IARC, 1993).

The present results revealed that ZEN induces severe stress on the testis and on the endocrine function in mice including the testis itself and indirectly on the pituitary gland. The treatment with either parsley or phoenix ginseng alone or in combination resulted in significant improvements in all the tested parameters meanwhile, the combined treatment was more effective than

the individual treatment.. Parsley or/and ginseng alone or in combination caused a significant decrease in total chromosomal aberrations, sperm abnormalities and increase in testosterone level and sperm counts and motility. The antioxidant activity of parsley has been reported previously. Zheng *et al.,* (1992) reported that parsley oil is rich in myristicin which showed a high activity as an inducer of the detoxifying enzyme GST in the liver and small intestinal mucosa of female mice. Reduction of myristicin yielded dihydromyristicin that retained the GST-inducing activity. Fejes *et al.* (1998) indicated that parsley oil contain flavonoids (apiin, luteolin-, apigenin-glycosides), essential oil (apiol, miriszticin), cumarines, (bergapten, imperatorin) and vitamin C. The protective role of parsley oil may be attributed to its higher content of these flavonoids which either scavenge free radicals or increase the production of GST. Ozsoy-Sacan *et al.* (2006) concluded that, parsley extract probably, due to its antioxidant property, has a protective effect against hepatotoxicity caused by diabetes and have free radical scavenging and membrane protective effects (Fejes et al., 2000). In the same regards, Nielsen *et al.,* (1999) indicated that treatment with parsley oil resulted in increased levels of glutathione reductase and SOD activity. The protective effects of falvonoids may occur through inhibitory effect on CYP1A1/2 among CYP enzymes involved in ZEN metabolism by rat microsomes as well as the decreased DNA damage and activating the phase II enzymes glutathione S-transferase (GST) and GSH peroxidase (GSH-Px). These results suggest that parsley oil is capable of counteracting ZEN toxicity by suppressing cytochromes P-450 mediated bioactivation of the mycotoxin (Abdel-Wahhab and Aly, 2003, 2005). The increased level of testosterone reported in the current study accompanied with the increase of sperm counts is supported by the previous reports of (Hileman and Jackson 1999; McDonald and Capen, 1989) who stated that the testosterone hormone promotes the growth development and secretory activity of the accessory sex organs of the male.

The current study revealed that phoenix ginseng exhibits a protective role against ZEN-induced disturbance in testicular function. Kumar *et al.,* (2003) and Kang *et al.,* (2002) observed that ginseng extract provides the protection of the testis by reflecting a significant decrease in testicular acid phosphatase activity, LPO level and significant increase in alkaline phosphatase activity. It has been reported that ginseng extract contains active components which are well known to suppress lipid peroxidation and reduce the cellular damage. Moreover, Kim *et al.,* (1993) reported that water fraction and alkaloid fraction of ginseng may reduce cell damage, especially the damage to DNA molecules, caused by gamma rays and thus playing a role in the repair of regeneration process of damaged cells. They also concluded that it is possible that ginseng reduces DNA damage by antiradical action. Thus the present study suggests that ZEN toxicity may be reduced through the inhibition of lipid peroxidation by phoenix ginseng extract, which in turn may reflected by a decline of testicular acid phosphatase and LPO level and elevated alkaline phosphatase activities. Generally, the protective and therapeutic effects of Phoenix ginseng on atrophy and testicular damage induced by ZEN, providing evidence that ginseng might be a useful agent in preventing and treating testicular damage induced by environmental pollutants (Kim *et al.*, 1999).

Our results indicated that phoenix ginseng improved the sperm count and sperm motility. In this regards, Hwang *et al.,* (2004) reported that ginseng improves the survival rate and sperm quality in guinea pigs exposed to TCDD and stimulates the spermatogenesis (Yamamoto *et al.*, 1977). This action may be attributed to the increase in LH secretion which acts directly on the pituitary gland (Tsai *et al.,* 2003). Furthermore, Fahim *et al.,* (1982) reported that rats that received 5% ginseng experienced a significant increase in blood testosterone level.

In conclusion, the current results revealed that ZEN induced a stressful on the testis function included, increased in chromosomes aberration and sperm abnormality, decrease in sperm counts and motility and testosterone concentration. Both parsley oil and phoenix ginseng proved to have a

protective effect singly or in combination and the combined treatment was more effective. Their protective role may be attributed to the higher ability to scavenge free radicals and protection the cell membranes from the lipid peroxidation and their effect on endocrine system as well as the antagonistic effects to ZEN. Generally, the protective role of both herbs can be explained by two mechanisms, the first one is the antioxidant effect of parsley by acting as a radical scavenger (Fejes *et al.*, 2000; Gazzani, 1994 and Ramadan *et al.*, 2003) and the competition effect of ginseng for binding the ZEN receptors (Gray *et al.*, 2004). The efficiency of the ginseng was related to its binding to receptors, which are likely also to be saturated from the dose of ginseng.

References

1. **Abdel-Wahhab MA. and Aly SE. (2003):** Antioxidants and Radical Scavenging Properties of Vegetable Extracts in Rats Fed Aflatoxin-Contaminated Diet. J Agric. Food Chem., 51(8): 2409-14.
2. **Abdel-Wahhab MA. and Aly SE. (2005):** Antioxidant Property of *Nagilia Sativa* (Black cumin) and *Syzygium Aromaticum* (Clove) Rats during Aflatoxicosis. J. Appl. Toxicol., 25: 218-223.
3. **Abid-Essefi S, Ouanes Z, Hassen W, Baudrimont I, Creppy E. and Bacha H. (2004):** Cytotoxicity inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. Toxicology., 18: 467-474.
4. **Abid-Essefi S, Ouanes Z, Hassen W, Baudrimont I, Creppy E. and Bacha H. (2003):** DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and CaCo-2 cells: prevention by Vit. E. Toxicology., 192: 237-248.
5. **Astin JA. (1998):** Why patients use alternative medicine; results of a national study. JAMA, 279: 1548 – 1553.
6. **Baytop T. (1984):** Therapy with medicinal plants in Turkey (Past and Present), Istanbul University Yayinlari, No. 3255.
7. **Blumenthal M. (2000):** Herbal medicine expanded. Commission E Monographs. Newton, MA: Integrative Medicine Communications
8. **Boniel T. and Dannon P. (2001):** The safety of herbal medicines in the psychiatric practice. Harefuah, 140: 780 – 783.
9. **Campanella L, Bonanni A, Favero G. and Tomassetti M. (2003):** Determination of antioxidant properties of aromatic herbs, olives and fresh fruit using an enzymatic sensor. Analytical and Biochemistry, 375: 1011-1016.
10. **Cetin Y. and Bullerman LB. (2005):** Cyotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay. Food Chem. Toxicol., 43: 755-764.
11. **Choi SS, Lee JK, Han EJ, Han KJ, Lee HK, Lee J. and Suh HW. (2003):** Effect of ginsenoside Rd on nitric oxide system induced by lipopolysaccharide plus THF in C6 rat glioma cells. Arch. Pharm. Res., 26: 375-382.
12. **Creppy EE. (2002):** Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters., 127: 19-28.
13. **Dayan AD. and Paine AJ. (2001):** Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. Hum. Exp. Toxicol., 20: 439- 451.
14. **El-Makawy A, Hassanane MS. and Abd-Alla EAM. (2001):** Genotoxic evaluation for the estrogenic mycotoxin zearalenone, Reprod. Nutr., 41: 79-89.
15. **Ernst E. and Cassileth BR. (1999):** How useful are unconventional cancer treatments? Eur J Cancer, 35: 1608 – 1613.
16. **Etienne M. and Dourmad JY. (1994):** Effects of zearalenone or glucosinolates in the diet on reproduction in sows: a review. Livestock Production Science, 40: 99-113.
17. **Evans EP, Breckon G. and Ford CE. (1960):** An air drying method for meiotic preparation from mammalian tests, Cytogenetics, 3: 613-616.
18. **Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W. and Hafez ES. (1982):** Effect of Panax ginseng on testosterone level and prostate in male rats. Arch Androl., 8(4): 261-263.
19. **Fejes S, Blazovics A, Lemberkovics E, Petri G, Sz''oke E. and Kery A. (2000):** Free radical scavenging and membrane protective effects of methanol extracts from Anthriscus cerefolium L. (Hoffm.) and Petroselinum crispum(Mill.) nym. ex A.W. Hill. Phytother Res., 14(5): 362-365.
20. **Fejes S, Blazovics A, Lemberkovics E, Petri G, Szoke E. and Kery A. (2000):** Free radical scavenging and membrane

protective effects of methanol extracted fractions of parsley. Acta-Alimentaria, 29: 81-87.

21. **Fejes S, Kery A, Blazovics A, Lugasi A, Lemberkovics E, Petri G, and Szoke E. (1998):** Investigation of the *in vitro* antioxidant effect of Petroselinum crispum (Mill.) Nym. ex A. W. Hill. Acta Pharm Hung., 68(3): 150-156.
22. **Fejes S, Kery A, Blazovics A, Lugasi A, Lemberkovics E, Petri G. and Szoke E. (1998):** Investigation of the in vitro antioxidant effect of Petroselinum crispum (Mill.) Nym. ex A. W. Hill. Acta Pharm Hung., 68(3): 150-156.
23. **Ford CE. (1969):** Meiosis in mammals. In: "Comparative Mammalian Cytogenetics" K. Benirschke 91-106.
24. **Gajecki M. (2002):** Zearalenone—undesirable substances in feed. Pol J Vet Sci., 5(2): 117-22.
25. **Gazzani G. (1994):** Anti and prooxidant activity of some dietary vegetables. Rivista d i Scienza dell Alimentazione, 23: 413-420.
26. **Gray SL, Lackey BR, Tate PL, Riley MB. and Camper ND. (2004):** Mycotoxins in root extracts of American and Asian ginseng bind estrogen receptors. Exp. Biol. and Med., 229: 560-568
27. **Hagler WMJ, Tyczkowska K. and Hamilton PB. (1984):** Simultaneous occurrence of deoxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from the Midwestern United States. Applied and Environmental Microbiology, 47: 151-154.
28. **Hempel J, Pforte H, Raab B, Engst W, Bohm H. and Jacobasch G. (1999):** Flavonols and flavones of parsley cell suspension culture change the antioxidative capacity of plasma in rats. Nahrung, 43: 201-204.
29. **Hileman SM. and Jackson GL. (1999):** Regulation of gonadotropin-releasing hormone secretion by testosterone in male sheep. J. Reprod. Fertil., 54: 231-242.
30. **Hwang SY, Kim WJ, Wee JJ, Choi JS, and Kim SK. (2004):** Panax ginseng improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo- p-dioxin. BJU Int., 94(4): 663-668.
31. **IARC (International Agency for Research on Cancer), (1993):** Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins., 56: 397-444.
32. **Jeong TC, Kim HJ, Park JI, Ha CS, Kim SI, and Rho JK. (1997):** Protective effects of red ginseng saponins against carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats. Planta Med., 63: 136-140.
33. **Justesen U. and Knuthsen P. (2001):** Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. Food Chem., 73: 245-250.
34. **Kang J, Lee Y, No K, Jung E, Sung J, Kim Y. and Nam S. (2002):** Ginseng intestinal metabolite-I (GIM-I) reduces doxorubicin toxicity in the mouse testis. Reprod Toxicol.,16(3): 291-298.
35. **Kaur C. and Kapoor HC. (2002):** Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. of Food Sc. And Tech., 37: 153-161.
36. **Kim IH, Son HY, Cho SW, Ha CS. and Kang BH. (2003):** Zearalenone induces male germ cell apoptosis in rats. Toxicol Lett. 138(3): 185-192.
37. **Kim MK, Lee JW, Lee KY. and Yang D. (2005):** Microbial conversion of major ginsenoside Rb1 to pharmaceutically active minor ginsenoside Rd. J. Microbiol., 43(5): 456-462.
38. **Kim SH, Cho CK, Yoo SY, Koh KH, Yun HG. and Kim TH. (1993):** In vivo radioprotective activity of Panax ginseng and diethyldithiocarbamate Planta Medica., 7(5): 467-470.
39. **Kim W, Hwang S, Lee H, Song H. and Kim S. (1999):** Panax ginseng protects the testis against 2,3,7, 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. BJU Int., 83(7): 842-849.
40. **Kreydiyyeh SI, Usta J, Kaouk I. and Al-Sadi R. (2001):** The mechanism underlying the laxative properties of parsley extract. Phytomedicine, 8: 382-388.
41. **Kuiper-Goodman T, Scott PM, and Watanabe H. (1987):** Risk assessment of the mycotoxin zearalenone. Regul Toxicol. Pharmacol., 7: 253-306.
42. **Kumar M, Sharma MK, Saxena PS, Kumar A. (2003):** Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testes of Swiss albino mice. Biol Pharm Bull., 26(3): 308-12.
43. **Lee Y, Jin YR, Lim WC, Park WK, Cho JY, Jange S. and Lee SK. (2003):** Ginsenoside-RbI acts as a week phytoestrogen in MCF-7 human breast cancer cells. Arch pharmacology Res. 26: 58-63.
44. **Lioi M, Santoro R, Barbieri S. and Salzano M. (2004):** Ursini, Ochratoxin A and zearalenone: a comparative study on

genotoxic effects and cell death in bovine lymphocytes, Mut. Res., 557: 19-27.

45. **Lopez MG, Sanchez-Mendoza IR, and Ochoa-Alejo N. (1999):** Compartive study of volatile components and fatty acids of plants and in vitro cultures of parsley (*Petroselinum crispum* (Mill) nym ex hill). J. Agric. Food Chem., 47: 3292-3296.
46. **Manderfeld MM, Schafer HW, Davidson PM. and Zottola EA. (1997):** Isolation and identification of antimicrobial furocoumarins from parsley. J. Food Prot., 60: 72-77.
47. **Mannaa F, Abdel-Wahhab MA, Ahmed HH. and Park MH. (2006):** Protective role of panax ginseng extract stndardized with ginsensoisde Rg3 against acrylamide-induced neurotoxicity in rats. Accepted in J Appl. Toxicol.
48. **Matsuda, H., Namba, K., Fukuda, S., Tani, T. and Kubo, M. (1986).** Pharmacological study on Panax ginseng: Effects of red ginseng on experimental disseminated intravascular coagulation. Chem Pharm Bull (Tokyo), 34(5): 2100-2104.
49. **McDonald LE. and Capen CC (1989):** Introduction. In Veterinary Endocrinology and Reproduction. Philadelphia. Ed: McDonald, L.E. and Pineda, M.H. p. 1-19.
50. **Metzler M. and Pffeifer E. (2001):** Genotoxic potential of xenobiotic growth promoters and their metabolites. APMIS, 109: 89-95.
51. **Moe-Behrens G,H, Klinger FG, Eskild W, Grotmol T, Haugen TB. and DeFelici M. (2003):** Akt/PTEN signaling mediates estrogen-dependent proliferation of primordial germ cells in vitro. Mol Endocrinol., 17(12): 2630-2638.
52. **Molto GA, Gonzalez HHL, Resnik SL. and Gonzalez AP. (1997):** Production of trichotecenes and zearalenone by isolates of *Fusarium* spp. from Argentina maize. Food Additives and Contaminants, 14: 263-268.
53. **Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P. and Sandstrom B. (1999):** Effect of parsley intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. British J. Nutrition, 81: 447-455.
54. **Ouanes Z, Abid S, Ayed I, Anane R, Mobio T, Creppy E, and Bacha H. (2003):** Induction of micronuclei by zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of vitamin E. Mut. Res. 538: 63-70.
55. **Ouanes Z, Ayed-Boussema I, Baati T, Creppy EE, and Bacha H. (2005):** Zearalenone induces chromosome aberrations in mouse bone marrow: preventive effect of 17-estradiol, progesterone and Vitamin E. Mutat. Res. 565: 139-149.
56. **Ozsoy-Sacan O, Yanardag R, Orak H, Ozgey Y, Yarat A, and Tunali T. (2006)**: Effects of parsley (Petroselinum crispum) extract versus glibornuride on the liver of streptozotocin-induced diabetic rats. J Ethnopharmacol, 104 (1-2): 175-181
57. **Ozturk Y, Baser CHK, and Aydin S. (1991):** Hepatoprotective (antihepatotoxic) plants in Turkey. Proceedings of the 9th Symposium on Plant Drugs, Eskisehir, Turkey, May: 40-50.
58. **Parker RE. (1979):** Introductory statistics for Biology. 2nd ed. Arnold, London.
59. **Parker F. (1981):** Skin and Hormones. pp. 1080-1098. In: Text Book of Endocrinology. Williams, R.H. (Hrsg), W.B. Saunders Co., Philadelphia.
60. **Pfohl-Leszkowicz A, Chekir-Ghedira L and Bacha H. (1995):** Genotoxicity of zearalenone an oestrogenic mycotoxine: DNA adducts formation in female mouse tissues. Carcinogenesis 16: 2315-2320
61. **Placinta CM, D'Mello JPF, Macdonald AMC. (1999):** A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Animal Feed Science and Technology 78: 21-37.
62. **Platel K. and Srinivasan K. (1997):** Plant foods in the management of diabetes mellitus: vegetables as potential hypoglycaemic agents. Nahrung, 41: 68 -74.
63. **Ramadan MF, Kroh LW and Moersel JT (2003):** Radical scavenging activity of black cumin, coriander, and niger crude seed oils and oil fractions. J. Agric. and Food Chem. 51: 6961-6969.
64. **Russo A. (2000):** In vivo cytogenetics: Mammalian germ cells. Mut. Res. 455: 167-189.
65. **Schermer S. (1967):** The Blood Morphology of Laboratory Animals 3rd ed. P. 42. Philadelphia, F. A. Davi Co.
66. **Scheutwinkel M, Hude VDW, and Basler A. (1986):** Studies on the genotoxicity of the anabolic drugs trenbolone and ZENanol. Arch. Toxicol. 59: 4-6.
67. **Shi QQ, Hao Bouissac J, Lu Y, Tan S, and Lui B. (2005):** Ginsenoside-Rd from *Panax notoginseng* enhances astrocyte differentiation from neural stem cells. Life Scie. 76: 983-995.

68. **Tannant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspan W and Resnik MA. (1987):** Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science, 236: 933-941.
69. **Thomas MG, Carroll JA, Raymond SR, Matteri RL and Keisler DH. (2000):** Transcriptional regulation of pituitary synthesis and secretion of growth hormone in growing wethers and the influence of ZENanol on these mechanisms. Domest Anim Endocrinol. 18(3): 309-324.
70. **Tsai SC, Chiao YC, Lu CC, Wang PS. (2003):** Stimulation of the secretion of luteinizing hormone by ginsenoside-Rb1 in male rats. Chin J Physiol. 46(1):1-7.
71. **Tunali T, Yarat A, Yanardag R, Ozcelik F, Ozsoy O, Ergenekon G, and Emekli N (1999):** Effect of parsley (Petroselinum crispum) on the skin of STZ induced diabetic rats. Phytother. Res. 13: 138-141.
72. **Wannemacher RW, Brunner DL and Neufeld HA (2000):** Toxicity of trichothe-cenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, Eds. Mycotoxins and animal foods. Boca Raton: CRC Press.
73. **Whitehead SA, and Lacey M, (2003):** Phytoestrogens inhibit aromatase but not 17b-hydroxysteroid dehydrogenase (HSD) type 1 in human granulosa-luteal cells: evidence for FSH induction of 17b-HSD. H. Muman Reproduction 18(3): 487-494.
74. **Wong YYP, and Kitts DD, (2006):** Studies on the dual antioxidant and antibacterial properties of parsley and cilantro extracts. Food Chem. 97: 505-515.
75. **Wyrobek AJ., Watchmaker G. and Gordon L. (1984):** Sperm morphology testing in mice. Handbook of Mutagenecity Test Procedures. Amsterdam Elsevier Science pp 733-750.
76. **Yamamoto M., Kumagai A. and Yamamura Y. (1977):** Stimulatory effect of Panax ginseng principles on DNA and protein synthesis in rat testes. Arzneimittelforschung. 27(7): 1404-1405.
77. **Yokozawa T. and Dong E. (2001):** Role of ginsenoside-Rd in cisplatin-induced renal injury: special reference to DNA fragmentation.Nephron. 89(4): 433-8.
78. **Yokozawa T. and Owada S. (1999):** Effect of ginsenoside-Rd in cephaloridine-induced renal disorder. Nephron.81(2): 200-207.
79. **Zhao ZD, (1990):** Experimental treatment of smoke inhalation injury with anti-lipid peroxidation agents. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi. 6 (4): 294-298.
80. **Zheng GQ, Kenney PM, Zhang J and Lam LK (1992):** Inhibition of benzo[a]pyrene-induced tumorigenesis by myristicin, a volatile aroma constituent of parsley leaf oil. Carcinogenesis. 13 (10): 1921-1923.

التأثيرات المضادة للأكسدة للبقدونس ومستخلص الجينسينج المعاير بالجينسونزيد Rg3 ضد التغيرات المستحدثة في الوظائف التناسلية لذكور الفئران

عزيزة محمد حسن[1]، مسعد عطية عبد الوهاب[2]
[1]قسم بيولوجيا الخلية، و[2]قسم سموم و ملوثات الغذاء المركز القومي للبحوث- الدقي- مصر

تم في هذه الدراسة تقييم التأثير الوقائي لزيت البقدونس ومستخلص الجينسينج ضد التسمم الخلوي الناتج عن الزيرالينون حيث استخدمت عدد 108 من ذكور الفئران الناضجة قسمت إلى تسعة مجموعات شملت المجموعة المقارنة والمجموعة المعاملة بزيت البقدونس (0.6 ملجم/كجم وزن جسم) والمجموعة المعاملة بمستخلص الجينسينج (40 ملجم/كجم وزن جسم) والمجموعات المعاملة بزيت البقدونس ومستخلص الجينسينج مع أو بدون الزيرالينون (10 ميكروجرام/كجم وزن جسم). قسمت كل مجموعة تجريبية إلى تحت مجموعة (أ) استخدمت في قياس التستيرون في السيرم والتشوهات الكروموسومية وعوملت لمدة أسبوعين بينما استخدمت تحت المجموعة (ب) لدراسة التشوهات في الحيوانات المنوية وعوملت بجرعتين يوميا لمدة أسبوع ثم ذبحت بعد 30 يوما. أوضحت النتائج أن المعاملة بالزيرالينون نتج عنها نقص معنوي في تركيز التستيرون في السيرم والعدد الكلي للحيوانات المنوية بينما تسببت في زيادة معنوية في عدد الحيوانات المنوية المشوهة وعدد التشوهات الكروموسومية في الخلايا التناسلية . أظهرت النتائج أيضا أن الحيوانات المعاملة بزيت البقدونس أو مستخلص الجينسينج بمفرديهما كانت طبيعية ومماثلة للمجموعة المقارنة في كل القياسات محل الدراسة. كما أثبتت النتائج أن المعاملة بالزيرالينون مع كل من زيت البقدونس أو مستخلص الجينسينج أو كلاهما معا أدت إلى حدوث تحسنا معنويا في كل القياسات تحت الدراسة إلا أن المعاملة بزيت البقدونس كانت أكثر فعالية عن مستخلص الجينسينج كما أن المعاملة بالزيرالينون مع زيت البقدونس ومستخلص الجينسينج معا كانت أكثر فعالية من المعاملتين المنفردتين. نستخلص من هذه الدراسة أن كل من زيت البقدونس ومستخلص الجينسينج لهما تأثير وقائي فعال ضد التغيرات الناتجة عن الزيرالينون وأن المعاملة بالمستخلصين معا أدت إلى نتائج أكثر فعالية في الحماية ضد التأثيرات الناتجة عن الزيرالينون.