Ameliorative Effects of Vitamin E on Fenamiphos-Induced Reproductive Toxicity in Male Rats

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Abstract: Organophosphate pesticides have destroying properties on male reproduction and adversely affect the male reproductive system. This study aimed to investigate the induction of oxidative stress in the male reproductive system of adult rats exposed to the organophosphate pesticide, fenamiphos (Fen), and tried to establish the ameliorative properties of vitamin E with respect to reproductive reconstruction in them. Forty adult male wistar rats were divided into 4 groups of 10 animals each. Group I received corn oil (2mL/k), group II was given vitamin E only (100 mg/kg), group III was administered Fenamiphos only (0.163mg/kg~1/20LD_{50}), while group IV was pretreated with vitamin E and then exposed to fenamiphos 2 hours later. After this treatment period, sperm parameters, serum testosterone, and acid phosphatse were determined. Moreover, DNA damage, malondialdhyde (MDA), reduced glutathione (GSH) and histopathological changes were detected in testis. Oral administration of fenamiphos caused a reduction in the sperm count and sperm motility. While, the number of sperm abnormalities were increased. On the other hand, acid phosphatse activity and MDA levels was increased and there was decline in GSH level. Testis DNA damage were happened. Histopathological studied in testis revealed congestion in the blood vessels associated with deposition of homogenous material in the interstitial stroma. Supplementation of vitamin E to fenamiphos-treated rats reduced the toxic effect of fenamiphos. The findings data suggest that administration of vitamin E ameliorated the fenamiphos-induced oxidative stress and sperm toxicity in rat.

Keywords: Fenamiphos fertility, sperm count, sperm motility, oxidative stress, DNA damage, Histopathology.

1.Introduction:

The increase in human and animal population, especially in the last few decades, has led to the compelling need to increase food production. The development of high-yielding crop varieties and the formulation of more potent pesticides to aid in the elimination of pests that destroy crops are therefore imperative (Joshi, *et. al.*, 2005). Organophosphates (OP) insecticides are widely used for the control of insect pests (Vidyasagar, *et. al.*, 2004) as it account for 50% of global insecticide use (Casida and Quistad, 2004). Therefore, the tendency for human and animal exposure in both rural and residential environment is common (Ngoula, *et. al.*, 2007). The main mechanism of action of OP is inhibition of the activity of Acetyl Cholinesterase (AChE), an enzyme essential for normal neuronal transmission in living organisms (Abou-Donia, 2003).

Organophosphate (OP) insecticides are widely used for agriculture, vector control and domestic purposes. About 70% of the pesticides in current use are OP compounds which constitute a total consumption of around 90 million pounds per year. However, the uncontrolled application of these insecticides in agriculture and public health operations has increased the scope of ecological imbalance and thus many non-target organisms have become victim (Das and Mukherjee, 2000).

The primary effects of OPs on organisms are through the inhibition of acetylcholinesterase (AChE), the enzyme responsible for terminating the transmission of the nerve impulse. However, there is evidence indicates that oxidative stress may be implied in OP toxicity more than AChE inhibition. Since (Bagchi, *et. al.*, 1995) found that different classes of pesticides may induce *in vitro* and *in vivo* generation of Reactive Oxygen Species ROS, which react with biological macromolecules and produce enzyme inactivation, lipid peroxidation and DNA damage (Winston, 1991). The production of reactive oxygen species has long been regarded as a possible mechanism of pesticide-induced toxicity as evidenced by triggered oxidative stress in a number of studies (Modesto and Martinez, 2010). They also elicit their toxicities *via* other mechanisms including cytotoxicity, genotoxicity, immunotoxicity, delayed polyneuropathy, nephrotoxicity, hepatotoxicity, carcinogenicity and reproductive toxicity (Taylor, 1990, and Navarro, *et. al.*, 2001).

Fenamiphos is an organophosphate pesticide, a class of chemicals that was originally designed to function as a human nerve gas in World War II. Fenamiphos is relatively stable to hydrolysis and moderately persistent in water, although when exposed to light, it degrades rapidly. Fenamiphos has potential to contaminate and persist in groundwater, since sunlight does not penetrate underground water stores. California has listed fenamiphos as a pesticide of concern for groundwater contamination.

The global increase in infertility is becoming a source of concern (Pennings, *et. al.*, 2009). In conjunction with this are reports that indicate a significant decrease in the quality of human semen (Nelson and Bunge, 1974, and Bendvold, 1989). It is estimated that male-related disorders are

probably present in up to 40% to 50% of childless couples, alone or in combination with female factors (Bungum, 2012, and Tournaye, 2012). Environmental chemical contaminants, including pesticides such as organophosphates (OP) insecticides have been largely implicated in this reproductive deficit (Volk, *et. al.,* 2011). There are numerous studies indicating that organophosphate-induced ROS caused reproductive tissue damage and that it has an important role in the pathophysiology of testis, probably via inhibition several enzymes involved in DNA synthesis and pathology of membrane polyunsaturated fatty acids. (Bendvold, 1989 and Bungum, 2012) decreasing glutathione (GSH) level, GSH peroxidase activity and antioxidant vitamins. (Tournaye, 2012 and Volk, *et. a*l., 2011).

Reactive oxygen species (ROS) play an important and positive role by modulating cell proliferation, differentiation and function, but also may have negative effects because these species are highly reactive and may damage any cell structure, including the DNA molecule (Aitken, *et. al.* 2012) The formation of free radicals or oxidants is a well-established physiological event in aerobic cells, which convene enzyme and nonenzyme resources, known as antioxidant defenses, to remove these oxidizing species. An imbalance between oxidants and antioxidants, the two terms of the equation that defines oxidative stress, and the consequent damage to cell molecules constitutes the basic tenet of several pathophysiological states, such as neurodegeneration, cancer, mutagenesis, cardiovascular diseases, and aging.

Vitamin E is known as a fat soluble antioxidant which interrupts release of lipid peroxidation in the plasma membrane and thus maintains the integrity of the membrane. Many studies had showed that vitamin E inhibits oxidative stress and lipid peroxidation induced by OP pesticides in experimental animals (Aitken and Curry, 2011 and John, *et. al.,* 2001) and therefore, prevented the adverse effects of active oxygen radicals on spermatogenesis and sperm health (Sameni, *et. al.,* 2011). Furthermore, antioxidants have substantial role in the female reproductive system (Aitken, *et. al.,* 1994).

Testes are the main organ of male reproduction. So, the principle objective of the present study planned to evaluate the role of Vit. E. as a protective agent against Fen-induced testes toxicity, this was done *via* evaluation of male fertility indices DNA damage in testes and morpgological changes in testes of male rats. This study through light on testis oxidative stress markers, malondialdhyde (MDA) and reduced glutathione (GSH) of Fen treated–rats.

2. Materials and Methods:

2.1. Animals:

Forty albino rats aged 12-weeks (170±10g) were obtained from the breeding unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University. Animals were maintained at the animal care facilities of Central Agricultural Pesticides Laboratory (CAPL) in plastic cages under controlled temperature (23±2^{o}C), 12-h light/dark cycle and relative humidity (50±5%). Water and food were available *ad libitum*. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments.

2.2. Experimental Design:

After the period of acclimation, animals were divided into four groups with 10 animals each. The first group was used as control, hence these animals were orally given corn oil (4mL/kg). The second male group was orally treated with fenamifos (0.163mg/kg b.w.); about 1/20 LD_{50}. The third male group was orally treated with dissolved vitamin E (100 mg/kg b.w.) and fourth group was treated with combination of fenamifos (0.163 mg/kg b.w.) and Vit. E. (100mg/kg b.w.). The daily oral administration of the experiments lasts for 65-days which is needed for completion of the spermatogenic cycle and maturation of sperms in epididymis (Casida and Quistad, 2004).

2.3. Epididymal Sperm Characteristics:

2.3.1. Testicular Sperm Count:

Immediately after dissection, one testis of each rat was placed in 1ml phosphate buffer (pH 7.4). Tunica albuginea was cut by surgical blades, removed and the remaining semeniferous tubules were mechanically minced using surgical blades in 1ml phosphate buffer. The testicular cell suspension was pipetted several times to make a homogenous cell suspension. One drop of the suspension was placed on the "Haemocytometer chamber" (Neubauer improved, Feinoptik Bad Blankenburg, Germany). Testicular sperm suspension was evaluated as million sperm cells per ml of suspension under 200X magnification using phase contrast microscope and the sperm were counted manually. Testicular sperm count was measured by Uzunhisarcikli, *et. al*., (2007).

2.3.2. Sperm Motility Analysis:

The sperms were collected as quickly as possible after a rat was sacrificed. The cauda epididymis was placed in 1 ml of 37^{o}C phosphate buffer saline solution (pH 7.4). and cut by surgical blades into approximately 1 mm3 pieces. The solution was pipetted several times to homogenize the sperm suspension. One drop of the suspension was placed on a slide, covered by a cover slip, and evaluated under a phase contrast microscope at 200 magnifications. The sperms were categorized on the basis of their motility as ''motile" or ''immotile". The results were recorded as percentage of sperm motility. (Uzunhisarcikli, *et. al*., 2007).

2.3.3. Sperm morphology:

To determine the percentage of morphologically abnormal spermatozoa, one drop of the suspension was spread on a clean slide. the slides were stained with a mixture of 1.67% eosin and 10% nigrosin in 0.1M sodium citrate for viewing under a light microscope at 400-magnification. A total of 300 spermatozoa were examined on each slide (1800 cells in each group (n=6)), and the head, tail and total abnormality rates of spermatozoa were expressed as percentage (**Turk,** *et. al.*, 2008).

2.4. Testis DNA Fragmentation:

Testicular DNA damage was measured using a single-cell gel electrophoresis (comet) assay (Singh, *et. al.,* 1988), 1g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5min and filtered. Cell suspension (100µl) was mixed with 600µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture were spread on agarose-precoated slides. The coated slides were immersed in lyses buffer (TBE (0.045M), pH 8.4, which containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2V/cm for 2 hr and 100 m A. The slides were stained with ethidium bromide (20µg/mL) at 4°C and observed with the samples still humid. The DNA fragment migration patterns of 100 cells for each dose level were evaluated using a fluorescence microscope (with excitation filter 420-490nm [issue 510nm]). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-strained DNA using a 40x objective on a fluorescent microscope. Although any image analysis system may be suitable for the quantitation of SCGE data, we used komet 5 image analysis software developed by kinetic imaging, Ltd. (Liverpool, UK) linked to a CCD camera. To assess the quantitative and qualitative extent of DNA damage in the cells the length of DNA migration and the percentage of migrated DNA were measured.. Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

2.5. Oxidative stress parameters in testis:

Tissue preparation: Testes were removed from rats at the end of experiment (65-days) and washed with cold saline buffer. Washed tissues were immediately stored at -80°C. For obtaining enzymatic extract, tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH7.4) containing 0.1mM ethylendiaminetetraacetic acids (EDTA) yielding 10 % (W/V) homogenate. The homogenates were centrifuged at 12.000g for 30 min at 4°C. The supernatant samples were separated and used for biochemical markers of oxidative stress (MDA and reduced glutathione).

Lipid peroxidation (LPO) was measured by estimation of malondialdhyde (MDA) in testes by the method of Okahawa, *et. al.*, (1979). Reduced glutathione content (GSH) of supernatant estimation was performed by the method of Beutler, *et. al.,* (1963) Determination of GSH is based on the reaction of DTNB [5, 50-dithiobis-(2-nitrobenzoic acid)] with GSH and yield a yellow colored chromophore with a maximum absorbance at 412nm. The amount of GSH present in the testicular tissue was calculated as nmole/g tissue.

2.6. Serum testosterone and acid phosphatase activity:

The testosterone level in serum determinted by using radioimmunoassay technique according to the method of Yen and Jaffe, (1978**).** Moreover, serum acid phosphates activity by using method of Kind and Kind, (1954).

2.7. Histopathology Examinations:

Testicular tissues for histopathological examination were fixed in 10% buffered formalin overnight and then embedded with paraffin. When analyzed, all paraffin-embedded tissue was sectioned at 4 µm, deparaffinized in xylene, dehydrated by ethyl alcohol in decreasing concentrations (100, 95 and 70%), and stained with haematoxylin and eosin. These specimens were examined under bright-field optical microscopy using a light microscope and 40× magnification powers. Corresponding digital images were captured for later analysis (Banchroft, *et. al.*, 1996).

2.8. Statistical analysis:

Analysis of data was performed by using SPSS (Version 15) and Results are expressed as M±S.E. Statistical differences were determined by Duncan test for multiple comparisons after ANOVA. $P<0.05$ was considered statistically significant.

3. Results:

3.1. Epididymal Sperm Characteristics:

Among the potential hazards effect of pesticides, reproductive toxicity is of special concern (Dunnick, *et. al.*, 1984). Reproductive toxicity is a special concern among the potential hazards of pesticides in concerning the effect of fen on the reproductive toxicity of male rat. The results presented in table (1) showed that, both of sperm count and motility decreased significantly while, sperm abnormalities were increased in exposed male rats group to fen in comparing with control group. The supplementation with Vit E. prior to Fen intubation ameliorated the effect of the pesticides on spermatotoxicity (sperm count, motility and abnormalities).

3.2. Testis DNA Fragmentation:

The results presented in Table (2) indicate that, DNA damage was significantly increased in testis of exposed male rats group to Fen as evidenced by an increase in both tail length and moment and increase in tail DNA%. This damage in DNA was ameliorated with Vit. E. supplemented group prior to fen but still higher than control group.

Table 1. Effect of administration with Fenamiphos and Vit. E. on epididymal sperm characteristics in male rats.

Groups / Sperm Characteristics	Control (1mld.w./kg b.w)	Vit. E. (100mg/kg b.w)	Fen (0.16mg/kg b.w)	Fen & Vit. E. (0.16mg/kg b.w)& (100mg/kg b.w)
Sperm Count (10^6/ml)	**108.83** ±3.08	**116.00** ±4.42	**76.60** ±6.33[a,b]	**96.17** ±1.85[b]
Sperm Motility (%)	**68.00** ±3.07	**76.20** ±1.88	**41.60** ± 9.16[a,b]	**52.51** ±5.37[)]
Progressive Motility (%)	**22.50** ±2.14	**25.00** ±2.23	**11.00** ±1.87[a,b]	**15.00** ±1.29[a,b]
Abnormalities (%)	**20.00 ±3.87**	**22.00** ±3.74	**45.00** ±2.74[a,b]	**38.33** ±4.22[a,b]

Data expressed as mean±S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$. (b) Significant different from corresponding Vit E group by one-way ANOVA at $P \leq 0.05$.

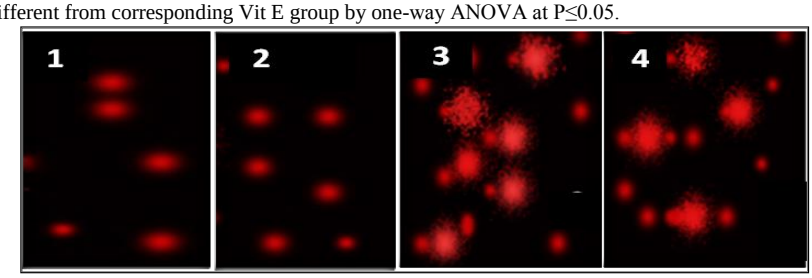

Plate 1: Effect of administration with Fenamiphos and Vit. E. on testis DNA fragmentation in male rats. (1) control, (2) Vit. E., (3) Fen, (4) Fen+Vit. E.

Table 2. Effect of administration with Fenamiphos and Vit. E. on testis DNA fragmentation in male rats**.**

Groups / DNA Fragmentation	Control (1mld.w./kg b.w)	Vit. E. (100mg/kg b.w)	Fen (0.16mg/kg b.w)	Fen & Vit. E. (0.16mg/kg b.w)& (100mg/kg b.w)
Tail length (µm)	**1.76** ±0.096	**1.77** ±0.114	**5.35** ±0.38[a,b]	**3.46** ±0.26[a,)]
Tail DNA (%)	**1.79** ±0.066	**1.83** ±0.084	**5.69** ±0.240[a,b]	**3.83** ±0.263[a,b]
Tail moment (Units)	**3.16** ±0.282	**3.27** ±0.332	**30.61** ±3.46[a,b]	**13.34** ±1.75[a,b]

Data expressed as mean±S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$. (b) Significant different from corresponding Fen group by one-way ANOVA at $P \leq 0.05$.

3.3. Oxidative stress parameters in testis:

In relation to oxidative stress markers in testes of Fen-exposed rats, the data presented in Table (3), showed that, malondialdhyde level (MDA) as the end product of lipid peroxidation was significantly increased in Fen-exposed group in comparing with control group. Meanwhile, supplementation Fen-exposed rat with Vit. E. reduced this damaging event but still significantly increased comparing with control. Also, the findings exhibited a marked decrease in reduced glutathione level (GSH) in the pesticide-exposed group. The same trend of decline in GSH was noticed in the group that supplemented with vitamin E prior exposing to pesticide.

The results presented in table (4) indicated that, acid phosphatase activity slightly changed in Fen-treated group and other groups comparing with control. On the other hand, testosterone significantly declined in rats-exposed to fen. Supplementation of rats with Vit. E. prior to Fen improved this decline in testosterone activity.

Table 3. Effect of fenamifos and vitamin E on testes MDA levels and GSH levels in male rats.

Groups / Biomarker	Cont (1mldw/kg bw)	Vit. E. (100mg/kg bw)	Fen (0.16mg/kg bw)	Fen & Vit. E. (0.16mg/kg bw)& (100mg/kg bw)
MDA (nmol/g tissue)	**35.64** ±0.78	**33.77** ±0.88	**53.15** ±0.83[a,b]	**41.44** ±0.71[a,b,c]
GSH (µmol/g tissue)	**7.77** ±0.46	**6.98** ±0.19	**6.52** ±0.25[a]	**6.58** ±0.19[a]

Data expressed as mean ± S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$. (b) Significant different from corresponding Fen group by one-way ANOVA at $P \leq 0.05$.

Table 4. Effect of fenamifos and vitamin E on serum testosterone levels and acid phosphatase activity in male rats.

Groups / Biomarker	Cont (1mLdw/kg bw)	Vit. E. (100mg/kg bw)	Fen (0.16mg/kg bw)	Fen&Vit. E. (0.16mg/kg bw)& (100mg/kg bw)
Testosterone (ng/ml)	**2.99** ±0.52	**4.28** ±1.2	**1.69** ±0.91[a,b]	**3.51** ±1.5[b]
Acid phosphatase (U/L)	**15.31** ±1.16	**14.99** ±0.68	**18.31** ±1.02	**15.84** ±0.52

Data expressed as mean ± S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$. (b) Significant different from corresponding vit. E, group by one-way ANOVA at $P \leq 0.05$.

3.4. Histopathology Examinations:

Histopathological examination of rat's testis was given in plate (2). Data showed no histological changes were observed in control (A) and Vit E (B) groups. While, there was congestion in the blood vessels (C1) associated with deposition of homogenous eosinophilic material in the interstitial stroma and degeneration in some seminiferous tubules (C2 and C3). Moreover, the group treated with Vit E prior the pesticide exhibited congestion in the blood vessels (D) associated with deposition of homogenous eosinophilic material in the interstitial stroma.

4. Discussion:

The exponential increase in the production and extensive use of pesticides has a profound impact on the environment and creates unforeseen hazards to any organism as well as human (Chia, 2000; Karallieda, *et. al*., 2003; and Karanthi, *et. al*., 2004). Organophosphates are among the most widely used synthetic pesticides. The wide spread use of organophosphate insecticides (OPIs) has a causable toxic effect on reproductive system (Joshi, *et. al*., 2007).

There are numerous studies indicating that organophosphate- induced ROS caused reproductive tissue damage and that it has an important role in the pathophysiology of testis. Thid damage is probably via inhibition several enzymes involved in DNA synthesis and pathology of membrane polyunsaturated fatty acids,(Kapoor, *et. al*., 2011, and Nagada and Bhatt, 2011) decreasing activity of glutathione peroxidase GSH level (El-Gendy, *et. al*., 2010 and Mandal and Das, 2012) and antioxidant vitamins.(Nagda and Bhatt, 2011 and Uzun *et. .al.*, 2009)

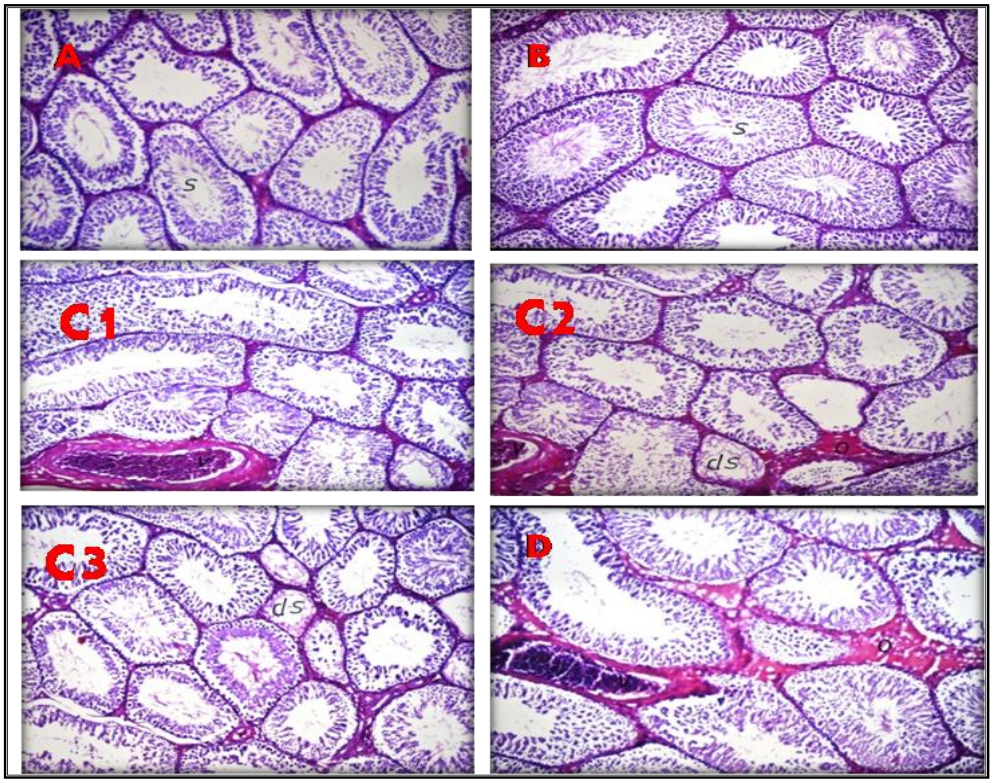

Plate 2: A Testes section of male rats control group. There was no histopathological findings and the normal histologixcal structure. **B:** Testes section of male rats Vit. E. group. There was normal histologixcal structure. **C1:** Testes section of male rats Fen group. There was sever congestion in blood vessels. **C2:** Testes section of male rats Fen group. Homogenous eosinephili. Material in interstitial stroma (O). **C3:** Testes section of male rats Fen group. There was degeneration and lose of spermatogenesis (ds) in some individual semmifenus tubules. **D:** Testes section of male rats Fen &Vit. E. group. Showing homogenous eosinophilic material in interstitial stroma with congestion in blood vessel.

The sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. According to our study the sperm morphological abnormalities were increased, sperm count and sperm motility were decreased due to Fen treatment. The decrease of sperm motility and count after oral treatment of Fen may be due to inadequacy of androgen (Chadhuary and Joshi 2003), which caused disruption of testicular functions by altering the activities of the enzymes which is causative for spermatogenesis (Siha, *et. al*. 1995). Similar type of results was found in profenofos treated rats for 60 days (EL-Kashoury, 2009). In addition the reduction in epididymal sperm count, observed in the Fen group is in concomitant with the result obtained by previous workers (Taylor, 1990). The lowered sperm in the Fen group may be partly due to induction of oxidative stress in the testis (Dirican and Kalender, 2011). Thereby is providing *un*conducive environment for spermatogenesis in the seminiferous tubules. Besides, the spermatozoa on their own are highly susceptible to oxidative damage by excessive ROS due to the high concentration of polyunsaturated fatty acids within their plasma membrane (Krishnamoorthy *et. al.*, 2007). Low concentrations of scavenging enzymes and glutathione (Dirican and Kalender, 2011 and Mariana, 2009).

It is established that, sperm motility is an important functional measurement to anticipate sperm fertilizing capacity (Aikten, *et. al*., 1984). Any negative impact on motility would seriously affect fertilizing ability of the organism (Murugavel, *et. al*., 1989). Low level of ATP content seriously affects the sperm motility. Sperm motility may be affected by alteration of the enzymatic activities of oxidative phosphorolytic process. Similarly oxidative phosphorolytic process is required for ATP production; it is a source of energy for the alleviated movement of spermatozoa (Joshi, *et. al*., 2007). Full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which is one of the major causes of infertility (Poon, *et. al*., 2004). A parallel essential indicator is sperm cell degeneration.

Comet assay is a widely applied method to measure DNA damage in a variety of cell types, sperm included (Singh and Stephens, 1998; Tice, *et. al.*, 2000). It has been applied in reproductive toxicological studies to evaluate the effects of physical and chemical agents on reproductive system of experimental animals (Cordelli, *et. al.*, 2005) as well as in epidemiological studies to examine the effects of environmental chemicals on DNA integrity (Belcheva, *et. al.*, 2004; Duty, *et. al*., 2003; Hauser, *et. al*., 2003; Migliore, *et. al.*, 2002; and Xu, *et. al.*, 2003).

Sperm quality, such as motility and DNA integrity, are important in male fertility and in the particular contribution to early embryonic development (Claassens, *et. al.*, 2000), which is also a sensitive and quick testing strategy for reproductive toxicology (Bendvold, 1989). DNA is a target for mutagens and carcinogens, which induce changes in DNA structure giving rise to mutations and/or cell death (Scott, *et. al*., 1991). Free radical generated following pesticide exposure may lead to extensive DNA damage (Hatjian, *et. al*., 2000). Sarabia *et. al.*, 2009) suggested that DNA damage is probably another mode of action of organophosphates on male reproductive function. Since sperm morphology is controlled by various autosomal and Y-specific genes (Amina, *et. al*., 2014) DNA damage may also reduce sperm motility. The possibility that would explain the increasing nuclear DNA damage is high concentrations of ROS (Wu and Cederbaum, 2003) and reduction in antioxidant defenses (Lecomte *et al.*, 1994). ROS is the major source of DNA damage, causing strand breaks, removal of nucleotide, and a variety of modifications of the organic bases of the nucleotides (Wu and Cederbaum, 2003). Oxidative stress can contribute to cell damage and may play important role in DNA damage in the germ cells, and increased oxidative stress is a well-accepted mechanism of pesticides-induced tissue injury, particularly in testis (Emanuele, *et. al.*, 2001).)

Lipid peroxidation is an identified cell damage mechanism in plants and animals, and it is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and break up into diverse complex forms; peroxides from polyunsaturated fatty acids produce malondialdehyde and 4-hydroxyalkenals, which are used as lipid peroxidation indicators (Siems, *et. al.*, 1998 and Esterbauer, *et. al.*, 1991). These lipoperoxidative changes couple with low antioxidant status represented by low level reduced glutathione in the pesticide exposed group is an indication of oxidative stress. Oxidative stress may therefore be partly responsible for the alteration in the synthesis of some sex hormones and other substances essential for sustaining reproductive activity. Therefore, oxidative stress may have been partly involved in the male reproductive (Reuber, *et. al.*, 1985 and Dirican and Kalender, 2001)

Reduced glutathione (GSH) is the major non-enzymatic antioxidant , the most abundant non-protein thiol source of cell (Sies, 1999) and it plays an important role in many biological processes of cells, including synthesis of proteins, DNA and protection against oxidative damage (Sies, 1999). The testis has high concentration of GSH, which plays an important role in spermatogenesis (Sahoo, *et. al.*, 2007). A glutathione deficiency can lead to instability of the mid-piece, resulting in defective sperm motility (Ursini, *et. al.*, 1999). Cells with high level of intracellular GSH are protected against oxidative damage caused by ROS. GSH either non-enzymatically reacts with ROS (Anderson, 1998) or directly scavenges them by neutralizing $OH^{\cdot}$ (Sies, 1986). Thiols such as thioredoxins (small disulphide-containing proteins) and reduced glutathione (GSH) can neutralize a hydroxyl radical (Sahoo, *et. al.*, 2007).

The testis secrete several male sex hormones, which are collectively called androgen, including testesterone, and dihydrostenedione. Testosterone is so much more abundant than the others that one can consider it the significant testicular hormone. Therefore, the global increase in infertility is becoming a source of concern (Pennings, *et. al.*, 2009). The decrease of testosterone levels induced by Fen exposure may indicate that Fen has a direct inhibitory effect on testosterone production from Leydig cells or disrupt the biosynthesis of testosterone in Leydig cells (Ramazan, *et. al.*, 2012)

The present study show from the first time that vitamin E ameliorated the reproductive toxicity of Fen. Vit. E administration reduced lipid peroxidation in Fen-treated rats. The effect of Vit. E in reducing lipid peroxidation might be related to high lipid solubility of Vit. E that allow it to localize in the cell membrane. Vit. E supplementation also resulted in significant protection of cell membrane damage. The protective mechanism of Vit. E is probably through its capacity to scavenge lipid peroxyl radicals. Furthermore, Vit. E can also normalize the level of glutathione, which is an important for intracellular free radical scavenging system, thus reducing the degree of oxidative damage (Nelson and Bunge, 1974). Also, Vit. E is another of the well-known antioxidants; since it is fat soluble, Vit. E can interact with and pass through cell membranes and effectively trap free radicals, prevent lipid peroxidation and forestall cellular damage (Traber, 1996). So, Vit. E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radicals reaction resulting in a relatively *un*reactive species of the antioxidant radicals (Galili, *et. al.*, 2007). Moreover, many insecticides are hydrophobic molecules that bind

extensively to biological membranes, especially phospholipid bilayers (Lee, *et. al.*, 1991), and they may damage the membranes by inducing lipid peroxidation. Since Vit E is known to be antioxidant, a number of studies have been performed to determine whether they can ameliorate the toxic effects of pesticides (Uzunhisarcikli, *et. al.*, 2007; Ogutcu, *et. al.*, 2008).

The activity of ALP is related to the mitosis of spermatogenic cells and glucose transport. ACP located in lysosome of leydig cells is involved in the protein synthesis by induction of sex hormones. Changes in the activity of ACP may be used as an indicator of spermatogenesis function. Changes in the activity of ACP in pesticide treated rats also reflect testicular degeneration, which may be a consequence of suppressed testosterone and indicative of lytic activity (Kaur, *et. al.*, 1999)

Siminferous tubules degeneration and congestion of blood vessels was observed in testis of Fen–exposed group as a histopathlogical changes. These results are concomitant with results of (Sarabia, *et. al.*, 2009) who found testicular degeneration in diazinon–exposed mice. It has been reported that OP pesticides can cross the blood-testis barrier and cause the degeneration of the spermatogenic and leydig cells. It has been demonstrated that OP pesticides caused testicular degeneration in experimental animals. (Kalender, *et. al.*, 2012).

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