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VITAMIN C AND HESPERIDIN ATTENUATE DELTAMETHRIN – INDUCED GENOTOXICITY, SPERM ABNORMALITIES AND BIOCHEMICAL ALTERATIONS IN RATS.

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ABSTRACT

The current study was performed to investigate the protective effect of Vitamin C and Hesperidin against genotoxicity, sperm abnormalities and biochemical toxicity induced by deltamethrin (DLM). Thirty six male albino rats were divided into six groups (6 rats each): Group I, used as control, group II received Vitamin C (20mg/kg b.wt), group III received hesperidin (50 mg/kg b.wt), group IV received deltamethrin (0.26 mg/kg b.wt.), group V received Vitamin C followed by deltamethrin and VI received hesperidin followed by deltamethrin with the same doses which are mentioned before orally by gavages for 60 days. DLM caused a significant enhancement in the frequencies of different types of chromosomal aberrations in bone-marrow cells and spermatocytes with significantly inhibited of mitotic index compared to control. While, when rats were treated with vitamin C or hesperidin prior to deltamethrin, the frequencies of chromosomal aberrations were dramatically reduced and recovered the mitotic activity compared to deltamethrin treated group. DLM caused a significant reduction in sperm count, serum testosterone level, activities of brain cholinesterase, and DNA & RNA contents in testes also; DLMtreated group showed a significant increase in sperm abnormalities. However, concurrent administrations of vitamin C or hesperidin with deltamethrin caused significant improvement in all parameters which were studied. Results indicate that DLM exerts significant harmful effects on chromosome and male reproductive

system and that the concurrent administration of vitamin C or hesperidin reduced the detrimental effects of DLM on male fertility.

KEYWORDS: Deltamethrin; Chromosomal aberration; Reproductive toxicity; Male rats; Vitamin C; Hespridin.

INTRODUCTION

Synthetic pyrethroids are modified derivatives of pyrethins, natural substances obtained from flowers of pyrethrum species (Luty et al., 2000). Pyrethroid insecticides are widely used in agriculture, domestic and veterinary applications than other insecticides, particularly organochlorine, organophosphate and carbamate insecticides (Pauluhn, 1999). Deltamethrin (DLM), a synthetic pyrethroid type II, is commonly used in Egypt for agriculture, veterinary and public health applications. In veterinary practice, it is used for the control of ectoparasites in domesticated animals and poultry. The most important sources of the animal and human exposure to DLM are polluted food and water, and it is readily absorbed by the oral route (Barlow et al., 2001).

DLM has a deleterious effect on male fertility (Abd El-Aziz et al., 1994; El-Gohary et al., 1999). During pyrethroid metabolism, reactive oxygen species (ROS) are generated and result in oxidative stress in intoxicated animals (Kale et al., 1999). In mammals, sperm plasma membranes have extremely high concentration of polyunsaturated fatty acids and insufficient antioxidant defenses; hence they are highly susceptible to lipid peroxidation (Aitken et al., 1993). The production of ROS is a normal physiological event in various organs including the testis controlling sperm capacitation, acrosome reaction and spermoocyte fusion. However, overproduction of ROS can be harmful to sperm and subsequently to male fertility (Akiyama, 1999). The natural antioxidants may be helpful in preventing or reducing the harmful effects of ROS on testes and semen quality (Yousef, 2010).

However, a number of studies have demonstrated genotoxic and immunotoxic effects of deltamethrin in mammalian species (Bhunya and Pati, 1990; Husain et al., 1996). Deltamethrin has been reported to induce micronuclei in human peripheral lymphocytes and mouse bone marrow cells (Surrales et al., 1995), and to induce sister chromatid exchanges in mouse chromosomes (Chauhan et al., 1997).

Many insecticides are hydrophobic molecules that bind extensively to biological membranes; especially to the phospholipid bilayers (Lee et al., 1991). Numerous studies showed that the antioxidant substances protect cells against deleterious effects of several environmental agents (Almeida et al., 1998). Antioxidants as vitamins, can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites, also the destruction of most free radicals rely on the oxidation of endogenous antioxidants mainly by scavenging and reducing molecules (Verma et al., 2007).

Vitamin C is a major circulating water-soluble antioxidant. It is well absorbed by the gastrointestinal tract and required for multiple biological functions and biochemical reactions in humans and animals and it is an important element for the body (Li and Schellhorn, 2007). In the male reproductive system vitamin C is known to protect spermatogenesis and it plays a major role in semen integrity and fertility both in men (Agarwal et al., 2005; Eskenazi et al. 2005) and animals, increases testosterone levels (Sönmez et al., 2005) and prevents sperm agglutination. It is an important chain-breaking antioxidant, contributing up to 65 percent of the total antioxidant capacity of seminal plasma found intracellulary and extracellulary (Makker et al., 2009). Shrilatha and Muralidhara, (2007) reported the protective effect of vitamin C on testicular oxidative stress, sperm oxidative stress and genotoxic effects using a diabetic mice model. Similarly, Naziroğlu, (2003) concluded that vitamin C acted as antioxidant in reproductive milieu. The effect of vitamin C against reproductive toxicity of pesticides was investigated (Allhaza and Bashandy, 1998; Uzunhisarcikli et al., 2007). Also, it is reported to neutralize ROS and reduce oxidative DNA damage and hence genetic mutations (Wang et al., 2008).

Hesperidin is a flavanone glycoside (flavonoid) found abundantly in citrus fruits. Hesperidin is believed to play a role in plant defense. It acts as an antioxidant according to *in vitro* studies (Monforte et al., 1995; Hirata et al., 2005). In human nutrition, it contributes to the integrity of the blood vessels. Various preliminary studies reveal novel pharmaceutical properties. Flavonoids are products of plant metabolism and have different phenolic structures (Ohtusuki et al., 2003; Guzmán and Navarrete, 2009). They are effective antioxidants because of their free radical scavenging properties and because they are chelators of metal ions (Trivedi et al., 2011); thus, they may protect tissues against free oxygen radicals and lipid peroxidation. Flavonoids may also be activated by mechanisms that apparently are not directly dependent on their antioxidative properties. A wide range of

different biological activities, including antibacterial, antithrombotic, vasodilator, antiinflammatory, and anticarcinogenic effects mediated by different mechanisms, are associated with flavonoid compounds (Middleton et al., 2000).

Hesperidin reduced cholesterol and blood pressure (Ohtsuki et al., 2003) in rats. In a mouse study, large doses of the glucoside hesperidin decreased bone density loss (Chiba et al., 2003). Hesperidin has anti-inflammatory effects (Galati et al., 1994; Kawaguchi et al., 2004). Hesperidin is also a potential sedative, possibly acting through opioid or adenosine receptors (Loscalzo et al., 2008). Hesperidin also showed the ability to penetrate the blood-brain barrier in an *in vitro* model. Infertility is one of the major health problems in life, and approximately 30% of infertilities are due to a male factor (Carlsen et al., 1992; Isidori et al., 2006). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production (Mosher et al., 1991).

Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood testis barrier stability (Jedlinska-krakowska et al., 2006). Evidence suggests that certain phytochemicals found in citrus sources, such as flavonoids and limonoids, play a major role in treating or retarding chronic diseases, including anti-oxidative, anti-carcinogenic, cardiovascular protective, neuro-protective, bone health promotion and anti-inflammatory diseases. Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men (Yang et al., 2006). Therefore, the role of nutritional and biochemical factors in reproduction and sub- fertility treatment is very important.

The present study was planned to assess the ability of citrus flavonoid Hesperidin and vitamin C to promote sperm parameters & spermatogenesis and modulate genotoxicity and oxidative stress which induced by synthetic pyrethroid deltamethrin.

MATERIALS AND METHODS

Chemicals

Commercially grade deltamethrin-based pesticide (Butox® 5%EC) (IntervetCo., France) was used in this study. Vitamin C and hesperidin were purchased from Sigma Chemical Company

(St. Louis, MO, USA). All other chemicals were analytical reagent grade and chemicals required for all biochemical assays were obtained from Sigma–Aldrich Chemicals Co (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Experimental animals

Thirty-six of adult male Wister albino rats weighing 120–150 g were obtained from the animal house colony of the National Research Centre, Egypt. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24.3°C) during the experimental period. The rats were provided ad libitum with tap water and fed with standard commercial rat chow. Animal procedures were performed in-accordance with Guidelines for Ethical Conduct in the Care and Use of Animals **Experimental design.**

After one week of acclimation, animals were divided into six groups (6 rats in each group). Group (1) served as untreated control, received saline orally by gavages once daily for 60 days. Group (2) received Vitamin C in a dose of 20mg/kg b.wt. orally by gavages for 60 days Manjula et al., 2006. Group (3) received hesperidin in a dose of 50 mg/kg b.wt orally by gavages once daily for 60 days (Balakrishnan and Menon, 2007). Group (4) received deltamethrin in a dose of 0.26 mg/kg b.wt (1/100 of the LD50) orally by gavages once daily for 60 days (Farouk, 2007). Group (5) received Vitamin C (20 mg /kg/day) followed by deltamethrin in a dose of 0.26 mg/kg b.wt orally by gavages once daily for 60 days. Group (6) received hesperidin in a dose of 50 mg/kg b.wt followed by deltamethrin in a dose of 0.26 mg/kg b.wt orally by gavages once daily for 60 days.

Blood and tissues collection

At the end of experimental period, blood samples were collected from the retro-orbital vein plexus and direct cardiac puncture, under ether anesthesia in sterile tubes and centrifuged at 3500 rpm for 15 min and Serum was separated for measurement of total testosterone. After the collection of blood samples all animals were sacrificed by cervical dislocation; the brain, testis and femur of each animal were dissected for cytogenetic and sperm morphology studies, DNA and RNA contents in the testis and cholinesterase levels in the brain.

Cytogenetic analyses: Two hours before sacrifice, the animals were injected i.p. with 10mg/kg body wt of colchicine.

(A) Chromosome analysis in somatic cells.

Chromosome preparation from bone-marrow cells (BMCs) was carried out according to the method of Yosida et al., (1971). BMCs were collected from both femora in 6–8 ml hypotonic solution (0.075 M) of KCl. The cell suspension was incubated for about 20 min at 37 ◦C, and then centrifuged at 1000 rpm for 10 min. The cells were re-suspended in cold fixative (methanol:acetic acid; 3:1, v/v), and centrifuged again for 10 min at 1000 rpm. The fixation step was repeated three times. Finally, the cells were spread by drop-ping onto cold slides. Dried slides were stained with 10% Giemsa in phosphate buffer for 40 min, washed for 10 min in phosphate buffer, and air dried. At least 100 well-spread metaphases were analyzed per rat.

Metaphases with gaps, chromosome or chromatid breakage, fragments, deletions as were recorded. The prepared slides for chromosomal aberrations were used to determine the mitotic index (MI), which based on the scoring of 1000 cells for each animal. The number of dividing cells including prophases and metaphases was recorded. The mitotic index (number of dividing cells/1000 cells) was calculated.

(B) Chromosome analysis in germ cells.

Spermatocyte cells were prepared according to **Brewen and Preston (1978)** for meiotic chromosomal analysis.

Sperm morphology: When the male rats were killed, both epididymis were also removed for sperm-morphology evaluation. The sperm smears were examined under light microscopy (600 x), and morphological abnormalities were assessed following the criteria of **Wyrobek and Bruce, (1975).** Four hundred sperm were scored for each animal.

Determination of nucleic acid (DNA and RNA) contents: Total DNA and RNA contents in the testis were determined according to **pears (1985).**

Determination of serum total testosterone levels: Testosterone level was estimated in serum by Elisa (Micro-well method) according to **Parker (1981).**

Determination of brain cholinesterase activities

Brain cholinesterase activities were determined according to **(Jakobs et al 1990).**

Statistical analysis

Data were expressed as means ± S. E. and analyzed statistically using Student's *t*-test. Results were considered to be statistically significant when $P < 0.05$.

RESULTS

The animals were treated with vitamin C and hesperidin showed insignificant change in the frequencies of chromosomal aberrations in rats bone-marrow cells in comparison to the control. However, the treatment with deltamethrin resulted in a significant enhancement in the frequencies of different types of chromosomal aberrations in bone-marrow cells such as gaps, breaks**,** centromeric attenuation**,** deletion and fragments compared with the control **(Table 1, Fig).** While, when rats were treated with vitamin C or hesperidin prior to deltamethrin, the frequencies of chromosomal aberrations were dramatically reduced compared to deltamethrin treated group. The mitotic index was significantly inhibited in deltamethrin treated group compared to control. The rate of bone-marrow proliferations were remarkably recovered in vitamin C /deltamethrin and hesperidin/deltamethrin groups, compared with deltamethrin treated group **(Table 1) Figure (1)**.

Table (1): Effect of vitamin C or hesperidin supplementation on structural chromosomal aberration in bone marrow induced by deltamethrin intoxication (n=6; mean± SE)

Groups	No. of examined cells	Structural chromosomal aberrations							Mitotic index
		Gap	Break	Centromeric attenuation	Deletion	Fragment	E t o E	Endomitosis	
Control group	300	0.833±0.44a	0.833±0.34a	1.33±0.462a	0.167±0.1a	0.0±0.0a	0.0±0.0a	1.0±0.566a	340.167 ±10.96a
Vit. C treated group	300	0.667±0.365a	0.5±0.245a	0.833±0.36a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.667±0.365a	365.5 ±6.967a
Hesperidin treated group	300	0.333±0.231a	0.333±0.23a	0.5±0.245a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.333±0.231a	370.1 ±8.187a
Deltamethrin treated group	300	2.667±0.365**b	1.83±0.267*b	3.0±0.4*b	1.5±0,244**b	1.33±0.231***b	1.0±0.233**b	2.67±0.365*b	189.5 ±3.792***b
Vit.C + deltamethrin treated group	300	1.5±0.369*c	1.0±0.261*c	1.5±0.217*c	0.5±0.374*c	0.667±0.165*c	0.333±0.116*c	0.833±0.337**c	275.167 ±7.252***c
Hesperidin+ deltamethrin treated group	300	1.0±0.283**c	0.5±0.244**c	0.833±0.44**c	0.333±0.231*c	0.5±0.845*c	0.116±0.182*c	0.5±0.295***c	298.35 ±4.230***c&d

Within each column, means superscript with the same letter are not significantly different.
(b) Significantly different from control group.
(c) Significantly different from deltamethrin treated group.
(d) Significantly different from Vit. C+ deltamethrin treated group
* P<0.05, ** P<0.01 and ***P<0.001

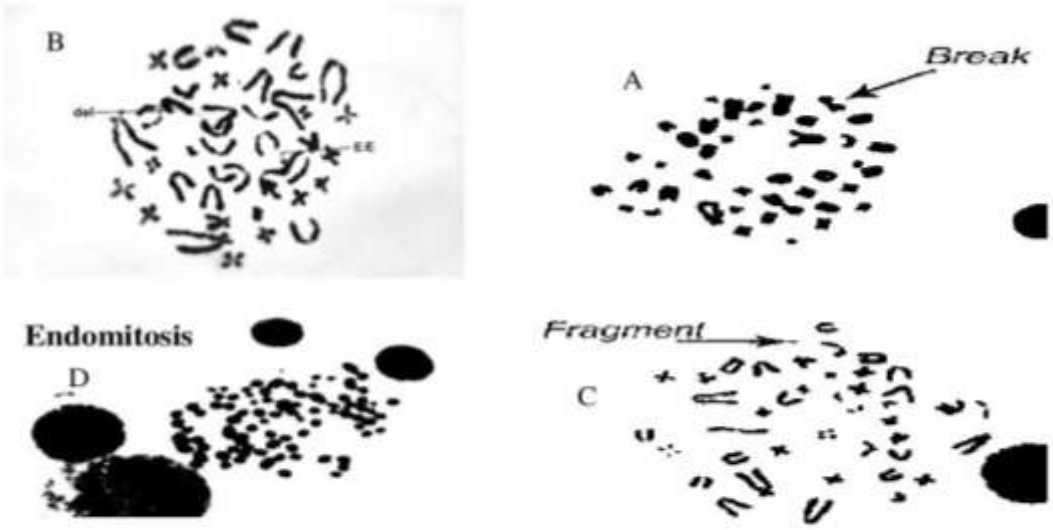

Figure (1): Showing (A) break – (B) Delction and End to End – (C) fragment – (D) Endomitosis

There was no significance differences in the frequencies of different types of chromosomal aberrations in rat's spermatocytes treated with vitamin C and hesperidin in comparison to the control. However, the animals treated with deltamethrin showed significance increases in the frequencies of chain, autosomal univalent, x-y univalent and total structural chromosome aberrations compared to control group **(Table 2).** However animals treated with vitamin C or hesperidin with deltamethrin showed significance decreases in all types of chromosomal aberrations in rat's spermatocytes compared with deltamethrin treated group **(Table2) (Figure 2)** The present findings indicated that hesperidin, reduced the cytotoxic effect of deltamethrin and recovered the mitotic activity more than vitamin C.

Table (2): Effect of vitamin C or hesperidin supplementation on chromosomal aberration in spermatocytes induced by deltamethrin intoxication (n=6; mean± SE)

Groups	No. of examined animals	No. of examined cells			Structural chromosomal aberrations	Total aberration
			X-Y Univalent	Chain	Autosomal Univalent	
Control group	6	300	0.333±0.231a	0.333±230a	0.666±0.365a	2.667±0.666a
Vit.C treated group	6	300	0.167±0.183a	0.167±0.182a	0.5±0.245a	1.666±0.667a
Hesperidin Treated group	6	300	0.0±0.0a	0.0±0.0a	0.333±0.231a	0.667±0.666a
Deltamethrin Treated group	6	300	2.5±0.245**b	2.5±0.469**b	3.167±0.523**b	16.333±1.033**b
Vit. C+ deltamethrin treated group	6	300	1.167±0.337*c	1.166±0.336*c	1.5±0.379*c	7.67±0.666***c
Hesperidin + deltamethrin treated group	6	300	0.667±0.230**c	0.5±0.244**c	0.833±0.337**c	4.0±0.577***c & d

Within each column, means superscript with the same letter are not significantly different.
(b) Significantly different from control group.
(c) Significantly different from deltamethrin treated group.
(d) Significantly different from Vit. C+ deltamethrin treated group
* P<0.05, ** P<0.01 and ***P<0.001

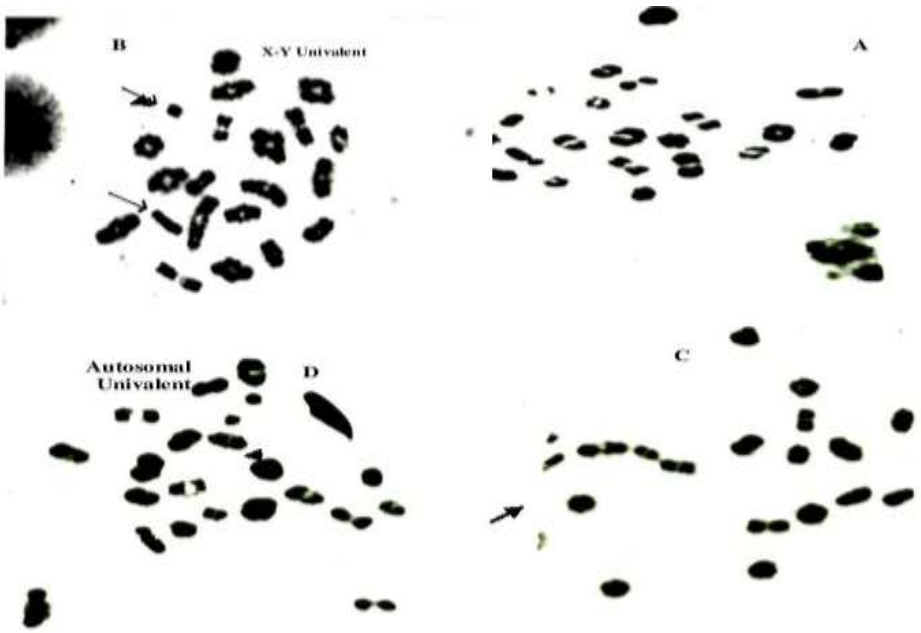

Figure (2): Showing (A) Control – (B) X-Y Univalent – (C) chain – (D) Autosomal Univalent

The mean sperm count in cauda epididymal plasma was found to be 31 ± 3.042 millions/gm testes in control rats. A significant ($P < 0.001$) reduction (20.625±0.209) in sperm count was observed in rats exposed to deltamethrin , when compared with the control rats **(Table 3)** All the sperms were apparently normal in control rats. Whereas in the rats exposed to deltamethrin, many morphologically-altered sperms were observed, in form of without hock head sperms, banana shaped head sperms, coiled tailed sperms and divided tailed sperms **(Table 3) (Figure 3).** However, administration of vitamin C or hesperidin prior to deltamethrin resulted in a significant recovery from testicular disorders, improved total sperm count and inhibit sperm abnormalities.

Serum testosterone levels was significantly lower in deltamethrin treated group ($P<0.01$), compared with the control group. Co-administration of vitamin C or hesperidin with deltamethrin resulted in a significant recovery from testicular disorders and increase serum testosterone levels **(Table 4).** Also**,** deltamethrin treatment was associated with low activities of brain cholinesterase**.** While**,** activities of brain cholinesterase significantly increased in vitamin C/ deltamethrin and hesperidin/ deltamethrin treated groups compared with deltamethrin treated group **(Table 4)**.

Table (3): Effect of vitamin C or hesperidin supplementation on changes in sperm count and morphology induced by deltamethrin intoxication (n=6; mean±SE)

	Tail		Head		Total	Total sperm count (millions/gm testes)
Groups	Divided	Coiled	Without hock	Banana		
Control group	1.0±0.566a	1.0±0.4a	0.833±0.439a	0.667±0.231a	3.5±0.548a	$31.042\times10^{6}\pm0.466a$
Vit.C treated group	0.5±0.245a	0.67±0.365a	0.5±0.245a	0.5±0.245a	2.67±0.462a	$31.75\times10^{6}\pm0.466a$
Hesperidin treated group	0.333±0.231a	0.5±0.244a	0.333±0.230a	0.33±0.231a	2.0±0.49a	$32.167\times10^{6}\pm0.383a$
deltamethrin treated group	2.67±o.365*b	2.5±0.469*b	2.5±0.469*b	1.833±0.336*b	9.5±1.158***b	$20.625\times10^{6}\pm0.209***b$
Vit.C+ deltamethrin treated group	1.167±0.367*c	1.0±0.4*c	1.0±0.47*c	0.833±0.3*c	4.0±0.693***c	$28.282\times10^{6}\pm0.278***c$
Hesperidin + deltamethrin treated group	0.667±0.231***c	0.666±0.365*c	0.5±0.244**c	0.5±0.244**c	2.5±0.678***c	$30.542\times10^{6}\pm0.259***c$

Within each column, means superscript with the same letter are not significantly different.
(b) Significantly different from control group.
(c) Significantly different from deltamethrin treated group.
* P<0.05, ** P<0.01 and ***P<0.001

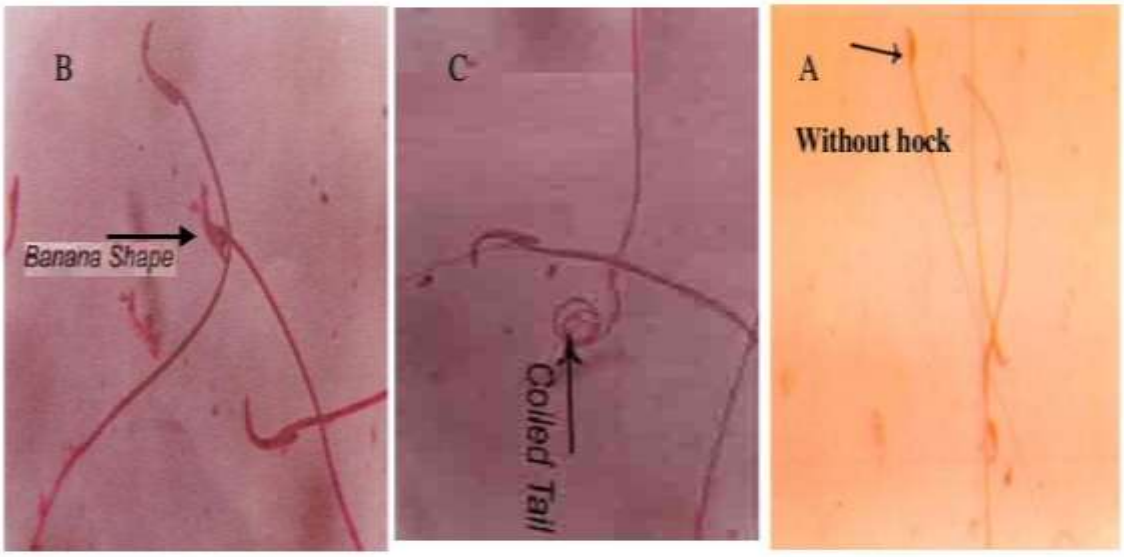

Figure (3) showing – (A) sperm without hock – (B) sperm banana shape 0 (C) colled tall

Table (4): Effect of vitamin C or hesperidin supplementation on changes in serum total testosterone levels as well as brain acetyl -cholinesterase activity induced by deltamethrin intoxication (n=6; mean± SE)

Groups	Serum testosterone levels (ng/ml)	Brain cholinesterase activities µmol/mg protein/ min
Control group	6.95±0.215a	7.059±0.124a
Vit.C treated group	7.217±0.182a	7.129±0.131a
Hesperidin treated group	7.483±0.151a	7.265±0.108a
Deltamethrin treated group	3.733±0.231***b	3.791±0.209***b
Vit.C + deltamethrin treated group	5.883±0.432**c	6.487±0.144***c
Hesperidin + deltamethrin treated group	6.35±0.209***c	6.766±0.069***c

Within each column, means superscript with the same letter are not significantly different.
(b) Significantly different from control group.
(c) Significantly different from deltamethrin treated group.
***P<0.001

Oral administration of deltamethrin (0.26 mg/kg body weight/ day) to male albino rats for 60 days caused significant, reduction in DNA and RNA contents in testes**.** However, concurrent administrations of vitamin C or hesperidin with deltamethrin caused significant improvement in all parameters which were studied **(Table 5).**

Table (5): Effect of vitamin C or hesperidin supplementation on changes in testis DNA and RNA contents induced by deltamethrin intoxication. (n=6; mean± SE)

Groups	No. of examined animals	Testis (mg/tissues)	
		DNA	RNA
Control group	6	0.421±0.008a	0.280±0.008a
Vit.C treated group	6	0.433±0.01a	0.292±0.008a
Hesperidin treated group	6	0.448±0.01a	0.297±0.009a
Deltamethrin group	6	0.230±0.016***b	0.150±0.004***b
Vit.C + deltamethrin treated group	6	0.355±0.01***c	0.233±0.006***c
Hesperidin+ deltamethrin treated group	6	0.403±0.004***c	0.264±0.007***c

Within each column, means superscript with the same letter are not significantly different.
(b) Significantly different from control group.
(c) Significantly different from deltamethrin treated group.
***P<0.001

DISCUSSION

The greatly increased agricultural uses of pesticides beside their vital role in public health, have introduced a serious and novel hazard to human and their live stocks. It causes modification of genetic material which may induce cancer or lead to germ cell alterations **(Williams and Burson, 1985).** Cytogenetic analyses of the **present study** revealed that oral administration of deltamethrin at a dose of 0.26 mg / kg b. wt. daily for 60 days possesses a genotoxic effect as it significantly increased the frequency of structural chromosomal aberrations of rat bone marrow cells spermatocytes and reduce the mitotic index (MI) of rat bone marrow cells. While, when rats were treated with vitamin C or hesperidin prior to deltamethrin, the frequencies of chromosomal aberrations were dramatically reduced and recovered rate of bone-marrow proliferations compared to deltamethrin treated group. The induction of significant genetic damage in the bone marrow cells of deltamethrin-treated rats in the present study is in concordance with the genotoxicity of deltamethrin shown in some previously published reports. **Ismail and Mohamed, (2012)**; **Fadlalla, et al (2014); Sekeroglu et al., (2011)**, **Chauhan et al., (2007)** revealed that, a significant increase in chromosomal aberrations and frequency of aberrant cells in from of chromatid breaks, deletions, fragments and gaps, inhibited the mitotic index in mouse bone marrow cells. and decrease serum total testosterone levels were noted in deltamethrin-treated rats as compared to the control group. Testosterone is needed for the development of male reproductive tract, spermatogenesis, maintenance of secondary sex characteristics and other sexual parameters such as libido **(McLachlan et al., 2002)**. Sister chromatid exchange frequency increased in mice administered orally with a single dose of 20 mg/kg DEL **(Dolara et al. 1992**; **Chauhan et al., 1997**). A single intraperitoneal dose of DLM (11.2 mg/kg) significantly decreased MI and significantly increased CA frequency in adult female rats at 24 h post-exposure **(Agarwal et al., 1994). Bhunya and Pati (1990)** reported that, technical deltamethrin (10-20 mg/kg body weight) to significantly increase chromosome aberrations and micronuclei in the bone marrow cells of mice.

The ameliorative effect of vitamin C may be ascribed to its anticlastogenic and antimutagenic effect as it tempers the negative influences of free radicals resulted from insecticide exposure. So it may modulate the oxidative DNA damage in mammalian cells and reduce the incidence of chromosomal aberrations **(Odin, 1997) Khan, et al (2015).** These results are parallel with **Abu-Aita and Yassa, (2008)** clarify that vitamin C plays an important role in modulating the genotoxicity, sperm abnormalities and serum biochemical alterations in deltamethrin exposed

rats. **Fatma and Omima, (2003)** stated that, simultaneous administration of vitamin C with deltamethrin inhibited the frequency of chromosomal aberrations**. Kan et al., (2012)** observed that co-administration of deltamethrin and vitamin E showed decrease in the frequency of micronucleus as compared to deltamethrin treated fish. **Salah et al., (2009)** suggested that treatment with mixtures of vitamins A,C, and E were decreased the frequency of chromosome aberrations in bone marrow cells as well as the frequency of sperm abnormalities of rats treated with tefluthrin and readjusted near to that of the healthy control animals, also mitotic index and sperm counts were increased significantly near to the control group after vitamins ingestion as treatments.

Hosseinimehr et al., (2009) reported that hesperidin at doses of 10, 50 and 100 µmol significantly reduced the micronuclei frequency in cultured Lymphocytes exposed to technetium-99m. **Ahmadi et al., (2008)** revealed that hesperidin at doses of (100, 200, and 400 mg/kg b.w.) for five consecutive days was significantly reducing frequency of micronucleated polychromatic erythrocytes induced by cyclophosphamide. **Hosseinimehr and Nemati, (2006)** demonstrated that hesperidin gives significant protection to mice bone marrow against the clastogenic effects of gamma irradiation. **Devi et al., (1998)** observed that, Orientin and Vicenin, two flavonoids, protect mice against chromosomal aberration induced by irradiation when administrated before 2 Gy c-rays. The exact mechanism for the optimum protection of hesperidin is unclear, still it could be suggested that at lower concentrations hesperidin might not be enough to quench all the radicals generated by ionizing radiation and at higher concentration hesperidin might have reacted with some other ligands in the system and thus might not be completely available for quenching the free radicals **(Kalpana et al., 2009).**

In the present study, there was significant reduction in sperm count, level of serum testosterone and significant increase in the percentage of abnormal sperm morphology in rats exposed to deltamethrin in comparison to the control. However, administration of vitamin C or hesperidin prior to deltamethrin resulted in a significant recovery from testicular disorders, improved total sperm count, inhibit sperm abnormalities and increase serum testosterone level. These results were in- agreement with **Ahmad et al., (2009&2012)**, **Poonam, et al., (2014)** who suggested decreased testicular & epididymal sperm counts and serum testosterone concentrations and increased percentage of abnormal spermatozoa (tailless, bent tails, coiled tailed) in dose and time dependent manner in cypermethrin –treated goats and

rabbits. **Odaa and El-Maddawy, (2011)** conducted that deltamethrin caused a significant reduction in sperm count and serum testosterone level. Deltamethrin -treated group showed a significant increase in sperm abnormalities. The concurrent administration of vitamin E/Selenium partly reduced the detrimental effects of deltamethrin on male fertility.

Abou El-Magd et al., (2011) demonstrated that serum testosterone level is significantly lower in pyrethroid exposed workers compared to the control group. **Shivaraj et al., (2011)** observed that, a dose-dependent reduction in the cauda epididymal sperm count was observed in rats exposed to Deltamethrin + Chlorpyriphos with increase percentage of abnormal sperms which is attributed to decreased spermatogenesis. It is well known that, cytotoxic drugs depress spermatogenesis in mammals by causing death of developing germ cells in the seminiferous tubules **(Wyrobek et al., 1983)**. This results in the elimination of active cells of spermatogenesis and thereby results in reduction in daily sperm production **(Lu and Meistrich, 1979). Ben Abdallah et al., (2010)** found that mice treated by deltamethrin was associated with a significantly decreased sperm count and significantly increased percent morphologically abnormal spermatozoa compared with the controls.

Meeker et al., (2008) observed that, reduce human semen quality and increased sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. These findings may be of concern due to increased pyrethroid use and prevalent human exposure. **Abd el-Aziz et al., (1994)** reported that deltamethrin increased the percentage of dead and morphologically abnormal spermatozoa of treated rats as well as decrease in the plasma testosterone level. A significant increase in the proportion of dead or abnormal sperm in mice was reported after exposure to deltamethrin **(Bunya and Pati 1990).**

Testis and sperm function are particularly vulnerable to the injury produced by ROS **(Shen and Sangiah, 1995).** During pyrethroid metabolism, superoxide anion, hydroxyl radicals and H2O2 were generated in intoxicated animals. The a-cyano pyrethroids, such as deltamethrin, form cyanohydrins which decompose to cyanides and aldehydes. Cyanide ions are mainly converted to thiocyanate and CO2. The major metabolic reactions are ester cleavage and hydroxylation at the 4-positon and formation of a lipophilic conjugate, 2[R]-2-(4-chlorophenyl)isovalerate. The aldehydes and other lipophilic conjugates may produce oxidative stress in pyrethroid toxicity **(Kale et al., 1999). Ahmad et al., (2012)** concluded that, pyrethroid exposure is responsible for endocrine disruption and decreases fertility in

both sexes of various non-target species and produces fetal mortality, which may be prevented by vitamin E supplementation due to its anti-oxidant potential.

Olorunshola et al., (2011) revealed that co-administration of ascorbic acid significantly caused improvement of rats sperm concentration, sperm motility and serum testosterone concentration which disturbed by Chlorpyrifos. **Khaki et al., (2011)** revealed that, administration of 600 mg/kg/day citrus extract significantly increased the sperm percentage, viability, motility. This suggested that citrus may be promising in enhancing sperm healthy parameters. Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood testis barrier stability **(Jedlinska-krakowska et al., 2006). Hozayen, (2012) stated** that, the pre-treatment of rats with hesperidin produced a potential increase of the lowered free testosterone levels as compared to doxorubicin treated group. **Trivedi et al., (2011)** showed Hesperidin protects testicular toxicity of doxorubicin in rat, prevention of oxidative stress, DNA damage and the cellular toxicity and protection against doxorubicin-induced germ cell toxicity was further evident from the sperm count and sperm head morphological evaluation.

Arafa et al., (2009) observed that pretreatment with the flavonoid hesperidin before benzo[α]pyrene improved the epididymal function as shown by the increased sperm count, motility, and production. **Goel, et al., (2006); Mi, et al., (2007); Izawa, et al., (2008)** demonstrated that, protective effects of other flavonoids in testicular tissue following exposure to various intoxicants. The protective effects of hesperidin could possibly be ascribed to its obvious antioxidant potentials observed in the current work. The flavonoid improved the antioxidant defense mechanism, both enzymatic and nonenzymatic, in testicular tissue. It increased the enzyme activities of SOD. Sperm count was apparently increased in rats following intake of two flavones **(Dhawan, et al., 2002). Roseff (2002)** has reported that the flavonoid pycnogenol was able to improve sperm morphology and function in sub fertile men. It was also reported by **Casini, et al. (2006)** that flavonoids could significantly improve sperm count, motility, and morphology in an oligospermic male.

In the present study, deltamethrin treatment was associated with low activities of brain cholinesterase**.** While**,** activities of brain cholinesterase significantly increased in vitamin C/ deltamethrin and hesperidin/ deltamethrin treated groups compared with deltamethrin treated group These results were in-accordance with **Vani et al., (2011)** who revealed acetylcholine esterase (AChE) is one of the most widely used enzymes as a biomarker for environmental

pollution. The highly decreased brain AChE activity of fish this might be due to the inhibitory effect of deltamethrin. Vitamin C can be effectively used to neutralize the toxic effect of deltamethrin on fish**. El-Zayat et al., (2008)** reported that acute deltamethrin intoxication induced marked alterations in the brain and blood acetylcholinesterase activity. Selenium supplementation, either individually or in antioxidant preparation, showed a considerable ability to attenuate some but not all signs of delayed deltamethrin neurotoxicity. **Tuzmen et al., (2007)** observed that deltamethrin causes brain damage via inhibition of AChE. **Wielgomas and Krechniak, (2007)** reported that, cholinesterase was markedly depressed to a different degree in plasma and brain of animals receiving chlorpyrifos alone or in combination with α-cypermethrin. **Yousef et al., (2006)** reported that, the activity of AChE in plasma was significantly decreased in rats treated with deltamethrin. Vitamin E in combination with deltamethrin alleviated its negative effect on the activities of the above measured enzymes. Mechanisms responsible for the neurotoxic effects of pesticides include changes in the entry in and exit from nerve cells of sodium and effects on cholinesterase activity (**Calore et al., 2000).** Activity of the indicated enzyme is reported to be inhibited in pyretroids intoxication **(Hossain et al., 2005). Szegletes et al., (1995)** reported that the AChE specific activity was significantly inhibited in the heart and intestine in deltamethrin treated group compared to control animals. **Hozayen, (2012) stated** that the pre-treatment of rats with hesperidin produced non-significant increase in the cholinesterase activity as compared to doxorubicin treated group.

Concerning DNA and RNA contents in testes**,** there was significant reduction in DNA and RNA contents in testes of rats treated by deltamethrin compared to control group. However, concurrent administrations of vitamin C or hesperidin with deltamethrin caused significant improvement in all parameters which were studied. These results were in-agreement with **Singh et al., (2010)** reported that deltamethrin caused a dose and time dependent significant reduction in the levels DNA and RNA in gonadal, and nervous tissue. Deltamethrin interacted with DNA to cause chromosomal damage, together with the repair of DNA lesions in premeiotic stages of spermatogenesis (spermatocytes and spermatogonia), which have reached, to some extent, spermatids and spermatozoa and produced mutagenicity **(Allen et al., 1995).**

Salah et al., (2009) revealed that, oral ingestion of tefluthrin caused a significant decrease in the content of RNA, DNA and **total soluble protein** of rat liver and testes. These abnormal

effects on nucleic acids system were reduced by the vitamins mixtures (A, C & E) induction into the pesticide intoxicated rat. **Giray et al., (2001)** indicated that, cypermethrin insecticide induces DNA damage may be due to the generation of reactive oxygen species (ROS)]. It is well known that ROS may cause DNA strand breaks. However, **Saxena et al., (2005)** reported that cypermethrin interacts with DNA via its acid moiety result of the strong polarization associated with chlorine atoms in structure of cypermethrin which lead to attaching of these pyrethroids to DNA by way of polarization may cause the destabilization of DNA structure and unwinding of DNA helix, inducing chromosomal damage.

In conclusion, the present studies suggested that deltamethrin is cytotoxic. The mechanism of such pathological facts may be prompted by the free radical release and the lipid peroxidation that they induce. The use of antioxidant vitamin C or citrus flavonoid hesperidin was ascertained to reduce the harmful effects of deltamethrin. However, sufficient feeding of vitamin C or hesperidin by human which regularly come in contact with the pesticides is benefit in combating the adverse effect of deltamethrin.

REFERENCES

1. Abd El-Aziz M I, Sahlab A M, Abdel-Khalik M. Influence of diazinon and deltamethrin on reproductive organs and fertility of male rats. Dtsch Tier¨arztl Wschr, 1994; 101: 230–2.
2. Abou El-Magd SA, Sabik LME, Shoukr A. Pyrethroid Toxic Effects on some Hormonal Profile and Biochemical Markers among Workers in Pyrethroid Insecticides Company. Life Science Journal, 2011; 8(1): 311-322.
3. Abu-Aita NA and Venes, F. Yassa VF. Impact of vitamin C on genotoxicity, sperm abnormalities and serum biochemical alterations in deltamethrin exposed rats. Egypt. J. Comp. Path. & Clinic. Path. 2008; 21(1): 168- 188.
4. Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. J. Androl, 2005; 26(6): 654-660.
5. Ahmad L,Khan A,Khan MZ, Hussain I, Mahmood F, Kashif Sleemi MK, Lodhi LA, Imran Abdullah I. Toxico-pathological effects of cypermethrin upon male reproductive system in rabbits. Pesticide Biochemistry and Physiology, 2012; 103: 194–201.
6. Ahmad M, Hussain I, Khan A, Najib-ur-Rehman Deleterious effects of cypermethrin on semen characteristics and testes of dwarf goats (Caprahircus), Exp. Toxicol. Pathol. 2009; 61: 339-346.

7. Ahmadi A, Hosseinimehr SJ, Naghshvar F, Hajir E, Ghahremani M. Chemoprotective effects of hesperidin against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Archives of Pharmacal Research, 2008; 31(6):794-7.
8. Aitken RJ, Harkiss DU, Buckingham DE. Analysis of lipid peroxidation mechanism in human spermatozoa. Mol Reprod Dev, 1993; 35:302–15.
9. Akiyama M. In vivo scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nippon Hinyokika Gakkai Zasshi, 1999; 90:421–8.
10. Allen JW, Ehling UH, Moore MM, Lewis SE. Germ line specific factors in chemical mutagenesis. Mutat Res, 1995; 330: 219–231.
11. Allhaza IM and Bashandy SA. Influence of Vitamin C on the Toxicity of PifPaf (Containing Permethrin) to Gonads of Male Rats. Saud. J. Biol. Sci. 1998; 5(1): 31-37.
12. Almeida MG, Ro¨pke CD, Davino SC, Nobre FAF, Santos E and Barros SBM. Modulation of hexachlorobenzene induced liver oxidative stress by a tocopherol. Toxicol Lett 1998; 95: 57.
13. Arafa HMM, Aly HAA, Abd-Ellah MF, El-Refaey HM. Hesperidin attenuates benzo[α] pyrene-induced testicular toxicity in rats via regulation of oxidant/antioxidant balance.Toxicology and Industrial Health, 2009; 25: 417- 427.
14. Balakrishnan A and Menon VP. Protective effect of hesperidin on nicotine induced toxicity in rats. Indian Journal of Experimental Biology, 2007; 45: 194-202.
15. Barlow SM, Sullivan FM, Lines J. Risk assessment of the use of deltamethrin on bed nets for the prevention of malaria. Food Chem Toxicol, 2001; 39:407–22.
16. Ben Abdallah R, Ben Slima A, DammakI, Keskes-Ammar L Mallek Z .(2010).
17. Bhunya SP and Pati PC. Effect of deltamethrin, a synthetic pyrethroid, on the induction of chromosome aberrations, micronuclei and sperm abnormalities in mice. Mutagenesis, 1990; 5: 229-232.
18. Brewen GJ, Preston JR. Analysis of chromosomal aberrations in mammalian germ cells.Chem. Mutagen, 1978; 5: 127- 150.
19. Calore EE, Cavaliere MJ, Puga FR, Calore NMP, Rosa AR, Weg R, Dias SS, Santos RP. Histologic peripheral nerve changes in rats induced by deltamethrin, Ecotoxicol. Environ. Saf, 2000; 47:82-86.
20. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ, 1992; 305: 609-613.

21. Casini ML, Gerli S, Unfer V. An infertile couple suffering from oligospermia by partial sperm maturation arrest: can phytoestrogens play a therapeutic role? A case report study. Gynecol Endocrinol, 2006; 22: 399-401.
22. Chauhan LKS, Agarwal DK, Sundararaman V. (1997): In vivo induction of sister
23. Chauhan LKS, Kumar M, Paul BN, Goel SK, Gupta SK. Cytogenetic effects of commercial formulations of deltamethrin and/or isoproturon on human peripheral lymphocytes and mouse bone marrow cells. Environmental and Molecular Mutagenesis, 2007; 48:636-643.
24. Chiba H, Uehara M, Wu J. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. J. Nutr. 2003; 133(6): 1892-1897.
25. Chromatid exchanges in mouse bone marrow following oral exposure to commercial formulations of alpha-cyano pyrethroids. Toxicol. Lett. 93: 153- 157.
26. Comparative effects of dimethoate and deltamethrin on reproductive system in male mice. Andrologia; 42:182-186.
27. Devi PU, Bisht KS, Vinitha M. A comparative study of radioprotection by ocimum flavonoids and synthetic aminothiol protectors. Br J Radiol, 1998; 71:782-4.
28. Dhawan K, Kumar S, Sharma A. Beneficial effects of chrysin and benzoflavone on virility in 2-year-old male rats. J Med Food, 2002; 5: 43-48.
29. Dolara P, Salvadori M, Capobianco T, Torricelli F. (1992): Sister chromatid exchanges in human lymphocytes induced by dimethoate,omethoate, deltamethrin, benomyl and their mixture. Mutat Res; 283:113–118.
30. Elbetieha A, Daas SI, Khamas W, Darmani H. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol, 2001; 41: 522-528.
31. El-Gohary M, Awara WM, Nassar S, Hawas S. Deltamethrin-induced testicular apoptosis in rats: the protective effect of nitric oxide synthase inhibitor. Toxicology, 1999 132:1–8.
32. El-Zayat E, Rawi S, Ismal N. Delayed Effects of Acute Deltamethrin Toxicity on Brain and Blood Monoamines and Free Amino Acids: Therapeutic Role of Selenium-Supplementation. Research Journal of Environmental Toxicology, 2008;; 2: 35-52.
33. Eskenazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Hum. Reprod, 2005; 20(4): 1006-1012.

34. Fadlalla, E; Ruixue, W; Zhihong, Z; Yuting, J; Shuang, C and Ruizhi, L influence of genetic abnormalities on semen quality and male fertility: A four – year prospective study. Iranian Journal of reproductive medicine, 2014; 2(2): 95-102.
35. Farouk, V.S. (2007): Clinico-pathological studies on the effect of exposure of rats to deltamethrin insecticide. M.V. Sc., Thesis Fac. Vet. Med., Cairo University.
36. Fatma MFand Omima IA. Cytogenetic effects of triazophos and deltamethrin in rat bone marrow. Egypt. J. Comp. Pathol. Clin. Path. 2003; 16: 16-33.
37. Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A,Tripodo MM. Biological effects of hesperidin, a citrus flavonoid. (Note I): antiinflammatory and analgesic activity. Farmaco, 1994; 40(11): 709-712.
38. Giray B, Gurbay A, Hincal F. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E and allopurinol. Toxicol. Lett, 2001; 118: 139-146.
39. Goel HC, Samanta N, Kannan K, Kumar IP, Bala M. Protection of spermatogenesis in mice against gamma ray induced damage by Hippophae rhamnoides. Andrologia, 2006; 38: 199-207.
40. Guzmán-Gutiérrez SL, Navarrete A. Pharmacological exploration of the sedative mechanism of hesperidin identified as the activeprinciple of Citrus sinensis flowers. Planta. Med. 2009; 75(4): 295-301.
41. Hirata A, Murakami Y, Shoji M, Kadoma Y, Fujisawa S. Kinetics of radical-scavenging activity of hesperetin and hesperidin and their inhibitory activity on COX-2 expression. Anticancer Res. 2005; 25(5): 3367-3374.
42. Hossain MM, Suzuki T, Sato I, Takewaki T, K. Suzuki K, Kobayashi H. (2005).
43. Hosseinimehr SJ, Ahmadi A, Beiki D,Habibi E, Mahmoudzadeh A. Protective effects of hesperidin against genotoxicity induced by 99mTc-MIBI in human cultured lymphocyte cells. Nuclear Medicine and Biology, 2009; 36: 863-867.
44. Hosseinimehr SJ, Nemati A. Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. Br J Radiol, 2006; 79: 415-8.
45. Hozayen WG. Effect Of Hesperidin and Rutin On Doxorubicin Induced Testicular Toxicity in Male Rats. International Journal of Food and Nutrition Science, 2012; 1(1): 31-41.
46. Husain R, Adhami VM, Seth PK. Behavioral, neurochemical, and neuromorphological effects of deltamethrin in adult rats. J. Toxicol. Environ. Health, 1996; 48: 467-475.
47. Isidori AM, Pozza C, Gianfrilli D, Isidori A. Medical treatment to improve sperm quality. J. Reprod Biomed, 2006; 12: 704-714.

48. Ismail MF and Mohamed HM. Deltamethrin-induced genotoxicity and testicular injury in rats: Comparison with biopesticide. Food and Chemical Toxicology, 2012; 50: 3421-3425.
49. Izawa H, Kohara M, Aizawa K, Suganuma H, Inakuma T, Watanabe G. Alleviative effects of quercetin and onion on male reproductive toxicity induced by diesel exhaust particles. Biosci Biotechnol Biochem, 2008; 72: 1235- 1241.
50. Jakobs DS, Kaisten Jr BL , Demmott wR , wolfson wl. (1990):Laborating test Hand Book, Lexi- Compand williams & wilkins Ed (2nd Edition).
51. Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B, Penkowski A. Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. J. Reprod. Dev. 2006; 52: 203-209.
52. Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocyte: a possible involvement of reactive oxygen species. Toxicol Lett, 1999; 105:197–205.
53. Kalpana KB, Devipriya N, Srinivasan M, Menon VP. Investigation of the radioprotective efficacy of hesperidin against gamma-radiation induced cellular damage in cultured human peripheral blood lymphocytes. Mutat Res, 2009; 676(1-2):54-61.
54. Kan Y, Cengiz EI, Ugurlu P, Mahmut Yanar M. The protective role of vitamin E on gill and liver tissue histopathology and micronucleus frequencies in peripheral erythrocytes of Oreochromis niloticus exposed to deltamethrin. Environmental Toxicology and Pharmacology, 2012; 34: 170-179.
55. Kawaguchi K, Kikuchi S, Hasunuma R, Maruyama H, Yoshikawa T, Kumazawa Y. A citrus flavonoid hesperidin suppresses infection-induced endotoxin shock in mice. Biol. Pharm. Bull. 2004; 27(5): 679-683.
56. Khaki A, Fathiazad F, Nouri M, khaki AA, Ghanbari Z, Ghanbari M, Ouladsahebmadarek E, Javadi L, Farzadi L. Anti-oxidative effects of citro flavonoids on spermatogenesis in rat. African Journal of Pharmacy and Pharmacology, 2011; 5(6): 721-725.
57. Khan, S; M.H. Jan; Kumar, D and AG. Telang Firpronil induced spermatoxicity is associated with oxidative stress, DNA damge and apoptosis in mals rats pestricide Bichemistry and physiology, 2015; 124: 8-14.
58. Lee A, East J and Balgaug P. Interactions of insecticides with biological membranes. Pestic. Sci. 1991; 32: 317–327.

59. Loscalzo LM, Wasowski C, Paladini AC, Marder M. Opioid receptors are involved in the sedative and antinociceptive effects of hesperidin as well as in its potentiation with benzodiazepines. Eur. J. Pharmacol. 2008; 580(3): 306-313.
60. Lu CC and Meistrich ML. Cytotoxic effect of chemotherapeutic drugs on mouse testis cells.Cancer Res, 1979; 39: 3575- 3582.
61. Luty S, Latuszynska J, Obuchowska-Przebirowska D, Tokarska M, Haratym-Maj A. Subacute toxicity of orally applied alpha-cypermethrin in swiss mice. Ann Agric Environ Med, 2000; 7:33–41.
62. Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. Indian J. Med. Res, 2009; 129(4): 357-367.
63. Manjula SD, Benjamin S, Bairy KL. Modulatory effect of vitamin C on genotoxic effect of endosulfan in developing albino rats. Iranian Journal of Pharmacology & Therapeutics, 2006; 5:113-116.
64. McLachlan R I., O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, Robertson DM. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys and man. Rec Prog Horm Res, 2002; 57:149-179.
65. Meeker JD, Barr DB, Russ Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Human Reproduction, 2008; 23(8); 1932-1940.
66. Mi Y, Zhang C, Taya K. Quercetin protects spermatogonial cells from 2,4-d-induced oxidative damage in embryonic chickens. J Reprod Dev, 2007; 53: 749-754.
67. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 2000; 52: 673-375.
68. Monforte MT, Trovato A, Kirjavainen S, Forestieri AM, Galati EM, Lo Curto RB. Biological effects of hesperidin, a Citrus flavonoid. (note II): Hypolipidemic activity on experimental hypercholesterolemia in rat. Farmaco, 1995; 50(9): 595-599.
69. Mosher WD, Pratt WF. Fecundity and infertility in the United States: Incidence and trends. J. Fertil. Steril. 1991; 56: 192-193.
70. Naziroğlu M. Enhanced testicular antioxidant capacity in streptozotocin-induced diabetic rats: protective role of vitamins C and E and selenium. Biol. Trace. Elem. Res. 2003; 94(1):61-72.
71. Neuromechanical effects of pyrethroids, allethrin, cyhalothrin and deltamethrin on the cholinergic processes in rat brain, Life Sci.; 77: 795–807.

72. Odaa SS and El-Maddawy ZK. Protective effect of vitamin E and selenium combination on deltamethrin-induced reproductive toxicity in male rats. Experimental and Toxicologic Pathology, 2012; 64: 813-819.
73. Odin AP. Vitamins as antimutagens: advantages and some possible mechanisms of antimutagenic action. Mut Re, 1997; 386: 39-67.
74. Ohtsuki K, Abe A, Mitsuzumi H. Glucosyl hesperidin improves serum cholesterol composition and inhibits hypertrophy in vasculature. J. Nutr. Sci. Vitaminol. 2003; 49(6): 447-450.
75. Olorunshola KV, Achie LN, Akpomiemie ML. Ascorbic acid ameliorates toxic effects of chlopyrifos on testicular functions of albino rats. British Journal of Pharmacology and Toxicology, 2011; 2(5): 262-269.
76. Parker F. Skin and Hormones. In: Text Book of Endocrinology, William, RH. (Hresg). W.B. saunders Co., Philadelphia, 1981; 1080 – 1098.
77. Pauluhn J. Hazard identification and risk assessment of pyrethroids in the indoor environment. Toxicol Lett, 1999; 107: 193–9.
78. Peares AGE. (1985): Histochemistry theoretical applied volume two. Anayltical technology, Churchill livingston, Fourth edition.Edinburgh, London Melbourne and NewYork.
79. Poonam, S; Kambir, S and Mysra, J Dose Dependent Effect of Deltamethrin in testies, liver and Kidney of Waster Rats. Toxicology International, 2014; 21(3): 131-139.
80. Roseff SJ. Improvement in sperm quality and function with French maritime pine tree bark extract. J Reprod Med, 2002; 47: 821-824.
81. Salah SH, Abdou HS, Abdel Rahim EA. Modulatory Effect of vitamins A, C and E mixtures against tefluthrin pesticide genotoxicity in rats. Research Journal of Agriculture and Biological Sciences, 2009; 5(6): 1070-1078.
82. Saxena PN,Chauhan LKS, Gupta SK. Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of Allium sativum: Spectroscopic basis of chromosome damage. Toxicology, 2005; 216(2–3): 244-252.
83. Sekeroglu V, Sekeroglu ZA, Kefelioglu H. (2011): Cytogenetic ffects of commercial formulations of deltamethrin and/or thiacloprid on wistar rat bone marrow cells. Environmental Toxicology; (wileyonlinelibrary.com) 22 Jul DOI: 10.1002/tox.20746.
84. Shen Y and Sangiah S. Na+, K (+)-ATPase, glutathione, and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice. Arch. Environ. Contam. Toxicol. 1995; 29: 174-179.

85. Shivaraj, David M, Ravi KB. Spermatotoxicity evaluation of deltamethrin 1% + chlorpyiphos 35% EC by oral gavage in Wister rats. International Journal of Pharma and Bio Sciences, 2011; 2(1): 262-268.
86. Shrilatha B and Muralidhara Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. Reprod Toxicol. 2007; 23(4): 578-87.
87. Singh RN, Kumar P, Singh VK, Singh DK. Toxic effects of Deltamethrin on the levels of biochemical changes in the snail Lymnaea acuminata. Journal of Pharmacy Research, 2010; 3(8):1739-1742.
88. Sönmez M, Türk G and Yüce A. The effects of ascorbic acid supplementation on sperm quality, lipid proxidation and testosterone levels of male Wistar rats. Theriogenology, 2005; 63(7): 2063-2072.
89. Surrales J, Xamena N, Creus A, Catalan J, Norppa H, Marcos R. Induction of micronucleus by five pyrethroids insecticides in whole blood and isolated human lymphocytes cultures. Mutat. Res. 1995; 341: 169-184.
90. Szegletes T, Polyhos CS, Bálint T, A. Rady A, Lang G, Kufcsák O, Nemcsók J. In vivo effects of deltamethrin on some biochemical parameters of carp (Cyprinus carpio L.) Environmental Monitoring and Assessment, 1995; 35(2):97-111.
91. Trivedi PP, Tripathi DN, Jena GB. Hesperetin protects testicular toxicity of doxorubicin in rat: role of NF_B, p38 and caspase-3. Food Chem. Toxicol. 2011; 49(4): 838-847.
92. Tüzmen MN, Candan N, Kaya E. The Evaluation of Altered Antioxidative Defense Mechanism and Acetylcholinesterase Activity in Rat Brain Exposed to Chlorpyrifos, Deltamethrin, and Their Combination, 2007; 17(9): 535-540. 25
93. Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A and Buyukkomurcu F. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pestic. Biochem. Phys, 2007; 87:115-122.
94. Vani T, Saharan N, Mukherjee SC, Ranjan R, Kumar R, Brahmchari RK. Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of Catla catla (Ham.) and their amelioration by dietary supplement of vitamin C. Pesticide Biochemistry and Physiology, 2011; 01:16-20.
95. Verma RS, Mnugya A, Srivastava N. In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins, Pest. Biochem. Physiol. 2007; 88: 191–196.

96. Wang FYZ, Wang BJY, Wang J, D Bai D. Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamin C and E, Exp. Toxicol. Pathol, 2008; 59: 415-423.
97. Wielgomas B, and Krechniak J. Effect of α-Cypermethrin and Chlorpyrifos in a28-Day Study on Free Radical Parameters andCholinesterase Activity in Wistar Rats. Polish J. of Environ. Stud. 2007; 16(1): 91-95.
98. Williams PL and Burson JL. Industrial Toxicolo-gy. Safety and Health Applications in the Work Place. Lifetime Learning Publications,Van Nostrand Reinhold Company, New York, 1985; 293-300.
99. Wyrobek A, Gordon L, Bukhart JG, Francis MW, Kapp RW, Letz G, Malling HV, Topham JC, Whorton MD. On evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. Mut. Res, 1983; 115: 1-72.
100. Wyrobek AJ and Bruce WR. Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. USA. 1975; 72: 4425-4429.
101. Yang HS, Han DK, Kim JR, Sim JC. Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. J.Korean Med. Sci. 2006; 21: 445-451.
102. Yosida TH, Truchiya k, Moriwaki K. Frequency of chromosome polymorphism of Rattus collection in Japan.Chromosome, 1971; 33:30 - 40.
103. Yousef MI, Awad TTI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E.Toxicology, 2006; 227: 240-247.
104. Yousef MI. Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. Food Chem Toxicol, 2010; 48:1152–9.
105. Zhang SY, Ito Y, Yamanoshita O, Yanagiba Y, Kobayashi M, Taya K, Li C, Okamura A, Miyata M, Ueyama J. Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. Endocrinology, 2007; 148: 3941-3949.