In Vitro Study of the Effect of Cypermethrin on Human Sperm Function, DNA Fragmentation and the Possible Protective Role of Vitamins C and/ or E

Adel Zalata[1], Hussein Abdelaziz Abdalla[1], Mohamed Saad Serria[1], Amal A. Aziz[2] Sahar A. El-Dakroory[3], Amal A. El-Bakary[3]

[1]Medical Biochemistry, Faculty of Medicine, Mansoura University,
[2]Clinical Pathology Dept. Faculty of Medicine, Cairo University,
[3]Forensic Medicine & Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

Pyrethroid pesticides are used preferably over organochlorines and organophosphates due to their high effectiveness, low toxicity to non-target organisms and easy biodegradability. Cypermethrin, a type II synthetic pyrethroid pesticide, is widely used in Egypt in pest control programs in agriculture and in public health as well. The objective of the present study was to evaluate the potential of cypermethrin genotoxicity in sperm and to investigate the possible ameliorative effects of vitamin C and/or E on cypermethrin toxicity. This study was done on semen samples collected from 10 healthy normozoospermic volunteers. Each semen sample was divided into six aliquots. One served as control negative aliquot (group I) that was not exposed to any treatments. The second aliquot (group II) was incubated with 20 mM vitamin C (ascorbic acid) and 2 mM vitamin E (α-tocopherol). The third aliquot was exposed to cypermethrin with a dose of 10 µM (group III) for 6 hours at 37°C, while the other three aliquots (IV, V, VI) were incubated with 20 mM vitamin C, 2 mM vitamin E and vitamin C & E (20 mM, 2 mM) respectively for 30 min before cypermethrin exposure. All aliquots were kept at room temperature. Unexposed and exposed aliquots were analyzed for sperm concentration, motility, and viability according to WHO guidelines. Hypo-osmotic Swelling (HOS) test and the modified alkaline comet assay were carried out on the prepared samples. There was statistically significant decrease in parameters of sperm motion, seminal functions and increase in sperm DNA damage parameters in cypermethrin group. With addition of antioxidant vitamins C and E either alone or combined there was statistically significant improvement in all of the parameters of sperm motion, seminal functions and DNA damage parameters and the maximal improvement was with the combined vitamin C and E. It could be concluded that cypermethrin can alter sperm function and induce genotoxic effect on sperms in vitro and that the antioxidant vitamins (C and E) might be useful in antagonizing the toxic effects of cypermethrin on sperm.

INTRODUCTION

Synthetic pyrethroid insecticides are among the most commonly available to consumers today. Pyrethroid usage has increased in recent years due to the need to replace common organophosphate insecticides following use restrictions. A consequence of the increased availability, use and broad-spectrum applicability of pyrethroids, there is widespread exposure among the general population[(1)]. Diet[(2)] and household dust[(3)] are the primary sources of exposure to pyrethroids among non-occupationally exposed individuals.

Data on altered reproductive or endocrine function resulting from pyrethroid exposure are limited, but animal and in vitro studies suggest that some pyrethroids or their metabolites might possess endocrine disrupting properties[(2,4-6)] **and adversely affect semen quality[(6-9)].**

Investigation of environmental impacts on sperm DNA damage is important since sperm DNA damage adversely affects male fertility, contributing to poorer embryo development and lower pregnancy rates[(10-14)].

The comet assay (single-cell gel electrophoresis, "SCGE") is a simple method for measuring DNA strand breaks in eukaryotic cells. The assay has applications in testing different chemical and physical agents for genotoxicity and monitoring environmental contamination with genotoxins[(15)]. Sensitivity and specificity of the test are considered to be very high [(16)].

The antioxidant vitamins are the most important free radical scavengers in extra-cellular fluids, trapping radicals in the aqueous phase and protect biomembranes from peroxidative damage [(17,18)]. However, no detailed study of the protective effects of antioxidants against the toxic effects of the pyrethroids in mammals is available.

Therefore, the present work aims to study the in vitro toxic effect of a synthetic pyrethroids pesticide "cypermethrin" on semen quality, sperm DNA measured by the comet against that insecticide toxicity.

MATERIAL AND METHODS

Chemicals:

Cypermethrin analytical standard was obtained from Sigma-Aldrich (Labor-chemikalien GmbH). Stock solution was prepared by dissolving cypermethrin powder as 1% in dimethylsulphoxide (DMSO). Normal and low melting point agarose, NaCl, NaEDTA, Tris, Triton X-100, DMSO, proteinase K, NaOH, Biggers-Whitten-Whittingham medium (BWW), ethidium bromide, vitamins C (Ascorbic acid) and E (α-tocopherol) were purchased from Sigma.

Sperm preparation:

Semen samples were collected from 10 healthy normozoospermic volunteers after taking their informed consent. All specimens were collected by masturbation after recommended abstinence period of sexual activity for 72 hours and allowed to liquefy completely for 15–30 min at 37ºC. After complete liquefaction, the semen samples were analyzed according to the

recommendation of **WHO[19].** Only ejaculates with normal parameters (sperm concentration > 20 millions/ml), total sperm motility >50%, normal sperm morphology >30%) were included in this study.

Experimental design:

Each semen sample from participants was divided into six aliquots. One served as control negative aliquot (group I) and was not exposed to any treatments. The second aliquot served as control positive (group II) and was incubated with 20 mM vitamin C (ascorbic acid) and 2 mM vitamin E (α-tocopherol)**[20].** The third aliquot was exposed to cypermethrin with a dose of 10 μM **[21]** (group III) for 6 hours at 37°C, while the other three aliquots (IV ,V,VI) were incubated with 20 mM vitamin C, 2 mM vitamin E and vitamin C&E(20 mM, 2 mM) respectively for 30 min before cypermethrin exposure. All specimens (aliquots) were kept at room temperature.

Semen analysis:

Computer assisted semen analysis (Autosperm, Fertipro, Belgium) was performed according to **Hinting et al.[22].** Sperm morphology was evaluated by phase contrast microscope and sperm Mac stain (Fertipro, Belgium). White blood cells (WBCs) were detected by Peroxidase stain **[23].** Spermatozoa were separated from WBCs by Sill-select gradient (Fertipro V.V., Industriepark Noord, Beerneme, Belgium) and the purified spermatozoa were used for assessment of acrosin activity by gelatinolysis and for assessment of the functional integrity of the sperm membrane by hypo- osmotic swelling (HOS) test.

Hypo-Osmotic Swelling test:

One ml of freshly prepared hypo-osmotic medium (0.735g. sodium citrate dihydrate $Na_3C_6H_5O_7.2H_2O$ and 1.351 g. fructose in 100 ml distilled water) was added to 0.1ml of liquefied semen and mixed well. The mixture was incubated at 37C º for 30 minutes. The sperms were then examined under phase contrast microscope, and the swelling of sperm tail was identified. The number of swollen cells was counted in duplicate in a total of 100 spermatozoa **[19,24].**

Preparation of gelatin-covered microslides and gelatinolysis of spermatozoa:

Gelatin-covered slides were prepared by spreading 20μL of 5% gelatin (Merck, Darmstadt, Germany) in distilled water on the slides. The slides were then air-dried, stored at 4°C overnight and fixed and washed in phosphate-buffered saline. Purified spermatozoa were diluted 1:10 in phosphate buffered saline (PBS) containing 15.7mM α-D-glucose. Semen samples were smeared on prepared slides and incubated in a moist chamber at 37°C for 2 hours. The halo diameter around any 10 spermatozoa shown to be representative of sperm present in the ejaculate was measured in phase contrast with an eyepiece micrometer. The halo formation rate was calculated per slide as the percentage of spermatozoa showing a halo. One hundred spermatozoa were evaluated. An acrosin activity index was calculated by multiplying the halo diameter by the halo formation rate **[25].**

Determination of malondialdehyde (MDA):

Proteins of the seminal plasma were precipitated by addition of trichloroacetic acid (TCA). Then, thiobarbituric acid (TAB) reacts with malondialdehyde (MDA) to form thiobarbituric acid reactive product which is measured at 534nm [(26)].

COMET assay:

The modified alkaline **comet** assay for sperm [(27)] was carried out on the prepared samples. Fully frosted slides were covered with 100 µl 0.5% normal melting point agarose and the agarose was allowed to solidify. Sperms in BWW (10 µl) were mixed with 90 µl 0.5% low melting point agarose and used to form the second layer. The slides were then placed in lysis buffer for 1 h (2.5 M NaCl, 100 mM NaEDTA, 10 mM Tris, 1% Triton X-100 and 10% dimethylsulphoxide, pH 10). The slides were then incubated overnight at 37°C in 100 µl/µl proteinase K added to the lysis buffer. Then the slides were placed in a horizontal electrophoresis unit filled with fresh alkaline electrophoresis solution containing 300 mM NaOH and 1 mM EDTA, pH 12.5, for 20 min to allow the DNA in the cells to unwind. Electrophoresis, for 10 min was performed at room temperature, at 2 V (0.714 V/cm) and 300 m.A, obtained by adjusting the buffer level. The slides were then washed with a neutralizing solution of 0.4 M Tris, pH 7, to remove alkali and detergents. After neutralization, the slides were stained with 50 ml 20 mg/ml ethidium bromide and covered with a cover-slip. All steps were carried out under yellow light to prevent further DNA damage.

Slides were examined through a 20× objective with a fluorescence microscope equipped with an excitation filter of 565 nm and a barrier filter of 590 nm. A digitalized camera was attached to the microscope and images of cells were processed by a computer-assisted image-analysis system (Comet Score, TriTek, USA) to determine the comet parameters. Fifty cells were scored from each replicate slide (one hundred cells in total). Results were expressed as tail length (TL; the distance that DNA migrated) and also percentage of DNA in tail ((% Tail; the density of migrated DNA), one of the most reliable measurements for detecting DNA damage[(28)] and tail moment (TM) (tail length x tail percentage of DNA/ 100).

Statistical Analysis:

Man-Whitney U test was applied for comparison between groups applying MedCalc. Data were expressed as range and median values. P-value of <0.05 was considered as statistically significant..

RESULTS

There was no statistically significant difference between the two control groups as regards sperm motility, functions, and comet parameters(Tables 1, 2, and 3).

There was statistically significant decrease in all parameters including; motility grade A (P = 0.0002), motility grade A&B (P = 0.0002), velocity (P = 0.0002), linear velocity (P = 0.0002) and linear- index (P = 0.0002) in cypermethrin group when compared to the control group. On addition of antioxidant vitamins, there was statistically significant improvement in

all of the sperm motility parameters and the maximal improvement was with combined vitamin C & E (group 6) including; motility grade A (P = 0.0002), motility grade A & B (P = 0.0002), velocity (P = 0.0005), linear velocity (P = 0.0002) and linear-index (P = 0.0002) when compared to cypermethrin group (table 1). However, there was significantly increase in MDA production in cypermethrin group compared with the control group. Also, addition of antioxidants was associated with significant decrease in MDA production compared with the control groups.

Table (2) shows statistically significant decrease in sperm function parameters including; acrosin activity index (P= 0.0002), halo diameter (P= 0.0002), halo percent (P = 0.0002), and HOST (P= 0.0002) in cypermethrin group when compared with the control group. Antioxidant vitamins produced statistically significant increase in all sperm function parameters and the maximal improvement was with adding combined vitamin C & E (group 6) including; acrosin index (P= 0.0002), halo (P= 0.0002), halo percent (P= 0.0002), host (P= 0.0002) when compared to the cypermethrin group (figure 1,2).

Also, in cypermethrin group there was statistically significant increase in comet parameters including; olive tail moment (P= 0.0002); tail length (P= 0.0002); DNA percentage in tail (P= 0.0002) when compared to the control group. Vitamins C and E induced statistically significant decline in all comet parameters and the maximal improvement was with adding both together (group 6) including; olive tail moment (P = 0.0002); tail length (P= 0.0002); DNA percentage in tail (P = 0.0002) when compared to the cypermethrin group (figure 3).

Table 1: Effect of cypermethrin on MDA production and semen motility parameters in different studied groups

	Group1 (Control)	**Group2 (Vitamins C&E)**	**Group3 (Cypermethrin)**	**Group4 (Cypermethrin and vitamin C)**	**Group5 (Cypermethrin and vitamin E)**	**Group6 (Cypermethrin and vitamins C&E)**
Motility parameters						
Grade A Range Median P P1	42.00-61.00 53.50	42.00-61.00 53.50 P = 0.8499	10.00-19.00 15.50 P = 0.0002	26.00-41.00 36.00 P = 0.0002 P_1 = 0.0002	22.00-34.00 26.50 P = 0.0002 P_1 = 0.0002	31.00-49.00 40.00 P = 0.0019 P_1 = 0.0002
Grade A&B Range Median P P1	62.00-77.00 68.00	53.00-76.00 66.50 P = 0.7050	15.00-29.00 22.50 P = 0.0002	38.00-49.00 44.50 P = 0.0002 P_1 = 0.0002	33.00-46.00 40.00 P = 0.0002 P_1 = 0.0002	44.00-60.00 50.00 P = 0.0002 P_1 = 0.0002
Velocity(µm/s) Range Median P P1	67.57-93.97 74.62	66.35-91.78 68.37 P = 0.1124	22.62-50.78 41.74 P = 0.0002	42.25-70.47 52.24 P = 0.0004 P_1 = 0.0126	36.41-60.18 52.92 P = 0.0002 P_1 = 0.0126	43.69-88.63 65.17 P = 0.0343 P_1 = 0.0005

Table 1 Cont.

Linear-velocity(µm/s) Range Median P P1	56.46- 82.70 65.35	53.96-82.77 63.08 P = 0.1509	13.91-37.69 25.69 P = 0.0002	34.18-60.02 41.47 P = 0.0002 P_1 = 0.0004	25.04-50.95 40.46 P = 0.0002 P_1 = 0.0015	39.34-75.00 53.85 P= 0.0191 P_1 = 0.0002
Linearity-index (%) Range Median P P1	83.56-92.38 87.46	79.19-92.42 87.30 P = 0.5967	53.47-74.22 62.72 P = 0.0002	75.12-85.16 80.27 P = 0.0003 P_1 = 0.0002	66.46-84.68 75.73 P = 0.0002 P_1 = 0.0009	75.55-90.82 83.39 P = 0.0284 P_1 = 0.0002
MDA mmol/ 10^8 spermatozoa Range Median P P1	3.09-8.09 6.42	3.7-8.51 6.4 P=0.9705	9.45-32.76 19.98 P<0.0001	4.88-14.65 10.93 P=0.0089 P1=0.0029	7.01-15.95 11.01 P<0.0001 P1=0.0029	3.99-12.02 7.91 P=0.0892 P1=0.0002

P is for comparison with group 1
P1 is for comparison with group 3.

Table 2: Effect of cypermethrin on sperm function parameters and the protective role of vitamins C &. E

	Group 1 (control)	**Group 2 (Vitamins C+E)**	**Group 3 (cypermethrin)**	**Group 4 (cypermethrin + Vitamin C)**	**Group 5 (cypermethrin + Vitamin E)**	**Group 6 (cypermethrin + Vitamins C+E)**
Acrosin activity index:						
Range	9.54-14.62	9.15-15.36	3.28-5.11	5.08-7.90	4.43-7.38	7.76-11.92
Median	11.84	11.85	3.92	6.55	5.68	9.06
P		P = 0.9118	P = 0.0002	P = 0.0002	P = 0.0002	P = 0.0019
P1				P1 = 0.0002	P1 = 0.0007	P1 = 0.0002
Halo diameter (nm):						
Range	12.39-17.49	12.53-17.85	8.54-11.26	9.51-13.07	9.26-12.24	11.59-15.02
Median	14.97	15.31	9.33	10.72	11.06	12.35
P		P = 0.6501	P = 0.0002	P = 0.0002	P = 0.0002	P = 0.0052
P1				P1 = 0.0233	P1 = 0.0284	P1 = 0.0002
Halo percent (%):						
Range	74.00-88.00	72.00-88.00	38.00-46.00	50.00-69.00	42.00-62.00	65.00-82.00
Median	79.00	79.50	41.00	62.00	56.00	70.00
P		P = 0.5700	P = 0.0002	P = 0.0002	P = 0.0002	P = 0.0046
P1				P1 = 0.0002	P1 = 0.0036	P1 = 0.0002
HOST (%):						
Range	84.00-96.00	83.00-95.00	23.00-45.00	55.00-80.00	56.00-74.00	62.00-84.00
Median	88.5	92.00	33.00	70.00	70.00	70.50
P		P = 0.8498	P = 0.0002	P = 0.0002	P = 0.0002	P = 0.0002
P1				P1 = 0.0002	P1 = 0.0002	P1 = 0.0002

P is for comparison with group 1
P1 is for comparison with group 3.

Table 3: Effect of cypermethrin on sperm COMT parameters and the protective role of vitamins C &. E

	Group 1 (Control)	**Group 2 (Vitamins C+ E)**	**Group 3 (cypermethrin)**	**Group 4 (cypermethrin + vitamin C)**	**Group 5 (cypermethrin + vitamin E)**	**Group 6 (cypermethrin + C and E)**
OTM Range Median P P1	0.9 – 1.37 1.07	0.76 – 1.39 1.11 P = 0.8534	1.80 – 4.53 3.18 P = 0.0002	1.46 – 2.06 1.72 P = 0.0002 P1 = 0.0005	1.50 – 2.29 2.10 P = 0.0002 P1 = 0.0082	1.11 – 1.47 1.30 P = 0.0343 P1 = 0.0002
Tail length Range Median P P1	0.22 – 0.82 0.58	0.20 – 0.76 0.51 P = 0.4057	1.42 – 2.73 1.65 P = 0.0002	0.80 – 1.40 1.13 P = 0.0002 P1 = 0.0002	0.93 – 1.66 1.31 P = 0.0002 P1 = 0.0082	0.32 – 0.89 0.56 P = 0.7055 P1 = 0.0002
DNA percentage in tail Range Median P P1	2.79-7.31 5.56	2.59 – 7.75 5.11 P = 1.0000	12.27 – 24.92 20.17 P = 0.0002	7.50 – 14.39 10.58 P = 0.0002 P1 = 0.0005	7.60 – 14.30 10.66 P = 0.0002 P1 = 0.0002	4.75 – 11.05 8.65 P = 0.0082 P1 = 0.0002

P is for comparison with group 1
P1 is for comparison with group 3.

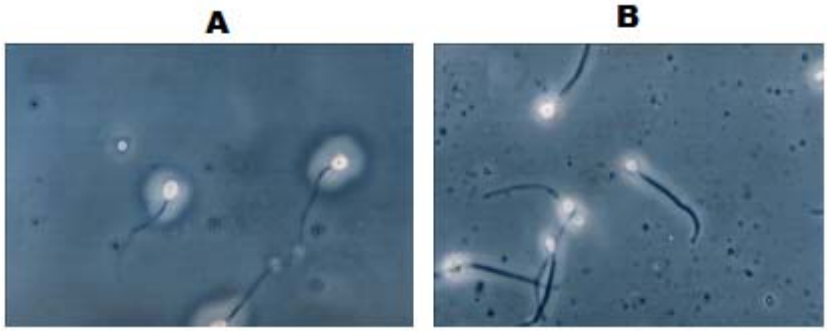

Figure (1): Acrosin activity: (500X) photomicrographs of human spermatozoa after 2 hours incubation on gelatin slides. (A) shows good acrosin activity and (B) shows poor and no acrosin activity

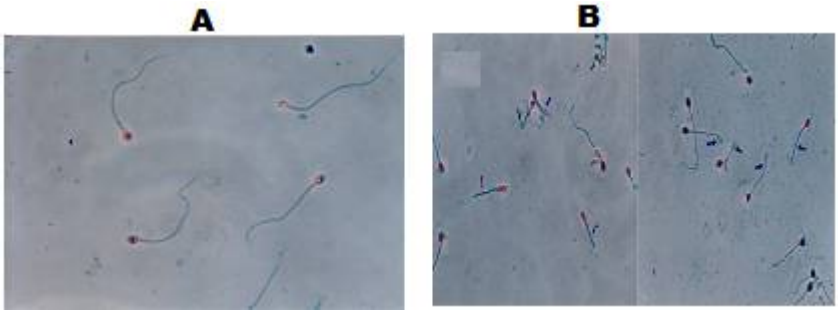

Figure (2): Hypo- osmotic swelling (HOS) test: Photomicrographs of (500X) sperm MAC stain spermatozoa. Acrosome, mid-piece, and tail are green in color, and the nucleus is red. A- Spermatozoa before HOS test (no tail swelling) B- Spermatozoa positive HOS test (arrows indicate different types of tail swelling).

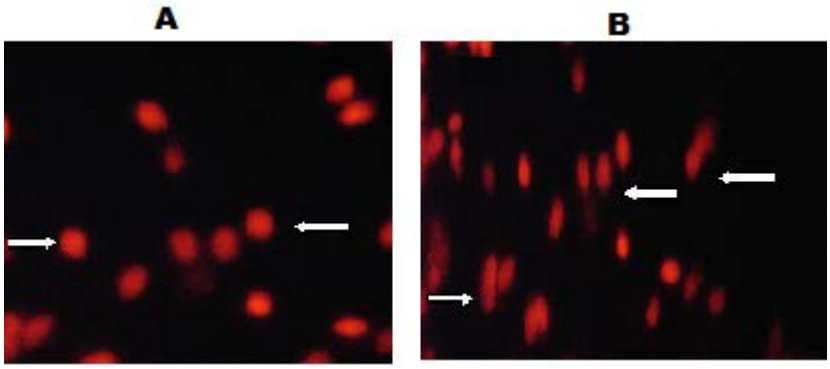

Figure (3): **Sperm nuclear DNA fragmentation test using comet Assay**(A) Comet assay showing normal sperms with undamaged DNA (without comet tail).. (B) Sperms with DNA damage and comet tails

DISCUSSION

Because of the extensive use of cypermethrin in different agricultural and public health purposes allover the world including Egypt, its genotoxic effects have considerable practical significance[15]. Also, environmental contamination and increased concentrations in different food products, therapeutic application and accidental/occupational exposure to pyrethroids are responsible for increasing oxidative stress in mammals[29].

The study revealed a statistically significant inhibition of all parameters of sperm motility and function by cypermethrin. This finding coincides with the result of **Yuan et al.** [30] who concluded that cypermethrin could reduce mature rat sperm motility via a direct interaction with sperm through unidentified mechanisms. Several hypotheses have been proposed to explicate how pesticides directly interact with sperm. Since vigorous motility of the sperm is dependent on the function of the intense transformation and energy expense produced in the mitochondria, pesticides may alter the mitochondria, producing a delay in motility and eventually leading to cell death[31]. Oxidative damage to mitochondrial membrane lowers the production of ATP[32] ultimately affecting the motility. The overall effect of membrane damage might be responsible for continuous decrease in motility and viability of spermatozoa after ejaculation[33]. Also, **Sun et al.**[34] reported that pyrethroids could act as anti-androgen in vitro. Besides testicular factors, epididymal dysfunction contributed also to abnormal sperm motility.

Concerning the sperm function parameters they were reduced by cypermethrin including acrosin index, halo percent and hypo-osmotic swelling test (HOS). HOS is a clear indicator of healthy status of membrane structure and function of sperm. Increased acrosin activity index promises the spermatozoa to penetrate through zona pellucida[35]. Spermatozoa are equipped with poor antioxidant defense system as compared to other cells[36]. Among environmental, genetic and physiological factors responsible for the poor sperm function, free radical induced oxidative stress gained much attention, due to its deleterious effects on sperm plasma membrane and DNA damage [37].

Cypermethrin preferentially gets localized in the hydrophobic core of the membrane, where it increases lipid packing and consequently decreases membrane fluidity [38].

The present study showed increased production of MDA as a marker of oxidative stress in particular with the addition of cypermethrin to human spermatozoa. In fact, this data was in harmony with the findings of **Atessahin et al.**[39] who revealed that administration of cypermethrin produces oxidative stress by generating reactive oxygen species and reducing the antioxidant defense systems. **Prasamthi et al.**[40] reported that oxidative damage, induced by pyrethroids might be due to their being lipophilic, whereby they could penetrate easily to the cell membrane and cause membrane lipid peroxidation.

This may explain the decline of sperm function parameters in the current study. Oxidative damage to plasma membrane, acrosomal and mitochondrial membranes in the form of lipid peroxidation results in the loss of functional membrane integrity and decreased production of ATP, which leads to infertility [35,41]. Furthermore, cypermethrin could induce impairments of the structure of seminiferous tubules and spermatogenesis in the male rats. The impairments could be attributed to the reduced AR expression [42].

As regards the effect of addition of vitamins C and E, there is overall improvement in all parameters of sperm motility, function and genotoxicity of cypermethrin with each vitamin alone. Higher protective effect was observed with vitamin C and maximal effect was shown with combined use of both vitamins. This finding agrees with **Lutsenko et al.**[43] results that directly support the hypothesis that vitamin C produces protection against oxidative DNA damage in human cells under oxidative stress. Also, it is consistent with results of **Abdou et al.**[44] who reported the protective role of vitamin C, as it reduced the number of abnormal sperms caused by tefluthrinin (a pyrethroid insecticide) in male albino rats. **Narayna et al.**[45] concluded that decreased ascorbic acid concentration in the testes was well correlated with decreased sperm count and increased sperm abnormalities, indicating a close relation between them. So, it could partly protect cells from the oxidative damage in the testis which might have affected the normal spermatogenesis.

Our results also coincides with the results of **Theng et al.** [35] who studied the effects of in vitro supplementation of antioxidant ascorbic acid on sperm plasma membrane integrity, acrosome intactness and mitochondrial activity index. There was highly significant improvement in these parameters that relate to the healthy state of the spermatozoa. **Abdou et al.**[44] results were consistent with the present results as regard to vitamin E which had a protective role in pyrethroid induced sperm genotoxicity. Also, **Raina e al.**[46] showed this ameliorative effect of vitamin E against oxidative stress of cypermethrin in rats. In another study, **Ben Abdallah et al.**[20] revealed that in vitro vitamin E improved the cytotoxic effects induced by dimethoate (an organophosphorous insecticide) on studied sperm parameters e.g. sperm motility and viability. Also, **Fulia et al.**[47] deduced the ameliorative effect of in vitro vitamin E on endosulphan-induced testicular toxicity in dwarf goats.

From the results of the current study, it could be concluded that the antioxidant vitamins (C and E) might be useful in alleviating the toxic effects of cypermethrin on sperm. So, administration of these vitamins to farmers, workers in pesticides factories or resident in exposed areas is recommended to protect them from sperm genotoxicity of these pollutants.

REFERENCES

1. **Meeker, J.D.; Barr, D.B. and Hauser, R. (2009):** Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men Reprod. Toxicol., 27(2):155-160.
2. **ATSDR. (2003):** Toxicological Profile for Pyrethrins and

Pyrethroids". Agency for Toxic Substances and Disease Registry; Atlanta, GA.

3. **Julien, R.; Adamkiewicz, G.; Levy, J.I.; Bennett, D.; Nishioka, M. and Spengler, J.D. (2008):** Pesticide loadings of select organophosphate and pyrethroid pesticides in urban public housing. J. Expo. Sci. Environ. Epidemiol. 18(2):167–174.
4. **Tyler, C.R.; Beresford, N.; van der Woning, M.; Sumpter ,J.P.; Thorpe K. (2000):"** Metabolism and environmental degradation of pyrethroid insecticides produce compounds with endocrine activities. Environ. Toxicol. Chem., 19(4):801-809**.**
5. **Mani, U.; Islam, F.; Prasad, A.K.; Kumar, P.; Suresh, K.V.;Maji, B.K.and Dutta, K.K. (2002)**: Steroidogenic alterations in testes and sera of rats exposed to formulated fenvalerate by inhalation. Hum. Exp. Toxicol., 21(11):593-597.
6. **Zhang,S.Y.; Ito, Y.; Yamanoshita, O.; Yanagiba, Y.; Kobayashi, M.; Taya, K.; Li, C., Okamura, A.; Miyata, M.; Ueyama, J. et al. (2007):** Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. Endocrinology, 148(8):3941-3949.
7. **Elbetieha, A;Da'as S.I.; Khamas, W.and Darmani ,H. (2001):** Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch. Environ. Contam. Toxicol., 41 (4):522-528.
8. **Yousef, M.I.; El-Demerdash, F.M.; Al-Salhen, K.S. (2003):** Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J. Environ. Sci. Health B, 38(4):463-478.
9. **El-Demerdash, F.M.;Yousef, M.I.; Kedwany, F.S.;Baghdadi, H.H. (2004):** Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats. J. Environ. Sci. Health B, 39(3):443-459.
10. **Duran, E.H.; Morshedi, M.; Taylor, S.and Oehninger, S. (2002):** Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum. Reprod., 17 (12):3122-3128.
11. **Morris, I.D.; Ilott, S.; Dixon, L.and Brison, D.R.(2002):** The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. Hum. Reprod.,17(4): 990-998.
12. **Agarwal, A. and Allamaneni, S.S. (2004)**: The effect of sperm DNA damage on assisted reproduction outcomes. A review. Minerva Ginecol., 56(4):235- 245.
13. **Lewis, .SE.and Aitken, R.J. (2005):** DNA damage to spermatozoa has impacts on

fertilization and pregnancy. Cell Tissue Res., 322(1):33-41.

14. **Borini, A.; Tarozzi, N.; Bizzaro, D.; Bonu, M.A.; Fava, L.; Flamigni, C. and Coticchio, G. (2006):** Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum. Reprod., 21 (11):2876-2881.
15. **El-Khatib,E.H.; Abdel-Aziz,M.; Badr,Y. and Kamal,N.(2005)** In-vivo genotoxicity of the synthetic pyrethroid pesticide "cypermethrin" in rat liver cells by comet assay .Arab J. Biotech, Vol. 8 (1): 67-82.
16. **Tice, R.R.; Agurell, E.; Anderson, D.; Burlinson, B.; Hartmann, A.; Kobayashi, H.; Miyamae, Y.; Rojas, E.; Ryu, J.C. and Sasaki, Y.F. (2000):"** Single cell gel/comet assay: guidelines for *in vitro* and *in-vivo* genetic toxicology testing". Environ. Mol. Mutagen., 35 (3): 206-221.
17. **Yavuz, T. ; Delibas, N.;Yildrim, B.; Altuntas, I.; Candri, O.; Cora, A.; Karahan, I. and Kustal, E. (2004):** Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamin E and C. Arch. Toxicol., 78(11):655-659.
18. **Sulak, O.; Altuntas, I.; Karahan, N.; Yildirim, B.; Akturk, O. ; Yilmaz, H.R. and Delibas, N. (2005):**Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamin E and C. Pestic. Biochem. Physiol., 83(1): 21-28.
19. **WHO (2010)**: WHO Laboratory manual for the examination of human semen and sperm-cervical mucus Interaction. 4th (ed.) Cambridge: Cambridge University Press.
20. **Ben Abdallah ,F.; H. Fetoui ,H.; . Zribi ,N.; Fakfakh,F. and Ammar-Keskes , L. (2012) :** Antioxidant supplementations *in vitro* improve rat sperm parameters and enhance antioxidant enzyme activities against dimethoate-induced sperm damages. Andrologia 44(Suppl.1):272-279.
21. **Sandal, S. and Yilmaz, B. (2011)**: Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. Environ. Toxicol., 26(5): 433–442.
22. **Hinting, A., Vermeulen, L. and Comhaire, F. (1988)** Evaluation of a simplified adenosine triphosphate release cytotoxicity test for the detection of sperm antibodies in human serum. J. Reprod.. Immunol., 13(2): 123–131.
23. **Endtz, A.W. (1972)** A direct staining method for moist urinary sediment and moist human sperm. Ned. Tijdschr .Geneeskd., 116(17): 681-5.
24. **24-Jeyendran R.S., Van der Ven H.H., Perez-Pelaez M., Crabo B.G., and Zaneveld L.J. (1984):** Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil., 70(1): 219-228.
25. **Henkel R., Muller C., Miska W., Schill W.B., Kleinstein J., and**

Gips H. (1995): Acrosin activity of human spermatozoa by means of a simple gelatinolytic technique: a method useful for IVF. J. Androl., 16(3): 272-277.

26. **Draper H. H., Squires E. J., Mahmoodi H., Wu J., Agarwal S., and Hadley M. A. (1993)**: A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radic. Biol. Med., 15 (4): 353 - 363.
27. **Hughes, C.M.; McKelvey-Martin, V.J. and Lewis, S.E. (1999)** Human sperm DNA integrity assessed by the Comet and ELISA assay. Mutagenesis, 14(1): 71–75.
28. **Kumaravel, T.S. and Jha, A.N. (2006)**: Reliable comet assay measurements for detecting DNA damage induced by ionizing radiation and chemicals. Mutat. Res., 605(1-2): 7–16.
29. **Yousef, M.I.; Awad, T.I. and Mohamed, E.H. (2006)**: Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. Toxicol., 227 (3): 240-247.
30. **Yuan C, Wang C, Gao SQ, Kong TT, Chen L, Li XF, Song L, Wang YB. (2010):** Effects of permethrin, cypermethrin and 3-phenoxybenzoic acid on rat sperm motility in vitro evaluated with computer-assisted sperm analysis. Toxicology in Vitro, 24(2):382–386.
31. **Betancourt, M.; Reséndiz, A.and Fierro, E.C. (2006):** Effects of two insecticides and two herbicides on the porcine sperm motility patterns using computer-assisted semen analysis (CASA) in vitro. Reproductive Toxicology 22(3):508–512.
32. **Wang, X.; Sharma, R.K.; Gupta, A.; George, V.; Thomas, A.J.Jr.; Falcone, T. and Agarwal, A. (2003):"** Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study". Fertil. Steril., 80 (Suppl. 2): 844-850.
33. **Calamera, J.C; Fernandez, P.J.; Buffone, M.G.; Acosta, A.A. and Dancel, G.F. (2001):** Effect of long- term in vitro in cubation of human spermatozoa: functional parameters and catalase effect. Andrologia 33(2): 79-86.
34. **Sun, H.; Xu, X.L.; Xu, L.C.; Song, L.; Hong, X.; Chen, J.F.;Cui, L.B.and Wang, X.R. (2007):** Anti-androgenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere, 66(3): 474–479.
35. **Theng A.D.; Gajare K.A. and Deshmukh A.A. (2009):** Protective effects of in vitro supplementation of ascorbic acid on plasma membrane, acrosomal membrane and mitochondrial activity index of human spermatozoa. Current Trends in Biotechnology and Pharmacy , 3 (3):291-296.
36. **Donnelly, E.T.; McClure, N. and Lewis, S.E. (2000):** Glutathione and hypotaurine in vitro: effects on human sperm motility, DNA integrity and production of reactive oxygen species. Mutagenesis 15 (1): 61-68.

37. **Agarwal, A.; Saleh,R.A. and Bedaiwy, M.A. (2003):** Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil. Steril., 79(4): 829-843.
38. **Gabbianelli R, Falcioni G, Nasuti C and Cantalamessa F. (2002):** Cypermethrin - induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. Toxicol. 175(1-3): 91-101.
39. **Atessahin A**, **Yilmaz S**, Karahan I, Ceribasi AO, Karaoglu A. **(2005)**: The effects of vitamin E and selenium on cypermethrin induced oxidative stress in rats. Turk J. Vet. Anim. Sci., 29(2-3):385-391.
40. **Prasamthi K., MuralidharaR., Rajini PS. (2005)**: Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. Food Chem. Toxicol., 43(2): 299-306.
41. **Zalata AA, Ahmed AH, Allamaneni SS, Comhaire FH, Agarwal A. (2004):** Relationship between acrosin activity of human spermatozoa and oxidative stress. Asian J. Androl., 6(4):313–318.
42. **Hu JX, Li YF, Li J, Pan C, He Z, Dong HY, Xu LC. (2011):** Toxic effects of cypermethrin on the male reproductive system: with emphasis on the androgen receptor. J. Appl. Toxicol.,: 10.1002/jat. 1769. [Epub ahead of print].
43. **Lutsenko, E.A.; Carcamo,J.M. and Golde, D.W. (2002):** Vitamin C Prevents DNA Mutation Induced by Oxidative Stress. J. Biol. Chem., 277(19): 16895–16899.
44. **Abdou H.S.; Salah,S.H. and Abdel Rahim, E.A.(2009):"** The Ability of Vitamins A, C and E as Antioxidants Against The Genotoxic Potential of Tefluthrin". Australian Journal of Basic and Applied Sciences 3(4): 4190-4198.
45. **Narayna, K.; Prashanthi N.; Nayanatara A., Kuma H.H.; Abhilash K. and Bairy K. L., (2005):** Effects of methyl parathion (o,o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutat. Res., 588(1): 28-34.
46. **Raina, R.; Verma, P.K.; Pankaj, N.K. and Kant, V. (2009):** Ameliorative effects of _-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats. International Journal of Medicine and Medical Sciences 1(9):396-399.
47. **Fulia, A., Chauhan, P.K. and Sharma, R.K. (2011):** Ameliorating effect of vitamin E on testicular toxicity induced by endosulphan in *Capra hircus in vitro*. J. Pharmacol. Toxicol., 6: 133-140.

دراسة خارج الجسم للتأثيرات السمية لمبيد السيبرميثرين علي الحيوانات المنوية والدور الوقائي المحتمل لفيتامين ج وهـ

عادل زلطة[1] - حسين عبد العزيز[1] محمد سرية[1] - أمال أ عزيز[2] -سحر الدكرورى[3]- آمال عبد السلام[3]

قسم الكيمياء الحيويه[1]- كلية الطب – جامعة المنصورة - قسم الباثولوجيا الاكلينيكيه[2] – كليه الطب – جامعه القاهره

[3] قسم الطب الشرعي والسموم الإكلينيك – كليه الطب – جامعة المنصورة

تعتبر المبيدات الحشرية البيروثرويدية من المبيدات المفضلة في الإستعمال عن مركبات الكلورالعضوية والفوسفورية العضوية نظرا لفعاليتها العالية وسميتها المنخفضة على الكائنات الحية غير المستهدفة، وسهولة التحلل البيولوجي. ويستخدم السايبرمثرين ، النوع الثاني من المبيدات الحشرية البيروثرويدية المصنعة، في مصر بكثرة في برامج مكافحة الآفات في الزراعة والصحة العامة أيضا. وتهدف هذه الدراسة إلي تقييم إمكانية السايبرمثرين في إحداث السمية الوراثية في الحيوانات المنوية بإستخدام تحليل المذنب القلوي المعدل و التحقق من الآثار التحسينية المحتملة لفيتامينات ج وهـ علي تلك السمية.وقد أجريت هذه الدراسة على عينات من السائل المنوي تم جمعها من عشرة متطوعين أصحاء (normozoospermic). ثم تم تقسيم كل عينة من السائل المنوي إلى ستة أقسام (مستخلصات) .القسم الأول استخدم كعينة ضابطة سلبية التي لم تتعرض إلى أي علاج (المجموعة الأولى). القسم الثاني استخدم كعينة ضابطة إيجابية وقد حضنت مع ٢٠ مللي مول فيتامين ج (حمض الاسكوربيك) و ٢ مللي مول فيتامين هاء (ألفا-توكوفيرول). أما القسم الثالث تم تعريضه لمبيد السايبرمثرين بجرعة ١٠ ميكرو مول لمدة ٦ ساعات في درجة حرارة ٣٧ درجة مئوية (المجموعة الثالثة). في حين تم تحضين المستخلصات الثلاثة الأخرى (المجموعة الرابعة والخامسة والسادسة) مع فيتامين (ج) بجرعة ٢٠ مللي مول ، ٢ مللي مول فيتامين هـ وفيتامين ج مع هـ (٢٠ مللي مول، ٢ ملل مول) على التوالي لمدة ٣٠ دقيقة قبل التعرض للسايبرمثرين. وأبقي كل مستخلص في درجة حرارة الغرفة. وقد تم تحليل المستخلصات المعرضة والغير معرضة من حيث تركيز الحيوانات المنوية، والقدرة على الحركة، وقدرتها على البقاء وفقا لتوجيهات منظمة الصحة العالمية.وتم عمل تحليل المذنب القلوي المعدل.وقد أسفرت النتائج عن انخفاض ملحوظ إحصائيا في حركة الحيوانات المنوية ووظيفتها في مجموعة السايبرمثرين. وكان هناك تحسن كبير إحصائيا في كل من حركة الحيوانات المنوية، ووظيفتها و تلف الحمض النووي مع اضافة الفيتامينات المضادة للأكسدة ج وهـ إما كل علي إنفراد أو مع بعضها البعض وكانت درجة التحسن القصوى مع فيتامين ج وهـ سويا وتخلص النتائج إلى أن السايبرمثرين يمكن أن يغير من وظائف الحيوانات المنوية وإحداث تأثير سمي جيني على الحيوانات المنوية ، وإلى أن الفيتامينات المضادة للأكسدة (ج و هـ) يمكن أن تكون مفيدة في تحسين التأثيرات السامة من السايبرمثرين على الحيوانات المنوية.