TOXIC EFFECTS OF CYPERMETHRIN ON SPERMS AND THE POSSIBLE PROTECTIVE ROLE OF VITAMINES C AND E : AN INVITRO STUDY

BY

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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 vitamins C and 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 (?-tocopherol). The third aliquot was exposed to cypermethrin with a dose of 10 μM (group III). 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 for 6 h at 37℃. 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 motility and seminal function and increase in sperm DNA damage parametrers in cypermethrin group. With addition of antioxidant vitamins C and E either alone or with each other; there was statistically significant improvement in all of the parameters of sperm motion, seminal function and DNA damage parametrers and the maximal improvement was with both vitamins C & E. It can be concluded that cypermethrin can alter sperm function and induce genotoxic effect on sperms in vitro and that the antioxidant vitamins (C and E) can be useful in improving the toxic effects of cypermethrin on sperm.

Key words: *sperm, invitro, cypermethrin, DNA damage, semen function.*

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 (Meeker et al., 2009). Diet (ATSDR, 2003) and household dust (Julien et al., 2007) 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 may possess endocrine disrupting properties (Tyler et al., 2000; Mani et al., 2002; ATSDR, 2003; Zhang et al., 2007) and adversely affect semen quality (Elbetieha et al., 2001; Yousef et al., 2003; El-Demerdash et al., 2004; Zhang et al., 2007).

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 (Duran et al., 2002; Morris et al., 2002; Agarwal and Allamaneni, 2004; Lewis and Aitken, 2005; Borini et al., 2006).

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 (El-Khatib et al., 2005). Sensitivity and specificity of the test are considered to be very high (Tice et al., 2000).

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 (Yavuz et al., 2004 and Sulak et al., 2005). 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 and sperm DNA measured by the comet assay and the possible protective role of vitamines C and E as antioxidant agents against this insecticide toxicity.

MATERIAL AND METHODS

Chemicals :

Cypermethrin analytical standard was obtained from Sigma-Aldrich (Laborchemikalien GmbH). Stock solution was prepared by dissolving cypermethrin powder as 1% in dimethyl sulphoxide (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 h and allowed to liquefy completely for 15-30 min at 37ºC.

After complete liquefaction, the semen samples were analyzed according to WHO (2010). 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 treatment. 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) (Ben Abdallah et al., 2012). The third aliquot was exposed to cypermethrin with a dose of 10 μM (Sandal and Yilmaz, 2011) (group III). While the other three aliquots (IV ,V,VI) were incubated with 20 mM vitamin C, 2 mM vitamin E and vitamins C & E (20 mM, 2 mM) respectively for 30 min before cypermethrin exposure. All specimens (aliquots) were kept for 6 h at 37ºC.

Semen analysis:

Computer assisted semen analysis (Autosperm, Fertipro, Bilguim) was performed according to Hinting et al., (1988). Sperm morphology was evaluated by phase contrast microscope and sperm Mac stain (Fertipro, Bilguim). White blood cells (WBCs) were detected by Peroxidase stain. Spermatozoa were separated from WBCs by Sill-select gradient (Fertipro V.V., Industriepark Noord, Beerneme, Bilguim) 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 (HOS) test for assessment of the functional integrity of the sperm membrane:

One ml of freshly prepared hypo-osmotic medium (0.735 g sodium citrate dihydrate 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 37ºC 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 (Jeyendran et al., 1984; WHO, 2010).

Preparation of gelatin-covered microslides and gelatinolysis of spermatozoa:

Gelatin-covered slides were prepared by spreading 20 μL 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 PBS containing 15.7 mM 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 (Henkel et al., 1995).

Comet assay:

The modified alkaline comet assay for sperm (Hughes et al., 1999) was carried out on the prepared samples. Fully frosted slides were covered with 100 ml 0.5% normal melting point agarose and the agarose was allowed to solidify. Sperms in BWW (10 ul) were mixed with 90 ul 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% dimethyl sulphoxide, pH 10). The slides were then incubated overnight at 37°C in 100 ml/ml 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 mA, 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 coverslip. 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), percentage of DNA in tail ((% Tail; the density of migrated DNA), one of the most reliable measurements for detecting DNA damage (Kumaravel and Jha, 2006) and tail moment (TM) (tail length x percentage of DNA in tail / 100).

Statistical Methods:

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

1- Regarding the control groups (group I and II):

There was no statistically significant difference between the two groups in the semen quality parameters, sperm motility parameters, seminal function analysis and sperm DNA damage measures (Tables 1, 3, 5).

2- Regarding cypermethrin treated group:

a- Semen quality parameters (Table 1):

The concentration and morphology showed no statistical difference in comparison to control group.

b- Sperm motility parameters (Table 1) :

There was statistically significant decrease (P<0.05) in all parameters including; motility grade A (median=15.50), motility grade B (median=8), motility grade A & B (median=22.50), velocity (median=41.74), linear velocity (median=25.69) and linear- index (median = 62.72) when compared to the control group which showed (medians=53.5, 17.5, 68.0, 74.62; 65.35; 87.46 for those parameters respectively).

c- Seminal function parameters (Table 3):

There was statistically significant decrease (P<0.05) in all parameters including; acrosin index (median=3.92), halo (median=9.33), halo percent (median=41.00), host (median=33.00) when compared to the control group which showed (medians =11.85; 14.97; 79.00; 88.5 for those parameters respectively).

d- Sperm DNA damage measures:

There was statistically significant increase (P<0.05) in all parameters including; olive tail moment (median=3.18); tail length (median=1.65); DNA percentage in

tail (median=20.17) when compared to the control group which showed (medians =1.07; 0.58; 5.56 for those parameters respectively) (Figure 1).

3- Regarding cypermethrin plus antioxidants groups; vitamin C, E, C&E:

a- Semen quality parameters (Table 2):

There was no statistically significant difference (P>0.05) in these parameters when compared to cypermethrin and control groups.

b- Sperm motility parameters (Table 2):

There was statistically significant improvement (P<0.05) in all of the parameters and the maximal improvement was with both vitamins C & E (group VI) including; motility grade A (median = 40.00), motility grade B (median = 11,50), motility grade A & B (median grade = 50.00), velocity (median = 65.17), linear velocity (median = 53.85) and linear- index (median = 83.39) when compared to cypermethrin group (medians = 15.50,8, 22.50, 41.74; 25.69 ; 62.72 for those parameters respectively).

c- Seminal function parameters (Table 4):

There was statistically significant increase (P<0.05) in all parameters and the maximal improvement was with adding both vitamin C&E (group VI) including; acrosin index (median=9.06), halo (median=12.35), halo percent (median=70.00), HOS test (median=70.50) when compared to the cypermethrin group which showed (medians =3.92; 9.33; 41.00, 33.00 for those parameters respectively).

d-Sperm DNA damage measures(Table 6):

There was statistically significant decline (P<0.05) in all parameters and the maximal improvement was with adding both vitamin C&E (group VI) including; olive tail moment (median=1.30); tail length (median=0.56); DNA percentage in tail (median =8.65) when compared to the cypermethrin group which showed (medians =3.18; 1.65; 20.17 for those parameters respectively).

DISCUSSION

Because of the extensive use of cypermethrin in different agricultural and public health purposes all over the world including Egypt, its genotoxic effects have considerable practical significance (El-Khatib et al., 2005). 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 (Yousef et al., 2006).

This study aimed to investigate whether cypermethrin has genotoxicity on human sperms by a simple sensitive test; the comet assay and to study the role of two antioxidants; vitamin C and E

in counteracting this toxicity.

Our study revealed a significant statistical inhibition of all parameters of sperm motility and function by cypermethrin. This coincides with the results of Yuan et al, (2010) 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 (Betancourt et al., 2006). Oxidative damage to mitochondrial membrane lowers the production of ATP (Wang et al, 2003) ultimately affecting the motility. The overall effect of membrane damage might be responsible for continuous decease in motility and viability of spermatozoa after ejaculation (Calamera et al, 2001).

Also, Sun et al., (2006) reported that pyethroids could act as antiandrogen 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 hypoosmotic 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 (Theng et al, 2009).

Spermatozoa are equipped with poor antioxidant defense system as compared to other cells (Donnelly, 2000). 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 (Agarwal, 2003).

Cypermethrin preferentially gets localized in the hydrophobic core of the membrane, where it increases lipid packing and consequently decreases membrane fluidity (Gabbianelli et al., 2002). The administration of cypermethrin has been shown to produce oxidative stress by generating reactive oxygen species and reducing the antioxidant defense systems (Atessahin et al., 2005). Prsanthi et al. (2005) reported that oxidative damage, induced by pyrethroids might be due to their lipophilieity, whereby they could penetrate easily to the cell membrane and cause membrane lipid peroxidation.This may explain the decline of sperm function parameters in this study. Oxidative damage to plasma membrane, acrosomal and mito-

chondrial membrane in the form of lipid peroxidation results in the loss of functional membrane integrity and decreased production of ATP, which leads to infertility (Theng et al, 2009).

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 agrees with Lutsenko et al. (2002) results that directly support the hypothesis that vitamin C protects against oxidative DNA damage in human cells under oxidative stress. Also, it is consistent with Abdou et al. (2009) results that 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. (2005) 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 can partly protect cells from the oxidative damage in the testis which could have affected the normal spermatogenesis.

Our results also coincides with the results of Theng et al. (2009) who studied the effects of in vitro supplementation of non-enzymatic 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. (2009) results were consistent with our results as regard to vitamin E which had a protective role in pyrethroid induced sperm genotoxicity. Also, Raina e al. (2009) showed this ameloriative effect of vitamin E against oxidative stress of cypermethrin in rats.

In another study, Ben Abdallah et al. (2011) 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., (2011) deduced the ameliorative effect of invitro vitamin E on endosulphan induced testicular toxicity in dwarf goats.

From the results of the current study, it can be concluded that the antioxidant vitamins (C and E) can be useful in improving 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.

Table (1) : Effect of cypermethrin on semen quality parameters and sperm motility parameters.

	Group I (Control)	Group II (Vitamins C&E)	Group III (Cypermethrin)
Semen quality			
Concentration (10^6 /ml)			
Range	22.00-28.00	22.00-29.00	23.00-29.00
Median	24.00	23.50	24.50
P		0.7028	0.5392
Morphology (%normal)			
Range	38.00-44.00	39.00-44.00	38.00-43.00
Median	41.50	42.00	39.00
P		0.9093	0.1267
Sperm motility:			
Grade A			
Range	42.00-61.00	42.00-61.00	10.00-19.00
Median	53.50	53.50	15.50
P		0.8499	0.0002
Grade B			
Range	12.00-20.00	11.00-19.00	5.00-10.00
Median	17.50	16.00	8.00
P		0.1712	0.0002
Grade A&B			
Range	62.00-77.00	53.00-76.00	15.00-29.00
Median	68.00	66.50	22.50
P		0.7050	0.0002
Velocity (µm/s)			
Range	67.57-93.97	66.35-91.78	22.62-50.78
Median	74.62	68.37	41.74
P		0.1124	0.0002
Linear-velocity (µm/s)			
Range	56.46-82.70	53.96-82.77	13.91-37.69
Median	65.35	63.08	25.69
P		0.1509	0.0002
Linear-index (%)			
Range	83.56-92.38	79.19-92.42	53.47-74.22
Median	87.46	87.30	62.72
P		0.5967	0.0002

P is for comparison with group I. P-value of <0.05 is considered as statistically significant.

Table (2) : Evaluation of the protective effect of Vitamins C &. E against cypermethrin toxicity as regards semen quality and sperm motility parameters.

	Group I (Control)	Group III (Cypermethrin)	Group IV (Cypermethrin and vitaminC)	Group V (Cypermethrin and vitamin E)	Group VI (Cypermethrin and vitamins C&E)
Semen quality					
Concentration (10^6/ml)					
Range	22.00-28.00	23.00-29.00	22.00-29.00	23.00-29.00	22.00-29.00
Median	24.00	24.50	27.00	26.00	25.00
P			P = 0.0932	P = 0.1883	P = 0.4475
P1			P_1 = 0.2547	P_1 = 0.5392	P_1= 0.6493
Morphology (%normal)					
Range	38.00-44.00	38.00-43.00	38.00-44.00	38.00-44.00	38.00-44.00
Median	41.50	39.00	39.50	39.50	41.00
P			P = 0.1710	P = 0.3234	P = 0.6485
P1			P_1 = 0.8478	P_1 = 0.7307	P_1 = 0.1832
Sperm motiltiy:					
Grade A					
Range	42.00-61.00	10.00-19.00	26.00-41.00	22.00-34.00	31.00-49.00
Median	53.50	15.50	36.00	26.50	40.00
P			P= 0.0002	P = 0.0002	P = 0.0019
P1			P_1 = 0.0002	P_1 = 0.0002	P_1 = 0.0002
Grade B					
Range	12.00-20.00	5.00-10.00	6.00-13.00	8.00-15.00	8.00-15.00
Median	17.50	8.00	8.50	12.00	11.50
P			P = 0.0002	P = 0.0008	P = 0.0006
P1			P_1 = 0.3057	P_1 = 0.0009	P_1 = 0.0101
Grade A&B					
Range	62.00-77.00	15.00-29.00	38.00-49.00	33.00-46.00	44.00-60.00
Median	68.00	22.50	44.50	40.00	50.00
P			P = 0.0002	P = 0.0002	P = 0.0002
P1			P_1 = 0.0002	P_1 = 0.0002	P_1 = 0.0002
Velocity (µm/s)					
Range	67.57-93.97	22.62-50.78	42.25-70.47	36.41-60.18	43.69-88.63
Median	74.62	41.74	52.24	52.92	65.17
P			P = 0.0004	P = 0.0002	P = 0.0343
P1			P_1 = 0.0126	P_1 = 0.0126	P_1 = 0.0005
Linear-velocity (µm/s)					
Range	56.46-82.70	13.91-37.69	34.18-60.02	25.04-50.95	39.34-75.00
Median	65.35	25.69	41.47	40.46	53.85
P			P = 0.0002	P = 0.0002	P= 0.0191
P1			P_1 = 0.0004	P_1 = 0.0015	P_1 = 0.0002
Linear-index (%)					
Range	83.56-92.38	53.47-74.22	75.12-85.16	66.46-84.68	75.55-90.82
Median	87.46	62.72	80.27	75.73	83.39
P			P = 0.0003	P = 0.0002	P = 0.0284
P1			P_1 = 0.0002	P_1 = 0.0009	P_1 = 0.0002

P is for comparison with group I and P1 is for comparison with group III. P-value of <0.05 is considered as statistically significant.

Table (3) : Effect of cypermethrin on seminal function parameters.

	Group I (Control)	Group II (Vitamins C&E)	Group III (Cypermethrin)
Acrosin index:			
Range	9.54-14.62	9.15-15.36	3.28-5.11
Median	11.84	11.85	3.92
P		0.9118	0.0002
Halo:			
Range	12.39-17.49	12.53-17.85	8.54-11.26
Median	14.97	15.31	9.33
P		0.6501	0.0002
Halo percent:			
Range	74.00-88.00	72.00-88.00	38.00-46.00
Median	79.00	79.50	41.00
P		0.5700	0.0002
HOS test:			
Range	84.00-96.00	83.00-95.00	23.00-45.00
Median	88.5	92.00	33.00
P		0.8498	0.0002

P is for comparison with group I**.** P-value of <0.05 is considered as statistically significant.

Table (4) : Evaluation of the protective effect of vitamins C and E against cypermethrin toxicity as regards seminal function parameters.

	Group I (Control)	Group III (Cypermethrin)	Group IV (Cypermethrin and vitaminC)	Group V (Cypermethrin and vitamin E)	Group VI (Cypermethrin and vitamins C&E)
Acrosin index:					
Range	9.54-14.62	3.28-5.11	5.08-7.90	4.43-7.38	7.76-11.92
Median	11.84	3.92	6.55	5.68	9.06
P			P = 0.0002	P = 0.0002	P = 0.0019
P1			P1 = 0.0002	P1 = 0.0007	P1 = 0.0002
Halo:					
Range	12.39-17.49	8.54-11.26	9.51-13.07	9.26-12.24	11.59-15.02
Median	14.97	9.33	10.72	11.06	12.35
P			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	38.00-46.00	50.00-69.00	42.00-62.00	65.00-82.00
Median	79.00	41.00	62.00	56.00	70.00
P			P = 0.0002	P = 0.0002	P = 0.0046
P1			P1 = 0.0002	P1 = 0.0036	P1 = 0.0002
HOS test:					
Range	84.00-96.00	23.00-45.00	55.00-80.00	56.00-74.00	62.00-84.00
Median	88.5	33.00	70.00	70.00	70.50
P			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 I and P1 is for comparison with group III.

P-value of <0.05 is considered as statistically significant.

Table (5) : Distribution of sperm DNA damage measures in control and cypermethrin groups (assessed by comet assay).

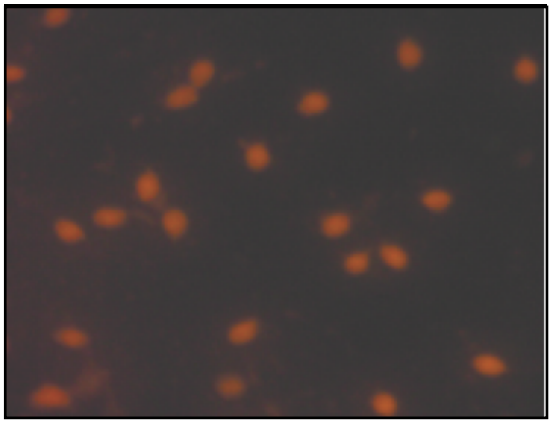
	Group I (Control)	Group II (Vitamins C&E)	Group III (Cypermethrin)
Olive Tail Moment(OTM)			
Range	0.9 – 1.37	0.76 – 1.39	1.80 – 4.53
Median	1.07	1.11	3.18
P		0.8534	0.0002
Tail length			
Range	0.22 – 0.82	0.20 – 0.76	1.42 – 2.73
Median	0.58	0.51	1.65
P		0.4057	0.0002
DNA percentage in tail			
Range	2.79- 7.31	2.59 – 7.75	12.27 – 24.92
Median	5.56	5.11	20.17
P		1.0000	0.0002

P is for comparison with group I. P-value of <0.05 is considered as statistically significant.

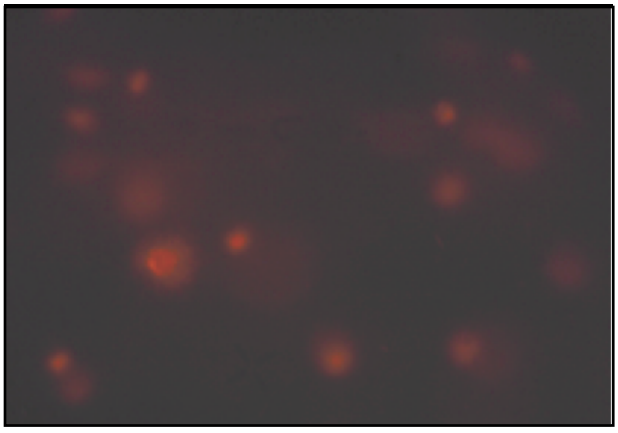
Table (6) : Distribution of sperm DNA damage measures in control, cypermethrin and antioxidants groups (assessed by comet assay).

	Group I (Control)	Group III (Cypermethrin)	Group IV (Cypermethrin and vitaminC)	Group V (Cypermethrin and vitamin E)	Group VI (Cypermethrin and vitamins C&E)
Olive Tail Moment(OTM)					
Range	0.9 – 1.37	1.80 – 4.53	1.46 – 2.06	1.50 – 2.29	1.11 – 1.47
Median	1.07	3.18	1.72	2.10	1.30
P			P = 0.0002	P = 0.0002	P= 0.0343
P1			P1 = 0.0005	P1 = 0.0082	P1 = 0.0002
Tail length					
Range	0.22 – 0.82	1.42 – 2.73	0.80 – 1.40	0.93 – 1.66	0.32 – 0.89
Median	0.58	1.65	1.13	1.31	0.56
P			P = 0.0002	P = 0.0002	P = 0.7055
P1			P1 = 0.0002	P1 = 0.0082	P1 = 0.0002
DNA percentage in tail					
Range	2.79-7.31	12.27 – 24.92	7.50 – 14.39	7.60 – 14.30	4.75 – 11.05
Median	5.56	20.17	10.58	10.66	8.65
P			P = 0.0002	P = 0.0002	P = 0.0082
P1			P1 = 0.0005	P1 = 0.0002	P1 = 0.0002

P is for comparison with group I and P1 is for comparison with group III.
P-value of <0.05 is considered as statistically significant.



a



b

Figure (1) : Comet assay of sperms from the control group showing no tailing (a) and from the cypermethrin group showing evident tailing in some cells (b).

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التأثيرات السمية لمبيد السيبرميثرين على الحيوانات المنوية والدور الوقائى المحتمل لفيتامين ج وهاء : دراسة خارج الجسم

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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 الثانى من المبيدات الحشرية البيروثرويدية المصنعة، في مصر بكثرة فى برامج مكافحة الآفات فى الزراعة والصحة العامة أيضا. وتهدف هذه الدراسة إلي تقييم إمكانية السايبرمثرين فى إحداث السمية الوراثية فى الحيوانات المنوية بإستخدام تحليل المذنب القلوى المعدل و التحقق من الآثار التحسينية المحتملة لفيتامينات ج وهاء على تلك السمية.وقد أجريت هذه الدراسة على عينات من السائل المنوى تم جمعها من عشرة متطوعين أصحاء (normozoospermic). ثم تم تقسيم كل عينة من السائل المنوى 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 مللى مول فيتامين ج (حمض الاسكوربيك) و ٢ مللي مول فيتامين هاء (ألفا-توكوفيرول). أما القسم الثالث تم تعريضه لمبيد السايبرمثرين بجرعة ١٠ ميكرو مول (المجموعة الثالثة). في حين تم تحضين المستخلصات الثلاثة الأخرى (المجموعة الرابعة والخامسة والسادسة) مع فيتامين (ج) بجرعة ٢٠ مللى مول ، ٢ مللى مول فيتامين هاء وفيتامين ج مع هاء (٢٠ مللى مول، ٢ ملل مول) على التوالى لمدة ٣٠ دقيقة قبل التعرض للسايبرمثرين. وأبقى كل مستخلص لمدة ٦ ساعات فى درجة حرارة ٣٧ درجة مئوية. 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 القلوى المعدل.وقد أسفرت النتائج عن انخفاض ملحوظ إحصائيا فى حركة الحيوانات المنوية ووظيفتها فى مجموعة السايبرمثرين. وكان هناك تحسن كبير إحصائيا في كل من حركة الحيوانات المنوية، ووظيفتها و تلف الحمض النووى مع اضافة الفيتامينات المضادة للأكسدة ج وهاء إما كل علي إنفراد أو مع بعضها البعض وكانت درجة التحسن القصوى مع فيتامين ج وهاء سويا وتخلص النتائج إلى أن السايبرمثرين يمكن أن يغير من وظائف الحيوانات المنوية وإحداث تأثير سمى جينى على الحيوانات المنوية، وإلى أن الفيتامينات المضادة للأكسدة (ج و هاء) يمكن أن تكون مفيدة فى تحسين التأثيرات السامة من السايبرمثرين على الحيوانات المنوية.