In-vivo genotoxicity of the synthetic pyrethroid pesticide "cypermethrin" in rat liver cells by comet assay

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ABSTRACT

The comet assay (single-cell gel electrophoresis, "SCGE") is a simple method for measuring deoxyribonucleic acid (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. The objective of the present study was to evaluate the genotoxic effects of the synthetic pyrethroid pesticide "cypermethrin", which is widely used in Egypt in pest-control programs in agriculture and in public health as well. Male rats were sacrificed 1, 7 or 14 days after administration of single oral dose 1/30, 1/10 or 1/5 LD_{50} of commercial formulation of cypermethrin. Single liver cell suspensions were prepared and Comet assay was performed. With the SCGE assay, a clear induction of DNA damage was observed. It is generally noticed that all pesticide treatments yielded statistically significant ($p<0.0001$) DNA damage. In conclusion, cyper methrin induced a clear significant positive dose-dependent increase in DNA damage in the rat liver cells exposed to cypermethrin as compared with controls. But the effects in the SCGE were generally decreased with time after treatments. The results of the present work suggested that comet assay might be a suitable and sensitive endpoint in genotoxicity evaluation of pesticides, but we confirm that various tests should be used for detecting the mutagenic activity of pesticides.

Key words: *Genotoxocity, cypermethrin, DNA damage, comet assay.*

INTRODUCTION

The induction of genetic damage is a critical step in the development of cancer, birth defects and other diseases. In particular, the multistage process of cancer development will be initiated by most of the chemical mutagens in the target organs. Therefore, a chemical genotoxicity in specific organs must be studied to properly evaluate its cancer-inducing potential (Miyamae *et al.*,1998). The number of chemicals applied in agriculture is constantly increasing, and although in general, it results in people benefiting from higher and better crop production, it might bring some genotoxic hazard to environment and human health too. Pesticides are very often applied in contemporary agriculture. For years, they have been considered to be potentially dangerous for human health (Sabbioni and Numann, 1990). Epidemiological data showed an increase in the number of cancer cases in persons involved in agricultural production using pesticides. According to (IARC, 1991), more than 25% of pesticides are classified as

carcinogenic. In recent years, the concept of malignant tumors developing after environmental contamination with chemicals has been advocated. Changes in genetic material are at the centre of this process because many environmental pollutants are chemical carcinogens and mutagens with the capacity of causing DNA damage. DNA damage assay was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants (Kornuta *et al*., 1996). Moreover, a potential genotoxic risk due to pesticide exposure was suggested recently by the findings of Zeljezic and Garaj-Vrhovac (2001) and Bolognesi *et al.* (2002).

The single cell gel electrophoresis method (SCGE), also called comet assay, is a rapid and sensitive tool to demonstrate the damaging effects of different chemical compounds or physical treatments on DNA at individual cell levels. The alkaline single cell gel electrophoresis (SCGE) assay does not require mitotic activity (Fairbairn *et al*., 1995). It is attractive for many reasons, apart from being a quick, simple, sensitive, reliable and fairly inexpensive way of measuring, it also produces appealing images. Cells with damaged DNA display increased migration of DNA fragments from the nucleus, generating a "comet" shape. When the technique is carried out in alkaline conditions (pH > 13), the distance of migration indicated the amount of cells with DNA single-strand breaks. Comet assay is increasingly used in genotoxicity testing and has various applications. Sensitivity and specificity of the test are considered to be very high and guidelines for the conduction of the comet assay have recently been published (Tice *et al*., 2000). DNA strand breakage is a sensitive marker of genotoxic damage, these strand breaks are potentially pre-mutagenic lesions (Kammann *et al*., 2001). In addition, it was suggested that occupational exposure to pesticides can cause DNA damage, and confirmed the sensitivity of the comet assay (Grover *et al*., 2003).

On the other hand, pyrethroids are broad-spectrum insecticides active to varying degree on an extensive range of arthropod species and used widely against insect pests in agriculture, veterinary and domestic outlets. Pyrethroids are known to induce toxic effects by the disruption and modulating neurotransmitter system (Reddy *et al*., 1994). The extensive worldwide efforts of structural modification of pyrethrins for better performances have resulted in successful development of a wide variety of synthetic pyrethroids with tremendously high efficacy, and knockdown activity (Miyamoto *et al*., 1995). Historically, synthetic pyrethroids classification system into two subclasses (Types I and II) is widely employed. This classification is based on chemical structure and the production of either the T (tremor) or CS (choreoathetosis with salivation) intoxication syndrome following intravenous or intracerebral administration to rodents (Soderlund *et al*., 2002). Synthetic pyrethroids pesticides were subjected to different genotoxic assays and were found to exhibit some mutagenic potentials (Ghosh *et al*., 1992; Amer *et al*., 1993; Surralles *et al*., *1995*; El-Khatib *et al*., 1998 and El-Khatib *et al*., 2001).

Cypermethrin, a Type II synthetic pyrethroid pesticide, was initially synthesized in 1974 and was first marketed in 1977 as a highly active synthetic pyrethroid pesticide, effective against a wide range of pests in agriculture, public health, and animal husbandry. In 1980, 92.5% of all the cypermethrin produced in the world was used on cotton. In 1982, world production was 340 tones of the active material. It is mainly used in the form of an emulsifiable concentrate, but ultra low volume concentrates, wettable

powders, and combined formulations with other pesticides are also available.

Because of the extensive use of cypermethrin in different agricultural and public health purposes all over the world and in Egypt, its genotoxic effects have considerable practical significance. With regard to mutagenicity, it was reported that cypermethrin is one of the potent insecticides. Cypermethrin has been found to be mutagenic in different test systems. It was reported that cypermethrin increased the induction of micronuclei and chromosome aberrations, in the proliferation of polychromatic erythrocytes in mouse bone marrow and the occurrence of sperm abnormalities (Bhunya and Pati, 1988). It affected the cell cycle causing a decrease in the proliferative rate index in human peripheral blood lymphocytes (Puig *et al.,* 1989). It has a clastogenic activity and/or damages the mitotic spindle, as manifested by a significant increase in the frequency of the micronucleated red blood cells using the micronucleus test in tadpoles of *Rana temporaria* and *Xenopus laevis* (Rudek and Rozek, 1992). It manifested genotoxic effect in mouse spleen and bone marrow as well as in cultured mouse spleen cells (Amer *et al.,* 1993). It increased the rates of the total chromatid and chromosome breaks in the peripheral lymphocytes of spraying workers by the end of the spraying season (Mohamed *et al.,* 1995). It induced chromosomal aberration and micronucleus in rat bone marrow cells (El-Khatib *et al.,* 1998). More recently, cypermethrin induced significant increase in the frequency of SCEs in mice bone marrow cells (Giri *et al.,* 2003).

In contrast to these reports, negative results were also obtained; some authors demonstrated that no significant genotoxic effects were induced by cypermethrin (Miyamoto *et al.,* 1995).

The present study was undertaken to evaluate the potential of cypermethrin to induce DNA damage in liver cells of male white rat *in vivo* using the single cell gel electrophoresis assay or "Comet assay".

MATERIALS AND METHODS

Tested pesticide

A commercial formulation of the cypermethrin (alpha-cyano-3-phenoxy-benzyl 3- (2,2- dichlorovinyl) 2,2- dimethylcyclo-propane-carboxylate), emulsifiable concentrate (EC 60 g/L) was used in the present study. It was obtained from the Central Agricultural Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Egypt. Cypermethrin was dissolved in distilled water for oral administration.

Tested animals

Thirty-three apparently healthy adult male Swiss albino rats were used in the present study. They were obtained from "The Egyptian Organization for Biological Products and Vaccines".

They were weighing 120 – 180 gm., approximately 4 months old rats were housed in appropriate conditions and allowed to acclimatize to the laboratory environment for two weeks prior to initiation of the study. Animals were fed a commercial balanced chow and allowed free access to fresh tap water throughout the acclimatization period and the experiment.

Animals were randomized by weight and were randomly divided into the following experimental groups (3 animals / group): (1) negative control (no treatment); (2) positive control (treated with H_2O_2); (3) nine treated groups with the tested pesticide. The cypermethrin treated animal groups were exposed orally to a single dose of one of three different dose levels (1/5, 1/10 or 1/30 LD_{50}),

and all groups were sacrificed at 1, 7, and 14 days after treatment.

Sampling

At the specified times after treatment, animals were anaesthetized using diethyl ether and were sacrificed by cervical dislocation. Liver was rapidly removed and quickly minced and suspended in chilled homogenization buffer (a 2 gm of a liver in 2 ml of cold HBSS containing 20 mM. EDTA, and 10 % DMSO), and then homogenized gently. Then, 1.5 ml cell sample in micro-centrifuge tube was centrifuged for 5 min at 5° C, at 3500 rpm.

Comet assay

The comet assay was carried out under alkaline conditions, basically as described by (Singh *et al.,* 1988). Ordinary microscopic slides were covered with 0.6 % normal melting point agarose (*Sigma)*. When this layer had solidified a second layer containing the sample mixed with 0.5 % low melting point agarose (*Sigma)* was placed on the slides. After 10 min of solidification on ice, slides were covered with a third layer of 0.5% normal melting point agarose. Slides were then immersed for 1 hr. in ice-cold freshly prepared lysing solution {2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris-HCl, 1 % sodium sarcosinate (*Sigma)*, pH 10}; with 1 % Triton X-100 (*Sigma);* and 10 % dimethyl sulfoxide freshly added to lyses cells and the DNA allowed to unfold. The slides were then placed on a horizontal gel electrophoresis tank, facing the anode. The unit was filled with fresh electrophoresis buffer {300 mM NaOH, 1 mM disodium EDTA, pH 13.0} and the slides were placed in this alkaline buffer for 20 min to allow DNA unwinding. Denaturation and electrophoresis were performed at 4° C. Electrophoresis was carried out for 20 min at: 25 V and 300 mA. After electrophoresis, the slides were rinsed gently three times with neutralization buffer {0.4 M Tris-HCl, pH 7.5} to remove excess alkali and detergents. Each slide was stained with ethidium bromide {20µg/ml} and covered with a coverslip. Slides were stored at 4° C in sealed boxes until analysis.

Evaluation of DNA damage

After application, the coverslip was removed, each slide was examined at 1200X magnification in a fluorescence microscope {excitation filter: 400 nm, with barrier filter: 590 nm}.

The endpoint, which was identified in this study, was the percentage of DNA in the tail or {the percentage of DNA damage}. A total of 100 randomly observed comets on each slide {3 slides/individual rat liver; 300 comets per individual rat liver sample; 900 comets per treatment}; were observed at a constant depth of the gel, avoiding the edges of the gel on each of three replicate slides. We followed the same system given by Cebulska-Wasilewsk,(2002) for scoring visually as belonging to one of nine predefined classes according to tail intensity and given a value of 9; 9 – 45; 45 ; 45 – 75; 75 ; 75 – 91 ; 91 ; 91 – 98; or 98 {from minimum observed damage 9 to maximum observed damage 98} . The frequency of tailed cells was scored, and the significance of increase was determined by Student's *t* test (Sendecore, 1969).

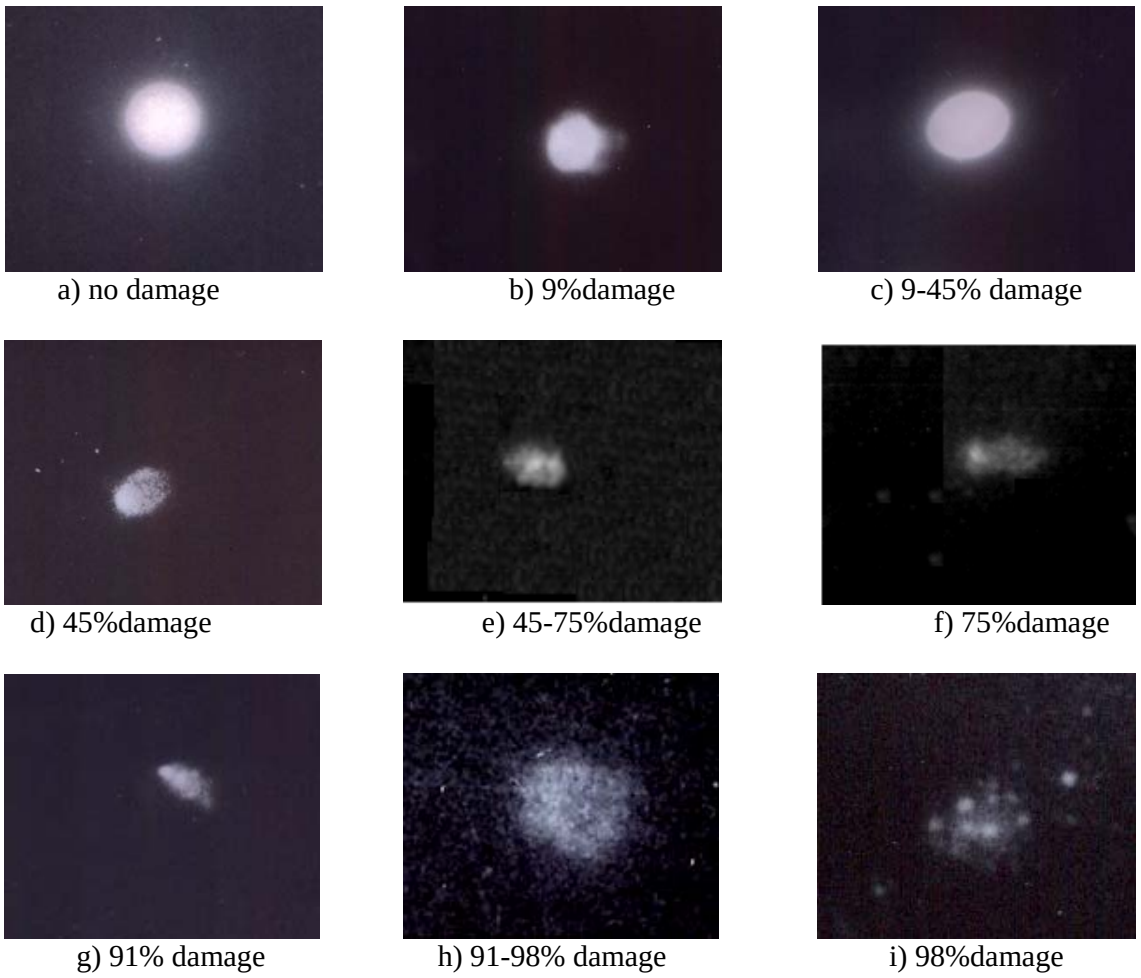
RESULTS

Applying the alkaline comet assay, the tested pesticide "cypermethrin" was examined for its capacity to induce DNA damage in rat liver cells.

In the present study, Hydrogen peroxide was used as positive control. This reference mutagen produced a statistical highly significant ($P<0.0001$) positive response

(DNA damage, 75.4 %) as compared to the negative control (DNA damage, 2.9 %) and the highest DNA damage among the different treatments.

We used the system for scoring the percentages of DNA damage shown in Fig. (1) to analyze the obtained here results of the alkaline comet assay for the "cypermethrin" exposed and control groups. Our results are summarized in Tables (1 and 2) and Figures (2 and 3).



a) no damage b) 9%damage c) 9-45% damage

d) 45%damage e) 45-75%damage f) 75%damage

g) 91% damage h) 91-98% damage i) 98%damage

Fig. (1): Images of rat liver cells showing the levels of DNA damage classes.

Data in Table (1) and Figure (2 – a, b, and c) give the values of DNA damages detected by comet assay after exposure to the different pesticide treatments. Data are presented in total number of DNA damaged cells and the level of DNA damage as percentages of rat liver cells, as belonging to one of none predefined classes according to tail intensity. Data were statistically analyzed using the t-test as shown in Table (2).

Moreover Figure (3 – a, b, and c) illustrates the results of the alkaline comet assay on rat liver cells exposed to different pesticide treatments (mean ± S.D. of DNA damaged cells).

Table (1): Values of DNA damages detected by Comet assay after exposure to different pesticide (cypermethrin) treatments (total No. of DNA damaged cells and level of DNA damage (%) of rat liver cells).

Cell Measurements		Control		Treatment								
				1 day			7 days			14 days		
		Negative Control	Positive Control H_2O_2	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}
Total No. of Examined Cells		900	900	900	900	900	900	900	900	900	900	900
Total No. of DNA Normal Cells		874	221	518	456	381	530	477	361	583	514	416
Total No. of DNA Damaged Cells		26	679	382	444	519	370	423	539	317	386	484
DNA Damaged Cells (%)		2.9	75.4	42.4	49.3	57.7	41.1	47	59.9	35.2	42.9	53.8
Level of DNA Damage (%)	9	19	34	320	347	335	294	279	295	249	343	371
	9-45	6	17	-	6	-	45	6	80	47	26	40
	45	1	57	45	47	104	21	61	52	16	11	37
	45-75	-	194	-	15	4	9	7	31	5	5	17
	75	-	119	17	22	40	1	39	39	-	1	12
	75-91	-	31	-	3	10	-	6	11	-	-	2
	91	-	105	-	4	17	-	21	17	-	-	5
	91-98	-	-	-	-	-	-	1	-	-	-	-
	98	-	122	-	-	9	-	3	14	-	-	-

As shown in Table (2) all pesticide treatments yielded statistically significant ($P<0.0001$) DNA damage. Exposure to 1/5 LD_{50} yielded the most pronounced DNA damage, compared to the other tested doses; the percentage of cells containing damaged DNA was: 57.7, 59.9 and 53.8 % for (1, 7 and 14 days after exposure, respectively). Moreover, one-tenth LD_{50} yielded highly significant DNA damage than 1/30 LD_{50} (49.3 %) one day after treatment, and decreased DNA damage percent was also observed at seven and 14 days (47 % and 42.9 %). Applying the 1/30 LD_{50} also yielded statistically significant DNA damage one day after treatment (42.4%) which decreased at 7 and 14 days to reach (41.1 % and 35.2 %), respectively.

Therefore, significant positive dose-response relationship was detected in the present study, but the effects in the SCG were generally decreased with time after treatments.

Table (2): Results of T-test of alkaline comet assay on rat liver cells exposed to different pesticide (cypermethrin) treatments (mean ±S.D. of DNA damaged cells).

Statistical Measurements	Control		Treatment								
			1 day			7 days			14 days		
	Negative Control	Positive Control H_2O_2	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}	1/30 LD_{50}	1/10 LD_{50}	1/5 LD_{50}
Mean* ± S.D.	2.89 ± 1.54	75.44 ± 3.28	42.44 ± 7.86	49.33 ± 9.89	57.66 ± 3.87	41.11 ± 3.41	47 ± 3.91	59.89 ± 2.37	35.22 ± 5.67	42.89 ± 1.36	53.78 ± 4.44
T-test		*T* = 0.05 *p* < 0.0001	*t* = 14.82 *p* < 0.0001	*t* = 13.93 *p* < 0.0001	*t* = 39.44 *p* < 0.0001	*t* = 30.68 *p* < 0.0001	*t* = 31.53 *p* < 0.0001	*t* = 60.56 *p* < 0.0001	*t* = 16.50 *p* < 0.0001	*t* = 58.40 *p* < 0.0001	*t* = 32.51 *p* < 0.0001

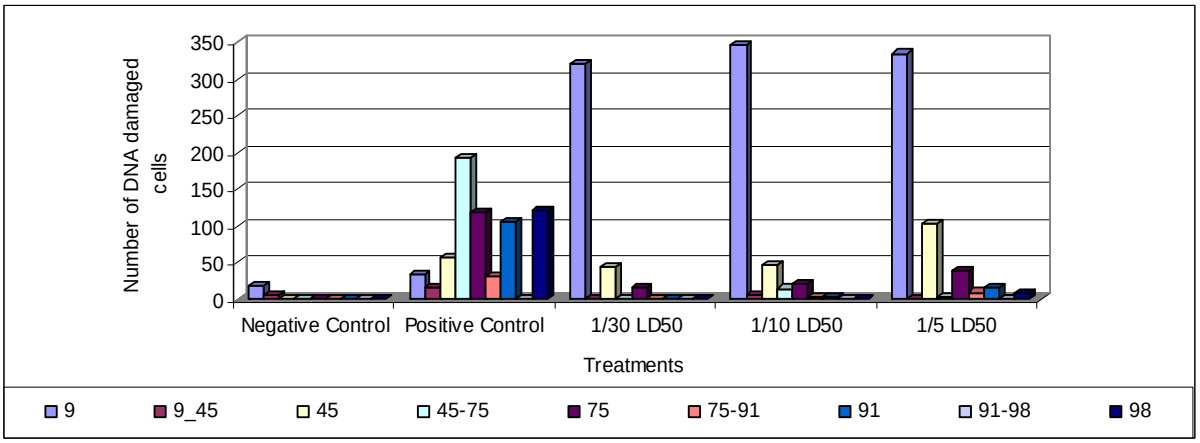


Fig. (2-a):Cypermethrin treatments {levels of DNA damage (%)} at one day.

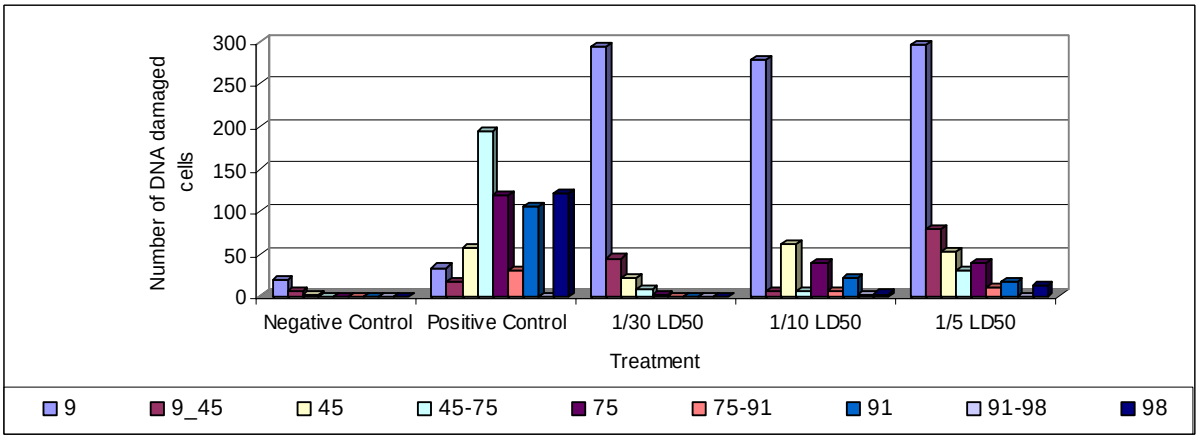


Fig. (2-b):Cypermethrin treatments {levels of DNA damage (%)} seven days.

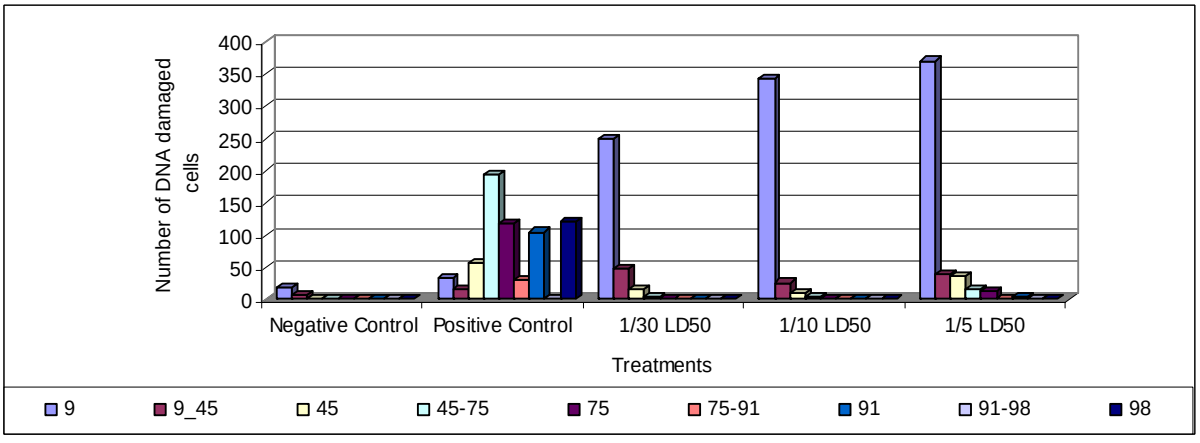


Fig. (2-c): Cypermethrin treatments {levels of DNA damage (%)} at 14 days.

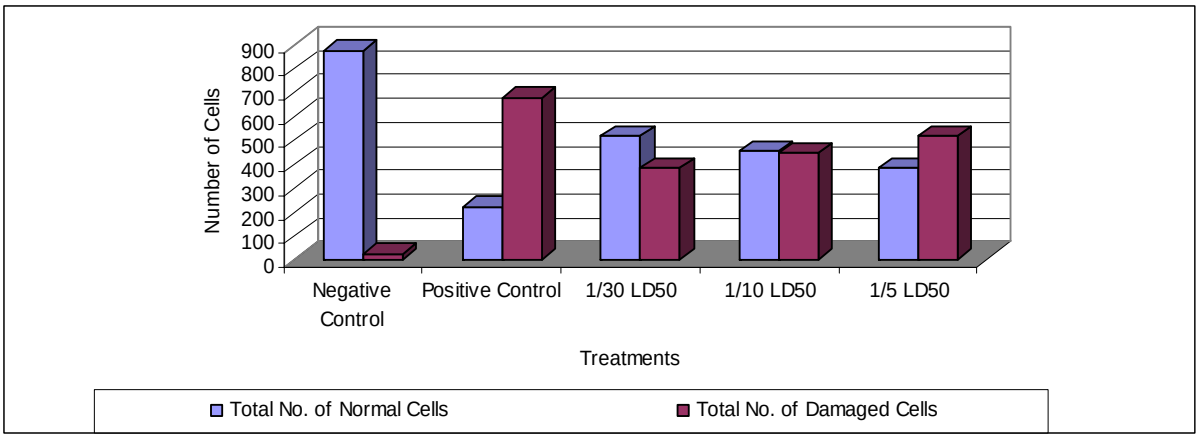


Fig. (3-a): Cypermethrin treatments at one day.

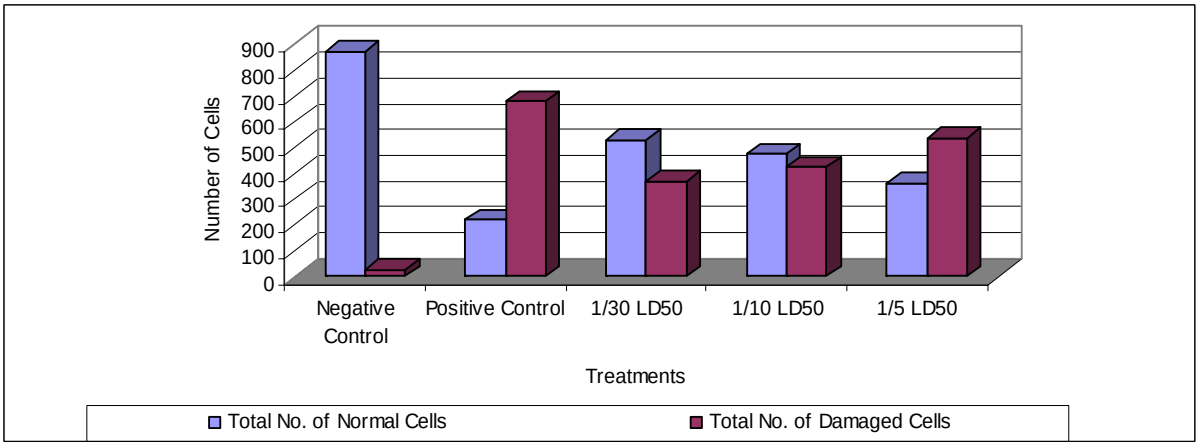


Fig. (3-b): Cypermethrin treatments at seven days.

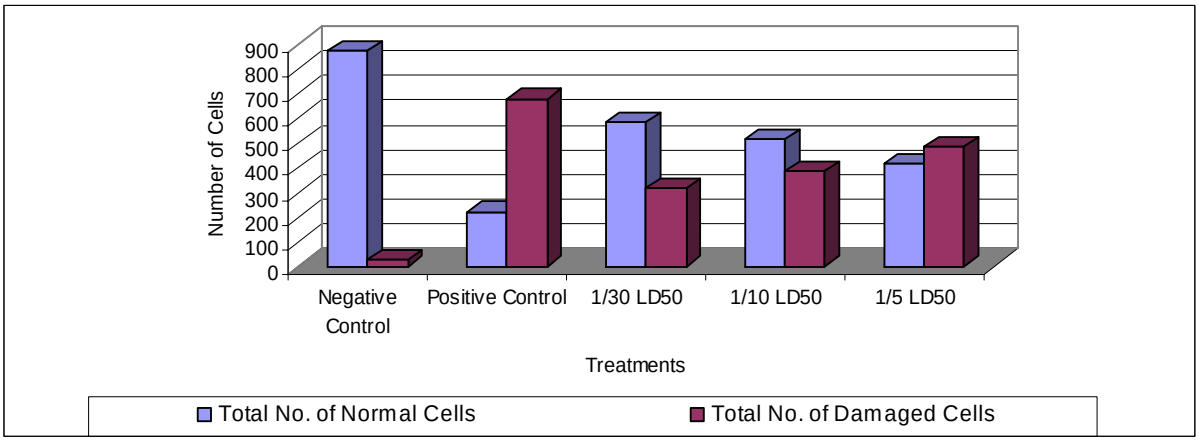


Fig. (3-c): Cypermethrin treatments at 14 days.

DISCUSSION

Inhibition of repair of induced DNA damage may cause DNA effects in the comet assay due to the persistence of DNA lesions, which leads to strand breakage, and/or because of an accumulation of DNA strand breaks related to ongoing DNA repair. Several authors reported that different chemical and physical agents might have an inhibitory effect on DNA-repair systems (Hartmann and Speit, 1996; Hartmann and Hartwig, 1998; Hartwig, 1998 and Shukla *et al*., 2002). Consequently, the increment in the length of migration observed could be reflecting DNA single-strand breaks as a consequence of incomplete excision repair sites. Therefore, the genotoxic effects (DNA damage) of cypermethrin treatments reported in the present study may be interpreted by the probable inhibitory effect of this agent on DNA repair systems.

The use of pesticides has been increasing in recent years, resulting in the need for increased production of pesticides. However, some pesticides may represent a hazard to human health, especially by causing cancer. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential for taking the appropriate measures to minimize its hazardous effects to man and his environment. Pesticides are highly biologically active chemicals. They may interact with DNA and damage its structure. Such interaction may be critical for the manifestation of carcinogenic properties of different chemicals (Kornuta *et al.,* 1996). Genotoxicity tests form an important part of cancer research and risk assessment of potential carcinogens (Grover *et al.,* 2003). So, the public health effects of pesticides cannot be denied, and. the undesired effects of chemical pesticides have been recognized as a serious public health concern during the past decades (Giri *et al.,* 2003).

Extensive studies have been carried out to investigate the mutagenic potential of various pesticides in different test systems ranging from bacteria to mammalian and human lymphocyte cultures. Chromosomal aberrations and micronuclei induction are commonly used cytogenetically to assess qualitatively the mutagenic potentiality of the environmental mutagens. Many attempts have been made to correlate primary lesions induced in DNA by mutagen to the cytological effects in an organism.

DNA strand breakage is a sensitive marker of genotoxic damage, these strand breaks are potentially pre-mutagenic lesions (Kammann *et al.,* 2001). Under standard conditions, the comet assay detects the amount of cells with DNA single strand breaks. These strand breaks could be a consequence of complete excision repair sites (Mouron *et al.,* 2001). Therefore, in the current study the potential DNA damage associated with exposure to pesticides was assessed using the single cell gel electrophoresis assay or comet assay.

It is generally believed that cell death leads to DNA fragmentation. So, to evaluate the chemical genotoxicity, therefore, positive responses in the single cell gel electrophoresis assay should be considered together with cytotoxicity data (Sasaki *et al.,* 1997).

Therefore, in the current study the potential DNA damage associated with exposure to a synthetic pyrethroids pesticide "cypermethrin" was assessed using the single cell gel electrophoresis assay. Our results showed that the pesticide treatment effects were generally decreased with time after treatments. These results might be interpreted in terms of decreased incidences of DNA damage as consequences of DNA repair. The effects in the comet assay test are influenced by DNA repair processes in a complex way. Similarly, it has been shown for various DNA-breaking agents that the effects in the comet test decrease with time after the end of the treatment (Singh *et al.,* 1988 and Tice *et al.,* 1990).

The obtained results strongly support earlier data demonstrating the genotoxicity of the tested pesticide "cypermethrin". The genotoxic potential of cypermethrin has been reported by many authors. Data on the genotoxicity and carcinogenicity of cypermethrin are rather controversial, depending on the genetic system or the assay used.

Cypermethrin has induced micronuclei in Chinese hamster V-79 cells and polychromatic erythrocytes of mouse bone marrow without metabolic activation (Bakhitova and Pashin, 1988). The genotoxicity of cypermethrin has been studied *in vivo* for inducing bone marrow chromosome aberrations, micronuclei test and sperm abnormalities, in mice and the results differed significantly from the respective control values (Bhunya and Pati, 1988). Significantly different increase in micronucleated polychromatic erythrocytes was observed at 24, 36 and 48 hrs. after administration of 1/2 LD_{50} (14 mg/Kg) of "Vastak", a commercial formulation of cypermethrin. Doses of 1/4 LD_{50} (7 mg/Kg) of Vastak showed no mutagenic activity as judged by the induction of micronuclei in polychromatic erythrocytes in mouse bone marrow (Benova *et al.,* 1989). However , Puig *et al.* (1989) reported that while cypermethrin did not increase the frequencies of chromosome-type aberrations and sister chromatid exchanges, it affected the cell cycle causing a decrease in the proliferative rate index in cultured human peripheral blood lymphocytes.

Cypermethrin was tested for the ability to induce micronuclei in both whole blood and isolated human lymphocyte cultures, by using the cytokinesis-block method cytochalasin B. The number of micronucleated cells slightly increased in lymphocyte cultures (Surralles *et al.,* 1995). *In-vivo* genotoxic potential of cypermethrin was evaluated through induction of sister chromatid exchange in mouse bone marrow cells. Sister chromatid exchange analysis in bone marrow metaphase chromosomes revealed modest induction with statistical significance as compared to the vehicle control group (Chauhan *et al.,* 1997). When the genotoxicological effects of

cypermethrin were studied on male rats, it increased the number of numerical chromosome aberrations of the bone marrow cells but did not change the number of structural aberrations (Institoris *et al.,* 1999). Cypermethrin was reported to cause free radical-mediated tissue damage and reduce total GSH in rats (Giray *et al.,* 2001).

On the other hand, Cypermethrin may be considered as environmentally safe and widely used in agriculture and veterinary medicine. To dispute the validity of this opinion Shukla *et al.,* (2003), evaluated its carcinogenic and co-carcinogenic (tumor initiating and tumor promoting) potential in mouse skin model of carcinogenesis. Their results revealed that cypermethrin possess complete carcinogenic as well as tumor initiating and promoting potential in both sexes of Swiss albino mice. In addition, the mutagenic potential of cypermethrin was investigated using the dominant lethal assay in male Swiss albino mice (Shukla and Taneja, 2002). The obtained results showed that cypermethrin has mutagenic activity, inducing dominant lethal mutations in male germ cells of mice and caution is recommended in the use of this insecticide. More recently, cypermethrin was reported to induce significant increases in the frequency of sister chromatid exchange in mouse bone marrow cells (Giri *et al.,* 2003).

The mutagenic potentiality of the synthetic pyrethroid pesticides may be attributed to their ability to perturb the membrane fluidity and its lipid content involving lipid peroxidation (Hebbel *et al.,* 1998). Peroxidation of lipids involves the reaction of oxygen with polyunsaturated lipids to form lipid-free radicals and semi-stable hydroperoxidase profile activity, which represents the most specific defense system against free radical oxidation. This is considered to be an important result ensuring the undifferentiated pattern of bone marrow cells. On the other hand, this strengthen the evidence of pyrethroids stimulation of the oxidative metabolism and free radical production (Andrews *et al.,* 1986). Free radicals, which may be liberated during treatment, are not candidates for DNA damage because of their extremely short half-lives. However, these radicals may interact with cellular macromolecules to form persistent regulators to inhibit gene transcription within these cells (Sellins and Cohen, 1987).

It is now well established that the sodium channel is the primary target site of pyrethroids, which leads to depolarization of the nerve membrane by prolonging the sodium current during excitation (Perry *et al.,* 1998). Metabolism of cypermethrin is considered to be both oxidation and hydrolytic processes, and both pathways involve esterase cleavage (Chang and Jordan, 1982 and Perry *et al.,* 1998). However, the mechanism of action of synthetic pyrethroids on nucleic acids is yet to be elucidated. Therefore, at present it is difficult to comment on the structure-genotoxicity relationship for cypermethrin. Independent of the nature of molecular interactions that may occur, the present data clearly indicate that cypermethrin possesses the potential, at least to a limited extent, to cause alterations in the cellular DNA in mammalian cells *in- vivo.*

In conclusion, the results of the present study indicate that cypermethrin has definite interactions with DNA in rats, resulting in DNA damage, indicating potential mutagenic and/or carcinogenic effects. Human exposure to this agent should be restricted. Our results confirmed that the single cell gel test is a sensitive genotoxicity test for DNA damaging chemical agents.

REFERENCES

Amer, S.M., Ibrahim, A.A. and El-Sherbeny K.M. (1993). Induction of chromosomal aberrations and sister chromatid exchange *in-vivo* and *in- vitro* by the insecticide cypermethrin. J. Appl. Toxicol. 13(5): 341-345.

Andrews, P.C. and Kinski, N.I. (1986). Human myeloperoxidase and hemiperoxidase. Meth. Enz. 132: 369-378.

Bakhitova, L.M. and Pashin, Iu.V. (1988). Cypermethrin induction of micronuclei in mammalian somatic cells Tsitol. Genet. 22(4): 32-35.

Benova, D.K., Rupova, I.M., Iagova, A.Kh. and Bineva, M.V. (1989). Mutagenic effect of pesticides fastac 10 EK and durs ban 4E studied in a micronucleus test in mouse bone marrow cells. Genetika. 25(12): 2266-2268.

Bhunya, S.P. and Pati, P.C. (1988). Genotoxic effects of a synthetic pyrethroid insecticide cypermethrin in mice *in-vivo*. Toxicol. Lett. 41: 223-230.

Bolognesi, C., Perrone, E. and Landini, E. (2002). Micronucleus monitoring of a floriculturist population from western Liguria, Italy. Mutagenesis, 17(5): 391-397.

Cebulska-Wasilewsk, A. (2002). Standard protocol applied in laboratory of the radiation and environmental Biology Department. NATO ARW, Karaków, Poland, June 23-27.

Chang, C.K. and Jordan, T.W. (1982). Penetration and metabolism of topically applied permethrin and cypermethrin in pyrethroid-tolerant *Wiseana cervinata* larvae. Pestic. Biochem. Physiol. 17: 196-204.

Chauhan, L.K., Agarwal, D.K. and Sundararaman, V. (1997). *In-vivo* induction of sister chromatid exchange in mouse bone marrow following oral exposure to commercial formulations of alpha-cyano pyrethroids. Toxicol Lett. 93(2-3): 153-157.

Chen, Y., Lin-Shiau, S. and Lin, J. (1998). Involvement of reactive oxygen species and caspase-activation in arsenite-induced apoptosis. J. Cell Physiol. 177: 324-333.

El-Khatib, E.N., Eweis, E.A., Ibrahim, M.A. and Kandil, M.A. (1998). Genotoxic effects of some pesticides in albino rat bone marrow micronucleus and chromosomal aberrations assays. Proceedings of the 26th Annual Meeting of Genetics, Alex. 29-30.

El-Khatib, E.N. and Rokaya, H.A. (2001). Genotoxic effects of two pesticides and their mixtures: *In-vivo* chromosomal aberrations and micronucleus assays. J.Union Arab. Biol. (16 A) Zoology: 355-380.

Fairbairn, D.W., Olive, P.L. and O'Neill, K.L. (1995). The comet assay: a comprehensive review. Mutat. Res., 339: 37-59.

Ghosh, A.K., Sharma, A. and Talukder, G. (1992). Cytotoxic effects of Sumicidin, a type II synthetic pyrethroid, on mice *in-vivo* at 6, 12 and 24 h after exposure. Cytobios. 71(285): 85-91.

Giray, B., Gürbay, A. and Hincal, F. (2001). Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. Toxicol. Lett. 118: 139-146.

Giri, S., Giri, A., Sharma, G.D. and Prasad, S.B. (2003). Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice *in-vivo*. Mutagenesis. 18(1): 53-58.

Grover, P., Danadevi, K., Mahboob, M., Rozati, R., Saleha B. and Rahman, M.F. (2003). Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay. Mutagenesis, 18(2): 201-205.

Hagmar, L., Brogger, A., Hansteen, I.L., Heim, S., Hogstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I., Reuterwall, Ch., Salomaa, S., Skerfving, S. and Sors, M. (1994). Cancer risk predicted by increased levels of chromosomal aberrations in

lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res. 54: 2919-2922.

Hartmann, M. and Speit, G. (1996). Effect of arsenic and cadmium on the persistence of mutagen-induced DNA lesions in human cells. Environ. Mol. Mutagen. 27: 98-104.

Hartmann, M. and Hartwig, A. (1998). Disturbance of DNA damage recognition after UV irradiation by nickel (II) and cadmium (II) in mammalian cells. Carcinogenesis. 19(4): 617-621.

Hartwig, A. (1998). Carcinogenicity of metal compounds: possible role of DNA repair inhibition. Toxicol. Lett. 102/103: 235-239.

Hebbel, R.P., Shalev, O., Poker, W. and Rank, B.H. (1986). Inhibition of erythrocyte Ca2+ - ATPase by activated oxygen through thiol and lipid-dependent mechanisms. Biochem. Biophys. Acta. 862: 8-13.

IARC (1991). Monograph on the "Evaluation of Carcinogenic Risks to Humans" Occupational exposure in insecticide application, and some pesticides, International Agency for Research on Cancer, Lyon. Mutat. Res. 260: 105-113.

Institoris, L., Undegeger, U., Siroki, O., Nehez, M. and Desi, I. (1999). Comparison of detection sensitivity of immuno- and genotoxicological effects of subacute cypermethrin and permethrin exposure in rats. Toxicology. 137(1): 47-55.

Kammann, U., Bunke, M., Steinhart, H. and Theobald, N. (2001). A permanent fish cell line (EPC) for genotoxicity testing of marine sediments with the comet assay. Mutat. Res. 498: 67-77.

Kornuta, N., Bagley, E. and Nedopitanskaya, N. (1996). Genotoxic effects of pesticides. J. Environ. Pathol. Toxicol. Oncol. 15(2-4): 75-78.

Miyamae, Y., Yamamoto, M., Sasaki, Y.F., Kobayashi, H., Igarashi-Soga, M., Shimoi, K. and Hayashi, M. (1998). Evaluation of a tissue homogenization technique that isolates nuclei for the *in-vivo* single cell gel electrophoresis (comet) assay: a collaborative study by five laboratories. Mutat. Res. 418: 131-140.

Miyamoto, J., Kaneko, H., Tsuji, R. and Okuno, Y. (1995). Pyrethroids, nerve poisons: how their risks to human should be assessed. Toxicol. Lett. 82-83: 933-940.

Mohamed, O., Walid, A.A. and Ghada, K. (1995). Chromosomal aberrations in human lymphocytes from two groups of workers occupationally exposed to pesticides in Syria. Environ. Res. 70 (1): 24-29.

Mouron, S.A., Golijow, C.D. and Dulout, F.N. (2001). DNA damage by cadmium and arsenic salts assessed by the single cell gel electrophoresis assay. Mutat. Res. 498: 47-55.

Perry, A.S., Yamamoto, I., Ishaaya, I. and Perry, R.Y. (1998). *Insecticides in Agriculture and Environment: Retrospects and Prospects*. Springer-Verlag, Berlin, Germany.

Puig, M., Carbonell, E., Xamena, N., Creus, A. and Marcos, R. (1989). Analysis of cytogenetic damage induced in cultured human lymphocytes by the pyrethroid insecticides cypermethrin and fenvalerate. Mutagenesis. 4: 72-74.

Reddy, P.M. and Philip, G.H. (1994). *In-vivo* inhibition of AChE and ATPase activation in the tissue of fresh water fish. *C*yprinus carpio exposed to technical grade of cypermethrin. Bul. Environ. Contam. Toxicol. 52(4): 619-626.

Rudek, Z. and Rozek, M. (1992). Induction of micronuclei in tadpoles of Rana temporaria and Xenopus laevis by the pyrethroid Fastac 10 EC. Mutat. Res. 298(1): 25-29.

Sabbioni, G. and Numann, H.G. (1990). Biomonitoring of arylamines: hemoglobin

adducts of urea and carbamat pesticides. Carcinogenesis. 11: 111-115.

Sasaki, Y.F., Saga, A., Akasaka, M., Nishidate, E., Akanuma, M.W., Ohta, T., Matsusaka, N. and Tusda, S. (1997). *In-vivo* genotoxicity of heterocyclic amines detected by a modified alkaline single cell electrophoresis assay in a multiple organ study in the mouse. Mutat. Res. 395: 57-73.

Sellins, K.S. and Cohen, J.J. (1987). Gene induction by gamma irradiation leads to DNA fragmentation in lymphocytes. J. Immunol. 139: 3199-3206.

Sendecore, G. (1969). Statistical methods. Aiwa state Univ. Press, Ames., USA.

Shukla, Y., Yadav, A. and Arora, A. (2002). Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin. Cancer. Lett. 182(1): 33-41.

Shukla, Y. and Taneja, P. (2002). Mutagenic potential of cypermethrin in mouse dominant lethal assay. J. Environ. Pathol. Toxicol Oncol. (3): 259-265.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell. Res. 175: 184-191.

Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Iccirillo, V.J., Sargent, D., Stevens, J.T. and Weiner, M.L. (2002). Mechanisms of pyrethroid neurotoxicity: implication for cumulative risk assessment. Toxicology. 171(1): 53-59.

Surrallés, J., Xamena, N., Creus, A., Catalan, J., Norppa, H. and Marcos, R. (1995). Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. Mutat. Res. 341: 169-184.

Tice, R.R., Andrews, P.W. and Singh, N.P. (1990). The single cell gel assay: a sensitive technique for evaluating intercellular differences in DNA damage and repair. Basic. Life. Sci. 53: 291-301.

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-vtro* and *in-vivo* genetic toxicology testing. Environ. Mol. Mutagen. 35: 206-221.

Zeljezic, D. and Garaj-Vrhovac, V. (2001). Chromosomal aberration and single cell gel electrophoresis (Comet) assay in the longitudinal risk assessment of occupational exposure to pesticides. Mutagenesis. 6(4): 359-363.

الملخص العربى

السمية الوراثية للمبيد البيريثرويد المصنع " سيبرمثرين " فى خلايا كبد الفئران باستخدام اختبار الكوميت

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* قسم سمية المبيدات على الثدييات ، المعمل المركزي الزراعي للمبيدات ، مركز البحوث الزراعية، وزارة الزراعة ، الدقي ، الجيزة. ج .

يعد اختبار الكوميت (التفريد الكهربى للخلايا المنفرده) طريقة بسيطة لقياس الكسور فى خيوط الحامض النووى "ألـدنا" فى خلايا الكائنات حقيقية الأنوية . ولهذا الاختبار تطبيقات متعدده فى الكشف عن مختلف المؤثرات الكيميائية و الفيزيائية مـن حيث قدرتها على احداث السمية الوراثية , فضلا عن استخدامه فى رصد التلوث البيئى بالمواد ذات السمية الوراثية . وتهـدف الدراسة الحالية الى تقييم التأثيرات السمية الوراثية لأحد المبيدات البيريثريدية المخلقة " سـيبرمثرين " وهـو أحـد المبيـدات المستخدمة فى مصر على نطاق واسع فى كل من برامج مكافحة الآفات الزراعية و الصحة العامة . حيث تم معاملة ذكور الفئران عن طريق الفم بجرعة واحدة من المستحضر التجارى للسيبرمثرين (توازى جزء من ثلاثين أو عشر أو خمس الجرعة القاتلة النصفية) ثم ذبحت الحيوانات بعد المعاملة بيوم او سبعة أو أربعة عشر يوما . وتم اعداد معلق من الخلايا المنفردة للكبد واجراء اختبار الكوميت والذى أوضح حدوث أضرار بالغة بالحامض النووى . حيث لوحظ بصفة عامة أن كافـة المعـاملات بالمبيد قد أدت الى حدوث أضرار معنوية أحصائيا بالحامض النووى "ألدنا". ونخلص الى أن التعريض لمبيد السيبرمثرين قـد يتسبب فى احداث أضرار بالحامض النووى"ألدنا" فى خلايا كبد الفئران , و تتناسب معدلات الضرر تناسبا طرديا مع مستويات الجرعة المستخدمة و ذلك مقارنة بالكنترول . ومن جهة اخرى فقد لوحظ أن تلك التأثيرات تتناقص مع مرور الوقـت عقـب المعاملة . وتشير نتائج هذا العمل الى أن اختبار الكوميت يعتبر طريقة مناسبة وحساسة لقياس وتقييم السمية الوراثية للمبيدات . مع التأكيد على على أهمية استخدام أختبارات متنوعة لتحديد القدرة الطفرية للمبيدات .