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Genotoxic effect of an organophosphorus pesticide "Ethephon"on somatic and germ cells of male mice

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ABSTRACT

Ethephon [(2-chloroethyl) phosphoric acid] is an organophosphorus pesticide "herbicide". Ethephon was tested for its genotoxic effect in both somatic (bone marrow) and germ (spermatocytes) cells of male mice. Three different dose levels (192, 240 & 480 mg/kg body weight representing 1/ 10, 1/8, 1/4 from the LD50) were orally administrated in male mice weighted 20-25 gm. An increase in chromosomal aberrations (structural & numerical) was observed in most treatment with ethephon in both somatic and gametic cells. In bone marrow cells, the significant structural chromosome aberrations were in the form of chromatid gaps & breaks, deletions, fragments and centric fusion, while in spermatocytes were in the form of chain, autosome univalent and x-y univalent. On the other hand, the numerical aberrations, such as aneuploidy (hypoploidy 2n- and hyperploidy 2n+), were below the significant level. Concerning the sperm shape abnormalities, there was no significant increase over the control groups. These observed results were attributed to the alkylating potency of the organophosphorus pesticide "herbicide" and also to its cytotoxic effects. From this study, it is possible to conclude that ethephon has clastogenic effects. The very scarce available information on mutagenicity of ethephon, in spite of its widespread use, emphasizes the need for further mutagenicity and carcinogenicity testing of this pesticide.

Key words: Genotoxic, Organophosphorus pesticide, Ethephon, Mice.

INTRODUCTION

Ethephon (plant growth regulator) is organophosphorus pesticide (OP) [Kidd and James, 1999, Haux , *et al.*, 2000]. Organophosphorus compounds are widely used throughout the world as insecticides [Kidd and James, 1999, Haux, *et al.*, 2000, Aurbek, *et al.*, 2006]. It was published that they inhibit acetylcholinesterase (AChE) [Brock, 1991]. Mouse plasma cholinesterase (ChE) *in vivo* and *in vitro*, are more sensitive to ethephon then any other esterases. All mouse liver esterase observed are less sensitive then plasma ChE to ethephon *in vivo* and *in vitro*. Thus, BChE inhibition continues to be the most sensitive marker of ethephon exposure [Haux, *et al.*, 2002]. However, the possibility that organophosphate toxicity is due to inhibition of targets other than acetylcholinesterase [Duysen, *et al.*, 2001].

The fundamental problem in pesticide development is to produce chemicals that are specifically effective against certain organisms without adversely affecting others [Kidd and James, 1999]. Because the similarities in the structure, metabolic and genetic components of life form, absolute species specificity is frequently difficult to attain. The usages of organophosphorus pesticides have been increased and represent a major class of agriculture pesticides today [Chen, *et al.*, 1982a, Chen, *et al.*, 1982b]. But the highly toxicity of OPs may be due to using them as chemical warfare agents (nerve agents) [Qing, 2007].

Several types of those pesticides have been reviewed as mutagenic (carcinogenesis and mutagenesis) compounds either *in vivo* or *in vitro* systems [Saleh, 1980, Garrett, *et al.*, 1986,[German], 1990, Qu, *et al.*, 2004]. Therefore, occupational

exposure of agriculture and industrial workers to pesticides possess several serious problems including genotoxic effects. Genotoxic effects of these agriculture chemicals are of special concern because of the generally irreversible nature of the process and the long latency associated with their manifestation.

The goal of this work was to screen the possible genotoxic effects of an organophosphorus pesticide "ethephon". It has been tested for its ability to induce chromosomal aberrations in both somatic (bone marrow) and germ cells (spermatocytes) of male mice *(Mus musculus)*. In addition to chromosomal aberrations, sperm abnormalities have also been investigated.

MATERIAL AND METHODS

Animals

Forty five adult male Swiss albino mice *(Mus musculus)*(20-25g) were maintained on feed and water *ad libitum*. Mice were obtained from the animal house of the King Fahad Research Centre. Five mice were grouped randomly in a cage and kept for treatment; a control group was kept parallel at the same conditions.

Ethephon

Etherel was purchased from HELLAFARM Company, Athens, Greece, as (48%) ethephon. It is diluted to the desired concentrations with sterile distilled water.

Dosage and treatment

The adult mice were weighted and divided into 3 major groups; each group was divided into three equal groups:the first three groups, animals which received the treatments (5 animals each) while the second is a control group which are animals received only distilled water in amount similar to that received by the treated animals.

So the first three treated groups received 192, 240, 480 mg/kg body weight from the ethephon representing 1/10, 1/8 and 1/4 from the LD50 reported by Holsing [Holsing, 1969]. These three doses acted as low, medium and high doses. All doses (low LD, medium MD, & high HD) were administered acutely, in a single oral administration, sacrificing the animals 24 hours of administration. The last fifteen animals were kept for sperm morphology essay and divided into three subgroups: the first received the high dose of the ethephon while the second received cyclophosamide that acts as a positive control. The third subgroup animals were kept parallel as a control. The drug was orally administrated using oral gavage needle in all the experiments for two successive weeks, the animals were killed in the day after.

Slide preparation and analysis

Mitotic and meiotic chromosomes were obtained from the same animal.

Animals were injected i.p. 2.5 hrs before killing with 0.2 ml colchicin (0.05%). Bone marrow chromosomes were prepared according to the method of Yoshida and Amano [Yoshida and Amano, 1965], while meiotic chromosome preparation from the testicles was prepared following the technique described by Brewen and Preston [Brewenand Preston, 1978]. For each animal, 50 metaphases were examined for both bone marrow and spermatocytes, where different types of abnormalities were recorded. For studying the sperm shape abnormalities, three groups of animals were used: the first group includes animals received high dose of ethephon. The second group was injected with 20 mg/kg cyclophosamide for 5 days that acts as a positive control. The third group was received sterile distilled water and acts as a negative control. Animals were sacrificed after 35 days following the first injection. Sperms were collected for examination of head and tail abnormalities following the method of Wyrobek and Bruce [Wyrobek and Bruce, 1978], more than 500 sperms per animal were examined. The obtained data were analyzed using standard Chi-Square test. But did not reach the significant level after treatment with various doses (LD, MD and HD) of ethephon

RESULTS AND DISCUSSION

From literature review, it is clear that information about chromosomal aberrations (in somatic and germ cells) and sperm abnormalities induced by ethephon unavailable either *in vivo* or *in vitro* systems.

The effect of ethephon on fertility is a dominant lethal mutations, because of its effect on chromosomal translocations at the first meiotic metaphase in male mice [Sovkovic, *et al.*, 1983].

In the present investigation an increase in chromosomal aberrations was observed in approximately all treatments (LD, MD & HD) with ethephon as discussed below.

The observed significant increase of structural chromosomal aberrations in bone marrow cells were in the form of chromatid gaps & breaks deletions, fragments and centric fusion. These aberrations were primarily chromatid gaps and breaks and deletions, an indication that chromosomal damage occurred following the G1 stage of cell cycle. Evans reported that gaps appears to represent a single hit effect on the chromosome, therefore, it is likely that, gaps indicate damage in the mutational sense [Evans, 1962]. One report correlates chromosome breaks to gene mutations, when they occur in somatic cells; they are potentially carcinogenic [Nichols, 1972]. Centric fusion (Robertosonian translocations) are stable aberrations, which can transmitted to new cell generations both in somatic and in gametic cells. Therefore, in addition to their carcinogenic effects, they add to the genetic load in future generation.

In present study, chromosomal aberrations were determined in:

Bone marrow cells

Table 1: Show the data obtained after treatment with various doses (LD, MD and HD) of ethephon. The data was written as (mean ± SD).

The significant ($P<0.01$) structural chromosomal aberrations observed were in the form of deletions, fragments and centric fusion after treatment with all doses (LD, MD, HD), chromatid gaps after treatment with MD & HD doses and chromatid breaks only after treatment with HD dose. These results showed that the total structural chromosomal aberration increased significantly at p. level 0.05 after treatment with LD dose and at p. level 0.01 after treatment with MD & HD. On the other hand, increase of numerical chromosomal aberrations such as aneuploidy [hypoploidy (zn-),hyperploidy(zn+)] were also observed.

The observed structural chromosomal aberrations produced by ethephon are suggestive of its mutagenicity and carcinogenicity depending on its effects on somatic or germ cells.

According to the chromosomal theory of carcinogenesis as reviewed, chromosomal aberrations are common pathway through which carcinogenic factors induce malignancy [de Grouchy and de Nava, 1968]. These factors are external and internal.

External factors are well documented; they include oncogenic viruses, carcinogenic chemicals and ionizing radiation, all of which are well known to produce chromosome damage.

Moreover, the increase in the chromosomal aberrations may be attributed to the alkylating potency of this organophosphorus pesticide, since it is chemically related to alkylating agents. According to Kihlman, alkylating agents have "delayed effect" i.e produce aberrations after many hours of treatment [Kihlman, 1966]. Compounds of this type could be presumed to produce aberrations in G1 and early S-phase and produce all chromatid types of aberrations.

This may offer an explanation for significant increase of chromatid type of aberrations observed after treatment with different doses (LD, MD & HD) of ethephon.

Spermatocytes

As in bone marrow cells, there was a significant increase in the total structural aberrations, where as the total numerical aberrations was below the significant level after treatment with different doses (LD, MD& HD) of ethephon. Table 2 represents the observed types of abnormalities in the spermatocytes. Four types of abnormalities were recorded (chain, autosomal, univalent and x-ray univalent). These abnormalities were significantly increased over the control level at p level 0.01 after treatment with all doses (LD, MD& HD), except for chain abnormally was significant only after treatment with MD and HD doses of ethephon.

Sperm morphology

Concerning abnormalities observed in

Table 1: Shows the effect of ethephon on the frequency of chromosomal aberrations induced by low, medium and high doses in bone marrow cells of mice

Treatment		No of treated	No of examined	Structural abnormalities							Numerical abnoramalities		
				Chro-matid gap	chro-matid gap	Deletion	Fragment	Centric	Endomi	Total	n^2+	n^2-	Total
Low Dose	Control	5	250	0.2 ± 0.447	0.0 ± 0.0	0.2 ±0.447	0.4 ± 0.547	0.6 ± 0.548	0.0 ± 0.0	1.4 ± 1.673	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Treated	5	250	1.0 ± 1.0	0.6± 0.548	2.0 ± 1.581**	2.4 ± 2.302**	3.4 ± 1.673**	0.8 ± 0.837	10.2 ± 6.573**	0.6 ± 0,548	0.4 ± 0,547	1.0 ± 1.0
Medium Dose	Control	5	250	0.2 ± 0.447	0.0 ± 0.0	0.2 ± 0.447	0.4 ± 0.547	0.6 ± 0.548	0.0 ± 0.0	1.4 ± 1.673	0.0± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Treated	5	250	1.4 ± 10140**	1.2 ± 1.304	3.4 ± 1.140**	4.4 ± 3.362**	5.0 ± 3.742**	1.4 ± 1.140**	16.8 ± 9.679**	1.4 ± 1.140	1.0 ± 1.225	2.4 ± 2.302
High Dose	Control	5	250	0.2 ± 0.447	0.0 ± 0.0	0.2 ± 0.447	0.4 ± 0.547	0.6 ± 0.548	0.0 ± 0.0	1.4 ± 1.673	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Treated	5	250	2.6 ± 1.140**	2.0 ± 1.581**	5.4 ± 1.817**	7.4 ± 3.130**	7.4 ± 3.130**	2.4 ± 1.517**	26.4 ± 6.656**	2 ± 1.581	1.6 ± 1.673	3.6 ± 3.049

*Significant at 0.05 **Significant at 0.01

Table 2: Shows the effect of ethephon on the frequency of chromosomal aberration induced by low, medium and high doses in spermatocyte of mice

Treatment		No of Treated mice	No of examined cells	Structural abnormalities					Numericalabnormalities		
				Chain	Autosom alunivalent	X-Yunivalent	poly ploidy	Total	n +	n-	Total
Low	control			0.0 ±	0.4 ±	0.2 ±	0.4 ±	1.o ±	0.0 ±	0.0 ±	0.0 ±
Dose		5	250	0.0	0.548	0.447	0.548	1.414	0.0	0.0	0.0
	treated	5	250	1.6 ±	3.2 ±	2.0 ±	3.4 ±	10.2 ±	0.4 ±	0.2±	0.6 ±
				1.517	2.049**	1.581**	1.673**	6.140**	0,548	0,448	0.894
Medium	control			0.0 ±	0.4 ±	0.2 ±	0.4 ±	1.0 ±	0.0 ±	0.0 ±	0.0 ±
Dose		5	250	0.0	0.548	0.447	0.548	1.414	0.0	0.0	0.0
	treated			2.8 ±	4.4 ±	3.4 ±	4.8 ±	11.8±	1.0 ±	0.6 ±	1.6 ±
		5	250	0.837**	1.817**	1.517**	2.168**	7.225**	1.414	0.894	2.191
High	control	5	250	0.0 ±	0.4 ±	0.2±	0.4 ±	1.0 ±	0.0±	0.0 ±	0.0 ±
Dose				0.0	0.548	0.447	0.548	1.414	0.0	0.0	0.0
	treated	5	250	4.4 ±	6.0 ±	5.4 ±	6.6±	22.4 ±	1.4 ±	1.0 ±	2.4±
				0.894**	2.345**	2.302**	2.302**	5.505**	1.346	1.414	2.510

*Significant at 0.05 **Significant at 0.01

Table 3: Shows the effect of ethephon on the frequency of sperm abnormalities induced by high dose in mice

Treatments	Sprem shape abnormalities				Total abnormalities sprem /100	
	Amorphous	Head Banana	Without hook	Tail Coiled	Mean±SE	Abnormal range
Untreated Negative control	33.8±5.8	5.0±0,89	3.4±0.48	5.0±1.0	47.2±7.2	36-58
Positive control 25 mg/kg cyclophosamide	65.4±5.5	14,4±2.6	13.0±3.7	20.6±3.8	113.4±7.3	102-121
High dose treatment	77.5±41.9*	21.5±8.2*	60.8±21.2*	23.8±14.7*	183.7±79.7*	91-36

*Significant at 0.05 **Significant at 0.01

germ (spermatocytes), autosomal and x-y univalents were the most frequent abnormalities observed of treatment with various doses(LD, MD & HD) of ethephon, followed by chain abnormality only observed after treatment with LD of ethephon. An increase in abnormal sperm morphology was found after treatment with various doses of ethephon was observed (table 3). The observed abnormalities were classified into two main types. The first was head abnormalities, while the second was tail abnormalities. The observed types of head abnormalities were in the form of amorphous, banana and without hook; while the only observed tail abnormality was in the form of coiled tail. Our observation concerning the abnormalities in germ cells, was agreed with those of Savkovic and his group who reported that the treatment of ethephon lead to the separation of x/y chromosome [Sovkovic, *et al.*, 1983].

Miller and Miller presumed that many but possibly not all mutagens are potential carcinogens [Miller and Miller, 1971]. They proved also that the damage cell having exchange figures or point mutations, could result in a tumor if a somatic cell is involved, or in a mutation that is evident in future generations if a germinal cell is involved. Since in the present investigation, ethephon produced chromosomal aberration in somatic and germ cells, the possibility that it is carcinogenic or mutagenic can not excluded. The later was supported by [Wang, *et al.*, 2004]. However, carcinogenic properties of ethephon, needs further specific carcinogenicity studies.

Finally, since the induction of chromosomal damage in somatic and gametic cells is closely related with clastogenic effect and with mutation, cell transformation and tumorigenesis in mammals, ethephon appear to be potent mutagen. This finding should be further evaluated, because the limited testing on mutagenicity of this pesticide has provided only inconclusive evidence so far. Therefore, further mutagenicity and carcinogenicity testing for this widely used pesticide (plant growth regulator) is necessary to elucidate the mechanisms by which it self or its metabolites induce genetic damage.

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