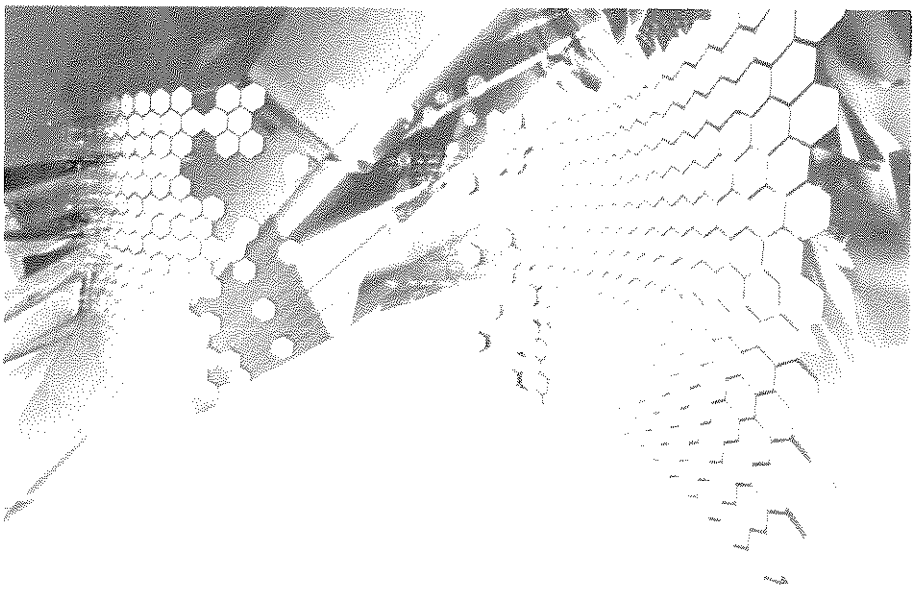

JBS
Journal of Biological Sciences

Journal of Biological Sciences 6 (6): 1041-1046, 2006
ISSN 1727-3048

Mutagenic Effects of Ethephon on Albino Mice

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Abstract: Ethephon (etherel) is a widely used as a plant growth promoter at low doses and a herbicide at high doses was evaluated for its toxigenic effects on albino mice. The study was assessed using 3 parameters, nucleic acids (DNA and RNA) concentrations in the liver and testis, protein content and cholinesterase activity in blood plasma. Ethephon was administrated to albino mice male with three doses level (1/4, 1/8 and 1/10 LD_{50} mg kg^{-1}). Ethephon was found to reduce the DNA and RNA concentrations. Similar results were observed for the protein content and cholinesterase enzyme activity. The results showed that ethephon could be a mutagenic in mice. It is recommended that a great attention should be paid towards the mutagenicity of ethephon to animals and human.

Key words: Ethephon, toxicity, mutagenicity, organophosphorus pesticides, enzyme activity

INTRODUCTION

Mutagenic pollution of the environment is a serious and general problem. Mutagenic chemicals occurring in various habitats can induce serious diseases, including cancer.

Therefore, detection of mutagenic pollution of the environment is an important issue. There are various tests in which different organisms are employed. (Beata, 2005). Organophosphorus pesticides are used in agriculture and public health programs. They are known as an important class of environmental carcinogenesis and mutagenesis (Saleh, 1980; El-Sabae *et al.*, 1981; Rama and Jaga., 1992; El-Fiky *et al.*, 1992; Rahman *et al.*, 2002). Therefore occupational exposure of agriculture and industrial workers to pesticides possess several serious problems including mutagenic effects. Organophosphorus pesticides which is the most important group of pesticides are known to react with DNA generally as alkylating agents and consequently, they are potentially mutagens and/or carcinogens. Some organophosphorus compounds were proved to be effective mutagens in a variety of organisms (Waters *et al.*, 1980).

Previous studies have revealed that at low doses, organophosphorus pesticides not only act as genotoxic agents but also affect several other biochemical pathways (Das *et al.*, 2006). The routine genotoxicity-testing is based on at least 20 years of development during which many different test systems have been introduced and used like *Salmonella typhimurium*, *Saccharomyces cerevisiae*, *Vicia faba* root-tip, barley and *Drosophila melanogaster*. Today, it is clear that no single test is capable of detecting all genotoxic agents. Additional test systems for the assessment of genotoxic and carcinogenic hazard and risk are urgently required. (Kramer, 1998).

The regulatory approval systems for plant protection products and therapeutic agents are based on a risk assessment approach, in which a demonstrated threshold effect for a genotoxic agent is likely to be an important factor in reaching a decision concerning authorisation of the product (Pratt and Barron, 2003).

On this base, all of chemical compounds used in agriculture either as pesticide or plant growth regulator must be tested for its mutagenic effects. Many plant regulator compounds have been tested for its mutagenicity. Miadokova *et al.* (1994) found that Rasti,m 30 DKV has increased rates of genetic changes in *Saccharomyces cerevisiae* and the carcinogenic effect of gibberellin A3 was investigated with Swiss albino mice, the results indicate that gibberellin A3 was carcinogenic in mice (El-Mofty *et al.*, 1994). The study on Maleic hydrazide (MH) proved to have inhibitory effect of biosynthetic activity (Marcano *et al.*, 2004), More recently, Sorensen and Danielsen (2006) have been conducted experiments to evaluate the plant growth regulator chlormequat, on reproductive function in male and female mice. These experiments showed that chlormequat had compromised fertilizing on reproduction in male mice.

Ethephon is organophosphorus pesticide and also used as a plant growth regulator, its use varies with plant species, chemical concentration, and time of application. Ethephon regulates phases of plant growth and development by application to various growth sites (Kidd and James, 1991). Ethephon's mode of action is manifested via liberation of ethylene, which is absorbed

by the plant and interferes in the growth process. It is also used in the acceleration of ripening of fruits and vegetables (Montogomery, 1993). Ethephon also inhibits the activity of plasma butyrylcholinesterase (BuChE) in humans, dogs, rats and mice (Brock, 1991; Haux *et al.*, 2000). Mouse plasma cholinesterase (ChE) *in vitro* and *in vivo* are more sensitive to ethephon than any other esterases. All mouse liver esterases observed are less sensitive than plasma ChE to ethephon *in vitro* and *in vivo*. Thus, BChE inhibition continues to be the most sensitive marker of ethephon exposure (Haux *et al.*, 2002).

A rodent model, the albino mouse, was used to investigate the toxicity and mutagenicity of organophosphorus *in vitro* and *in vivo* by detecting its capacity to inhibit enzymes (mainly ChE), measuring change of plasma protein content, and determination of DNA and RNA concentration (Mutch *et al.*, 1995; Williams *et al.*, 1997; Sparks *et al.*, 1999; Shukla and Taneja, 2000; Monteiro *et al.*, 2001; Seiler *et al.*, 2001; Quistad and Casida, 2004).

The present study aims to study the mutagenic effects of an organophosphorus pesticides ethephon by detecting the level of nucleic acids (DNA and RNA) in the liver and testis of albino mice and measuring the level of proteins and cholinesterase in plasma as sensitive parameters for mutagenicity.

MATERIALS AND METHODS

This study was done in the department of biology, College of science, King Abdulaziz university, Jeddah, Saudia Arabia during of the year 2005.

Animals: Twenty-eight adult albino male mice weighting between 25-30 g were used in this study. The animals were obtained from the animal house of King Fahad Research Centre. The male mice were divided into four groups each group have 7 animals (N = 28):

Group I: Seven animals were used as control.

Group II: Seven animals were orally with a dose of 1/4 LD_{50} of ethephon for two weeks.

Group III: Seven animals were orally with a dose of 1/8 LD_{50} of ethephon for two weeks.

Group IV: Seven animals were orally with a dose of 1/10 LD_{50} of ethephon for two weeks.

After 2 weeks from beginning of experiment blood plasma was collected from each animal group, animals were sacrificed, the liver and testis were removed for biochemical assay study.

Methods

Determination of Nucleic acid concentrations: For the determination of DNA and RNA, liver and testis tissues, are homogenized with distilled water (1 g tissue in 20% dH_2O) at 0°C one million of the previous tissue homogenate mixed with 10% cold trichloroacetic acid (TCA). The pellet obtained was washed once with cold TCA and twice 95% ethanol. The cell pellet was boiled in mixture of ethanol and diethyl ether.

The pellet was re-suspended in 5% cold TCA and heat in 90°C for 15 min. The supernatant was used for assessment of DNA and RNA.

Determination of DNA content according to Dische (1955) using diphenylamine reagent (1% acetic acid add to 2.75% concentrated H_2SO_4). DNA gives blue colour when heated with diphenylamine reagent was read at 600 nm using spectrophotometer.

The RNA content was determined using Orcinol method (1% concentrated HCl containing 0.5 g $FeCl_3$) according to Schneider (1957). Green colour was read at 660 nm using spectrophotometer.

Determination of protein: Protein was determined according to protein kit (Gornall *et al.*, 1949).

Bivert reagent: Twenty one m mol/L sodium potassium tartrate mixed with 750 m mol L^{-1} sodium hydroxide with potassium iodide 6 m mol L^{-1}.

In a clean test tube, 0.05 mL plasma of mice was taken, then 2 mL reagent solution were added. The optical density of resulting blue color was read at wavelength 550 nm.

Determination of cholinesterase activity: Cholinesterase enzyme was determined by using ChE Kit. The enzyme activity was tested in blood plasma of mice according to the method (Jakobs *et al.*, 1990).

The solution was prepared by mixing portion of Bulyrythiocholine iodide 420 m mol L^{-1} add with 20 parts of 5.5 dithiobis-2-nitrobenzoic acid 0.26 m mol L^{-1} with phosphate buffer 42.0 m mol L^{-1} this mixture with 3 mL of deionizer water.

The absorbance was read again after 30, 60 and 90 sec at wavelength 405 nm.

Statistical analysis: The results of DNA, RNA, protein content and enzyme activity were statistically analyzed using t-test (Sokall and Rohlf, 1969) and SSPS.

RESULTS

Changes in DNA concentrations: Table 1 showed the effect of ethephon on total DNA concentrations in treated mice liver and testis. The effect of the high and medium

Table 1: Effect of ethephon on total DNA concentrations in treated mice liver and testis

	DNA content in liver (mg g^{-1} tissue)		DNA content in testis (mg g^{-1} tissue)	
Treatments	Mean±SD	Percentage of reduction	Mean±SD	Percentage of reduction
Control	0.803± 0.03	-	0.531±0.034	-
High dose	0.514±0.056***	35.99	0.348±0.028***	34.46
Medium dose	0.62±0.057***	22.79	0.424±0.0319***	20.15
Low dose	0.737± 0.087*	8.22	0.478±0.046*	9.98

* Significant at 0.05 level, *** High significant at 0.001 level

Table 2: Effect of ethephon on total RNA concentration in the liver and testis of treated male mice

	RNA content in liver (mg g^{-1} tissue)		RNA content in testis (mg g^{-1} tissue)	
Treatments	Mean±SD	Percentage of reduction	Mean±SD	Percentage of reduction
Control	0.448±0.039	0	0.327±0.051	0
High dose	0.305±0.071***	31.92	0.226±0.037***	30.89
Medium dose	0.345±0.042***	22.99	0.261±0.067	20.18
Low dose	0.371±0.036**	17.19	0.27±0.0598	17.35

**Significant at 0.01 level, ***High significant at 0.001 level

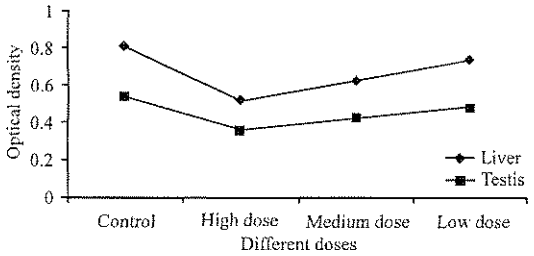

Fig. 1: DNA content in the liver and testis of male mice after treatment with ethyephon-pesticide

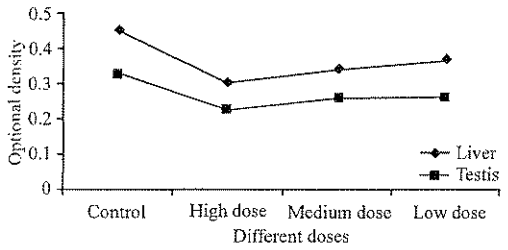

Fig. 2: RNA content in the liver and testis of male mice treated with ethephon-pesticide

doses on the total content of the liver DNA were highly significant comparing with the control, also the low dose effect was significant, It is therefore, apparent that the effect of all three doses of ethephon on the total content of the liver DNA was significant. The similar results were obtained for the effect of ethephon on the testis DNA content which were significantly reduced when compared to the control (Fig. 1).

Changes in RNA contents: Statistical analysis of total RNA content is presented in Table 2 and Fig. 2. The results showed that, there were significant differences between the mean value of different doses of treated mice and the control. The RNA content (using the three doses) in the testis were also decreased significantly when compared with the control mice.

Table 3: Effect of ethephon on protein content in blood plasma of treated male mice

	Protein content in blood plasma (g dL^{-1})	
Treatments	Mean±SD	% of reduction
Control	8.469±0.376	0
High dose	5.721±0.278 ***	32.45
Medium dose	6.053±0.666***	28.53
Low dose	7.057±0.69***	16.67

***High significant at 0.001

Change in protein content: The changes in protein content of blood plasma of animals treated with ethephon shown in Table 3 and Fig. 3. The reduction in plasma protein levels in the treated mice was correlated with dose increase. In all doses reduction in blood plasma were observed for treated mice, where it was highly significant in all three regimen of doses.

Changes in cholinesterase contents: The classical laboratory tests for exposure to organophosphorus toxicants are inhibition of cholinesterase enzymes (Haux *et al.*, 2002).

Results for measuring the changes in cholinesterase activity of blood plasma after treating mice with ethephon were detected in blood plasma of male mice treated with ethephon are shown in Table 4 and Fig. 4. It is noticed that the cholinesterase content was significantly decreased when high and medium doses were used. The low dose also showed a decrease of the enzyme activity comparing to the control but not significant.

Table 4: Effect of ethephon on cholinesterase activity in blood plasma of treated male mice

	Cholinesterase activity in blood plasma (U mL^{-1})	
Treatments	Mean±SD	% of reduction
Control	6.775±0.631	0
High dose	5.242±0.963***	22.63
Medium dose	5.605±0.857**	17.69
Low dose	6.010±0.774	11.29

Significant at 0.01 level, * High significant at 0.001 level

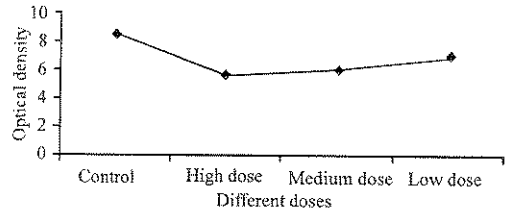

Fig. 3: Total protein in the blood plasma of male mice treated with ethephon

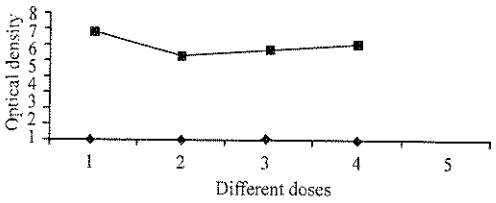

Fig. 4: Effect of ethephon on cholinesterase activity in the blood plasma of male mice

DISCUSSION

It has been settled that numerous environmental agents can induce genetic alteration in human and livestock cells. The changes (mutation) are deleterious to normal cell function. There are several examples of exposure to genotoxicants such as pesticides especially in agriculture area, and industrial emissions. The results of the present study presented in Table (1-4) reveal that the treated groups of animals showed a significant decrease of nucleic acids (DNA and RNA), protein and cholinesterase levels compared with control groups.

From the results, significant decrease in nucleic acids content in liver and testis were correlated with increasing doses of pesticide ethephon was observed. This assay is sensitive for the detection of mutagenicity caused by pesticides.

Also, the genotoxicity of organophosphorus pesticide (ethephon) could be attributed to many reasons such as the sensitivity of the treated animal itself. The way, time and the administrated dose of ethephon may be also reasons for such discrepancy.

Our results were in agreement with Rahman *et al.* (2002) who observed DNA damage at all doses with two organophosphorus in albino mice when compared with control. Because cellular RNA synthesis is a DNA depended process, thus significant decrease in RNA was recorded after treatment with several insecticides due to inhibition of DNA dependent RNA polymerase (Lafarge and Frayssinet, 1970; Maugh, 1974; De Hondt *et al.*, 1979). Our results were also in agreement with El-Fiky *et al.* (1992) who found a decrease in nucleic acids and total protein in liver and brain of mice treated with seven pesticides. The results also disagree with those informing that ethephon has no genotoxic effect, when tested for unscheduled DNA synthesis in the rat hepatocyte system (Barfknecht *et al.*, 1984).

Observation of a highly significant decreased of protein content in blood plasma of animals treated with ethephon in all doses may be due to the toxicity and mutagenicity of the pesticide. The potential for genetic toxicity of ethephon was evaluated in several assays. The compound was found to be mutagenic in strain TA-1535 with and without S9 activation in the Ames assay (Jagannath, 1987).

ACE determination in plasma has been used as a good method to evaluate exposure to organophosphorus pesticides (Eduardo and Patricio, 2003).

In the present study, the gradual decrease in cholinesterase (ChE) activity in blood plasma of male mice treated with ethephon was observed.

These results emphasize the positive correlation between mutagenic effect and toxicity, which was previously reported in mice by Haux *et al.* (2002), who found that ChE inhibition continues to be the most sensitive marker of ethephon exposure.

Kamal *et al.* (1990), who measured a change in the serum cholinesterase and total protein in Egyptian pesticide spray men, they recorded a significant decrease in the level of cholinesterase and protein than control Rupa *et al.* (1991) reported that pesticide induced significant increase in abnormal sperm in mice and indicated that this results due to the reduction in several biochemical parameters after treated with inhibitor and phosphorylating agent. Sakar (1985), Abiola *et al.* (1991) and Pope and Chakraborti (1992), observed inhibition of cholinesterase in blood plasma of treated animals after exposure to pesticides.

Depression of plasma cholinesterase level was recorded also by Rama and Gaga (1992), in 27% of farm workers exposed to pesticides.

Study of organophosphorus pesticides recorded inhibition in blood and brain cholinesterase of rats after single injection for four weeks (Bushnell *et al.*, 1993). Also, Sheets *et al.* (1997), studied the effect of six organophosphorus pesticides in rats, their results showed

an inhibition of cholinesterase activity. Lassiter *et al.* (1999) and Tang *et al.* (1999), found that this organophosphorus pesticide caused decrease in cholinesterase activity in treated mice and rat.

The inhibition rate is generally related to ethephon concentration; also ethephon acts as a phosphorylating agent in inhibiting ChE activity. This is agreement with Haux *et al.* (2000 and 2002) when they studied the specificity of ethephon as a butyrylcholinesterase inhibitor and phosphorylating agent. Therefore, the observed decrease in nucleic acids, protein and cholinesterase contents after treatment with different doses of ethephon, may be attributed to its pesticidal and phosphorylating activities.

Further studies are necessary to clarify the specificity and physiological significance of the effects induced by ethephon in mammalian cells.

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