Genotoxicity of Chlorpyrifos and the Antimutagenic Role of Lettuce Leaves in Male Mice

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Abstract

Chlorpyrifos [O O-diethyl-O-(3 5 6-trichloro-2-pyridyl)-phosphorothioate] is one of the most widely used organophosphate insecticides. Previous studies proved that chlorpyrifos, at different doses, induced genotoxicity. In Egyptian foods, the residual levels of pesticides are often higher than those found in developed country ones. So the aim of this research was to evaluate the genotoxicity of the insecticide chlorpyrifos at doses equal to its maximum residue limit (MRL) in the leafy vegetables, its double and quadruple (0.5, 1 and 2 mg/kg body weight) in somatic and germ cells of male mice. In addition to that, evaluating the role of lettuce leaves as antigenotoxic in reducing the genotoxic effects of chlorpyrifos tested doses when concurrently administrated to these animals. The study was conducted on adult male laboratory mice at three levels: bone marrow cells as a model for mitotic chromosome aberrations, spermatocytes as a model for meiotic chromosomes and sperm count and morphology. The results of the present study indicate that the treatment of male mice with chlorpyrifos by oral gavages for three months induced significant increase in the frequencies of total chromosomal aberrations in both somatic and germ cells in relation to control groups. Results of the sperm analysis showed that chlorpyrifos induced significant decrease in the sperm count when compared to negative control. Furthermore, it induced significant increase in head and tail sperm abnormalities, among which coiled tail was considered the most obvious sperm abnormality induced by chlorpyrifos. At the same time, the present study indicated that lettuce leaves feed concurrently with three doses of chlorpyrifos could not protect cells from damage.

***Key words*:** organophosphate, chromosome aberration, somatic cells, germ cells, sperm analysis

Genotoxidade de Chlorpyrifos e papel antimutagênico das folhas de alfaces em ratos

Resumo

Chlorpyrifos [O O-diethyl-O-(3 5 6-trichloro-2-pyridyl)-phosphorothioate] é um dos inseticidades a base de organofosfato mais utilizados. Estudos anteriores provaram que chlorpyrifos, em doses diferentes, induziu a genotoxidade. Em alimentos egípcios, os níveis residuais de pesticidas são normalmente mais elevados que aqueles encontrados em países desenvolvidos. Então, o objetivo dessa pesquisa foi o de avaliar a genotoxidade do inseticida chlorpyrifos em doses iguais ao limite residual máximo (MRL) em vegetais com folhas, seu dobro e quádruplo (0.5, 1 and 2 mg/kg peso corpóreo) em células somáticas e germinativas de ratos. Além disso, avaliando o papel das folhas de alface como antígenos tóxicos na redução dos efeitos de genotoxidade em doses de chlorpyrifos testadas quando administradas a esses animais. O estudo foi conduzido em ratos adultos de laboratório, em três níveis: células ósseas como modelo para aberrações miótico-cromossômicas; espermatócitos como modelo meiótico-cromossômico, contagem e morfologia de espermatozóides. Os resultados do presente estudo indicaram que o tratamento de ratos com chlorpyrifos, administrado em doses orais por três meses induziram um significativo aumento da freqüência de aberrações cromossômicas totais tanto em células somáticas quanto em células germinativas se relacionados aos grupos de controle. Os resultados da análise de espermatozóide demonstraram que chlorpyrifos induziu uma diminuição significativa na contagem de espermatozóides quando comparado ao grupo de controle negativo. Além disso, também houve a indução de um aumento significativo em anomalias na cabeça e na cauda dos espermatozóides, dentre as quais a cauda enrolada foi considerada a anomalia mais óbvia entre os espermatozóides induzidos por chlorpyrifos. Ao mesmo tempo, o presente estudo indicou que as folhas de alface alimentadas regularmente com três doses de chlorpyrifos não conseguiram proteger suas células de danos.

Palavras-Chave: organofosfato, aberração cromossômica, células somáticas, células germinativas, análise de espermática

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Introduction

Chlorpyrifos [O O-diethyl-O-(3 5 6-trichloro-2-pyridyl)-phosphorothioate] is one of the most widely used organophosphate insecticides (Donaldson et al., 2002). According to the U.S. Environmental Protection Agency, approximately 800 registered products on the market contain chlorpyrifos, and they are used for a number of purposes, including pest control for a variety of food crops, turf and ornamental plants, green houses and sod; indoor pest control; structural pest control; and pet collars (Smegal, 2002). The primary mechanism of action of chlorpyrifos involves the inhibition of acetylcholinesterase resulting in a wide range of neurotoxic effects in humans, such as sensory and motor neuropathy with permanent paralysis (Meggs, 2003).

Some experimental studies proved that chlorpyrifos induced genotoxicity (Patinaik & Tripathy, 1992; Rahman et al., 2002; Ali et al., 2008 & 2009), sister-chromatid exchanges (Amer & Aly, 1992; Sobti et al., 1982), and chromosomal loss (Woodruff et al., 1983). In addition to that, chlorpyrifos induced mitotic abnormalities and cytotoxicity (Roy et al., 1998), immunologic abnormalities such as an increased expression of the CD5 and CD8 surface markers (Blakley et al., 1999) the generation of reactive oxygen species, a DNA damage, and the lactate dehydrogenase leakage (Bagchi et al., 1995).

Residual levels of pesticides in Egyptian foods are often higher than those found in developed country ones, either because the numbers of skilled technicians available to enforce the laws concerning pesticide usage are inadequate or due to the lack of adequate financial resources (Dogheim et al., 1996). The highest levels of pesticide exposure occur in pesticide applicators, farm workers and people who live closer to heavily treated agricultural lands. In a study of the health profile of 300 occupationally exposed pesticide workers in the formulating industry in Egypt, Amer (1994) reported several disease conditions including hypertension, hepathomegaly, dermatosis, and chromosomal aberrations. Pesticide exposure has also been associated to an elevation of cancer risks, and reproductive dysfunctions in agricultural workers (Horrigan et al., 2002). A recent work on organophosphate pesticide illustrates that exposure to neurotoxic compounds, such as chlorpyrifos, at levels considered safe for adults could lead early children to permanent loss of brain function if exposed to pesticide residues through their diet (Slotkin, 2004).

Food is one of the most important routes of exposure to pesticide residues while considering human beings. Pesticides are applied to growing crops and fruits and if they are used close to harvest, contamination is very likely. If animals are fed on feedstuff treated with pesticides, these hazardous chemicals can be taken up in meat, milk and its products. Finally, food may be contaminated when treated with pesticides for prolonged storage (Saeed et al., 2005).

Pesticide use and residues on lettuce are of particular food safety interest. Lettuce is consumed fresh, so residues that may remain on the harvested produce are not removed by processing. Pesticide residues are detected in lettuce and other leafy vegetables more often than in other fresh vegetables, since lettuce is a major fresh-market vegetable crop (Yess, 1991.). Moreover, the exposure to pesticides through food besides the dermal or inhalation routes varies greatly among individuals of a population, due to different eating habits and the amount of food consumed. Hence, the long-term genetic hazard of pesticides on man cannot be ignored and it is, therefore, highly desirable to search for protective measures to minimize their harmful effects (Fan & Jackson, 1989).

Recently, there have been considerable efforts to search for naturally occurring substances that can inhibit, reverse or retard mutagenicity. A wide array of substances derived from edibles and medicinal plants have been reported to possess anti-carcinogenic and antimutagenic activities (Surh et al., 2001). Souri et al. (2004) reported that some vegetable extracts including savory, radish leaf, garden cress, spearmint, leek, chive, lettuce and dill have shown an antioxidant activity. Also, Llorach et al. (2004) and Nicolle et al. (2004) reported that lettuce consumption improves antioxidant status due to its richness in antioxidants, such as vitamins C and E and carotenoids.

Taking these considerations into account, the aim of this research was to evaluate the genotoxicity of the insecticide chlorpyrifos at tested doses and the role of lettuce leaves as antigenotoxic when concurrently administrated to animals with the tested insecticide. The study was conducted in adult male laboratory mice at three levels: bone marrow cells as a model for mitotic chromosome aberrations, spermatocytes as a model for meiotic chromosomes and sperm count and morphology.

Material and Methods

Experimental Animals

Seventy male adult Swiss albino mice weighing between 20-25 grams were used in this study. These animals were obtained from the animal house of the National Research Center, Giza, in Egypt. The animals were housed in plastic cages, ten per cage, and maintained on standard laboratory diet and water *ad libitum*. Animals were divided into two experiments. The first experiment had forty-five animals and was divided into nine equal groups. Animals of the first group were orally treated by gavages with distilled water daily and used as control. Animals of the second group were fed on 20% lettuce leave diet according

to Nicolle et al. (2004). Animals of group three were injected intraperitoneal with single dose of 25mg/kg body weight cyclophosphamide and used as positive control. In groups four, five and six animals were orally treated by gavages daily with chlorpyrifos, using doses of 0.5, 1 and 2 mg/kg, these doses were equal to the maximum residue limit of chlorpyrifos in leafy vegetables (MRL), its double and quadruple doses respectively. Animals of groups seven, eight and nine were orally treated by gavages daily with the three doses of chlorpyrifos (0.5, 1 and 2 mg/kg respectively) and concurrently fed on 20% lettuce leave diet. All animals were treated for 90 successive days and then they were sacrificed for chromosomal analysis of both somatic and germ cells. The second experiment had twenty-five animals, divided into five equal groups, as follows: animals of groups one, two and three were treated as those in the first experiment, animals of group four were orally treated by gavages daily with a dose of 2 mg/kg chlorpyrifos. Animals of group five were treated orally by gavages with a dose of 2 mg/kg chlorpyrifos daily and concurrently fed on 20% lettuce leave diet. All animals were treated for 90 successive days and sacrificed at the day 35 after the end of treatment for sperm analysis (Monesi, 1962).

Chromosomal Aberration Analysis

In the end of the treatment, animals of all treated groups of the first experiment were injected intraperitoneally with colchicine to arrest cell division at metaphase. Two hours after injection, animals were sacrificed by cervical dislocation for preparation of the chromosomes of bone-marrow and spermatocyte cells. Chromosomes of bone-marrow cells were prepared by using the methodology of Yosida & Amano (1965). Both femurs were dissected out and cleaned of any adhering muscle tissue. Bone-marrow cells were collected from both the femurs by flushing in saline solution and then incubated at 37°C in hypotonic solution (KCl 0.56%) for 35min, fixed in methanol-glacial acetic acid (3:1). The cells were resuspended in a small volume of fixative, dropped onto chilled slides, flame-dried, and stained with 10% buffered Giemsa (pH 6.8). Spermatocytes were prepared according to Brewen & Preston (1987). The tunica of the testis was removed and the tubules were transferred to a small Petri dish containing isotonic solution (2.2% sodium citrate) and teased out with curved forceps on a piece of mesh. The cell suspension was transferred into a conical tube and centrifuged. The supernatant was removed and the pellet was re-suspended in hypotonic solution (1.1% trisodium citrate) for 20 minutes and fixed in cold fixative. The cells were resuspended in a small volume of fixative, two or three drops of cell suspension were dropped on a clean slide stored in cold 70% ethanol and dried on hot plate at 60°C. Slides were stained with 10% buffered Giemsa (pH 6.8). One hundred good metaphases for somatic and germ cells of each animal were examined microscopically for scoring different types of aberration.

Sperm analysis

After 35 days of the last dose (duration of spermatogenesis), the animals of the second experiment were sacrificed by neck vertebra luxation. The epididymides from each mouse were removed and sperm was collected as quickly as possible when each mouse was dissected. To release sperm, the cauda epididymides were cut in a pre-warmed Petri dish containing 1ml of saline solution at 37°C. Spermatozoa were counted using heamocytometer and a drop of a homogenate smeared on a cleaned slide allowed to air dry and stained with approximately 0.05% aqueous eosin Y. The slides were coded and used for the examination of sperm head and tail abnormalities. For each animal 500 sperms were examined for morphological abnormalities according to the criteria of Jeong et al. (2005).

Statistical analysis

Statistical analyses were performed with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons between pairs. Results are reported as mean values ± S.D. and differences were considered as significant when ($P \leq 0.05$).

Results and Discussion

Widespread use of pesticides in agriculture is now a worldwide phenomenon. The rate of increase of pesticide use is relatively high in Africa, Central and South America, Far East Asia, and Middle East (Luke et al., 1988). While the sales of herbicides have been greater than those of insecticides in developed countries, the greatest proportion of pesticides used in developing countries are still insecticides, mainly used for agricultural crops. Awareness about the potential harmful effects of pesticide residues on human health has been growing (Dittus & Hillers, 1993).

So, in the present study, the genotoxicity of chlorpyrifos and the role of lettuce leaves as antigenotoxic were evaluated. The study was performed on three levels: bone marrow cells as a model for somatic cells, spermatocytes as a model for germ cells and sperm analysis assay. Chromosomal aberrations were structural and numerical; the observed structural chromosomal aberrations in bone marrow cells were chromatid gaps, chromatid breaks, deletions, centric fusion and centromeric attenuation figure 1B-F. The observed types of numerical aberrations were endomitosis and polyploidy figure 1G-H. The observed types of structural chromosomal aberrations in spermatocyte were X-Y univalent and autosomal univalent, while the observed type

of numerical aberrations and polyploidy (figure 2B-D).

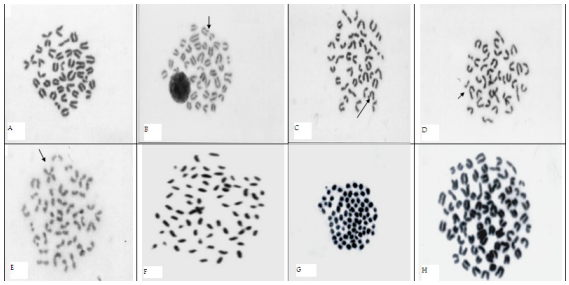

Figure 1. Different types of chromosomal aberrations induced in bone marrow cells: (A) normal; (B) chromatid gap; (C) chromatid break; (D) deletion; (E) centric fusion; (F) centromeric attenuation; (G) endomitose; (H) polyploidy

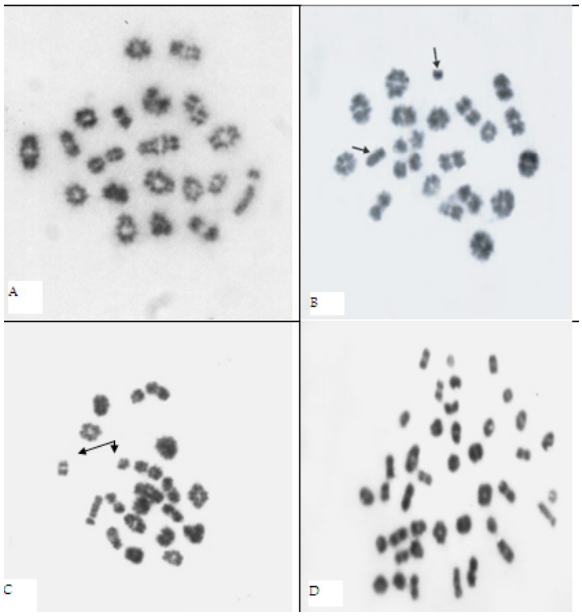

Figure 2. photographs showed different types of observed chromosomal abnormalities in spermatocytes: (A): normal; (B) x-y univalent; (C) autosomal univalent; (D) polyploid.

Tables 1 and 2 represent the genotoxic effect of chlorpyrifos, atdifferent doses, on bone marrow cells and spermatocytes of male mice and they also show the role of lettuce leaves against the genotoxicity of chlorpyrifos in all examined cells. The results show that chlorpyrifos causes dose dependent significant increases in the mean values of structural and numerical chromosomal aberrations in both somatic and germ cells when compared to negative and positive controls. In addition to that, the results demonstrate that lettuce leaves show no-significant decrease in the mean values of the individual and total chromosomal aberrations induced by the three doses of chlorpyrifos in bone marrow cells and spermatocytes of male mice.

The result of the present study seems to be in agreement with Waters et al. (1980) who reported that Chlorpyrifos depicted positive results for DNA damage in prokaryotic systems. De Hondt et al. (1983) reported a significant increase in the incidence of chromosomal abnormalities in the bone marrow cells of rats treated with a formulation of chlorpyrifos. Amer & Aly (1992) reported a significant increase in chromosome aberrations after *in vitro* treatment of primary cultures of mouse spleen cells for 4 h with chlorpyrifos. Woodruff et al. (1983) and Patnaik & Tripathy (1992) reported the genotoxicity of chlorpyrifos in the somatic and germ cells of Drosophila. Amer & Fahmy (1982); Benova et al. (1989) and Ni et al. (1993) reported that multiple intraperitoneal administration of chlorpyrifos induced mutagenic response in mouse bone marrow cells MNPCE assay. Furthermore, Ali et al. (2008 & 2009) studied the acute genotoxic effects of chlorpyrifos in different tissues of fish Channa punctatus (Bloch) by the use of micronucleus assay (MN assay) and alkaline single-cell gel electrophoresis (comet assay). They observed that chlorpyrifos increased the frequency of micronucleus induction and tail DNA in the lymphocyte and gill cells on 96 h,at all tested concentrations.

Some studies have also shown a positive association between genotoxicity and occupational exposure to pesticides. De Ferrari et al. (1991) indicated that organothiophosphorus insecticides showed significant increase in the incidence of chromosomal aberrations in lymphocytes of healthy individuals who works with flower production. Kourakis et al. (1996) found that there was a significant increase in the frequency of chromosome aberrations in peripheral blood

Table 1. Mean values of different chromosomal aberrations induced by chlorpyrifos without and with lettuce leaves in bone marrow cells of male mice.

Experimental groups	Structural chromosomal aberrations						Numerical aberrations			Total chromosomal aberrations
	Chromatid gaps	Chromatid breaks	deletions	Centric fusion	Centromeric attenuations	Total structure aberrations	endomitosis	polyploidy	Total numerical aberrations	
Negative control	1.80d±0.45	0.00e±0.00	0.00d±0.00	0.00b±0.00	1.20b±0.83	3.00e±1.23	2.20e±0.45	0.00b±0.00	2.20d±0.45	5.20e±1.30
lettuce	1.60d±0.55	0.00e±0.00	0.00d±0.00	0.00b±0.00	0.80b±0.45	2.40e±0.55	1.40f±0.55	0.00b±0.00	1.40e±0.55	3.80e±0.84
Cyclophosphamide	2.60c±0.55	1.20d±0.45	2.20c±0.45	0.00b±0.00	1.20b±0.84	7.20d±1.30	2.40de±0.55	1.20a±0.45	3.60c±0.55	10.80d±0.84
0.5 mg/kg chlorpyrifos	3.40b±0.55	2.80bc±0.447	3.40b±0.55	1.20 a±0.45	1.60b±0.548	12.40c±0.55	3.20c±0.45	0.40b±0.55	3.60c±0.55	16.00c±0.71
1 mg/kg chlorpyrifos	3.60ab±0.55	3.40b±0.55	4.20b±0.84	1.40 a±0.55	2.60a±0.548	15.20b±1.30	4.40b±0.548	1.20 a±0.45	5.60b±0.55	20.80b±1.30
2 mg/kg chlorpyrifos	4.40 a±.55	4.40 a±0.55	5.20a±0.84	1.60 a±0.55	2.60a±0.548	18.20a±1.30	5.40a±0.548	1.40a±0.55	6.80 a±0.45	25.00a± 1.414
0.5mg/kg chlorpyrifos ± lettuce	3.20bc±0.84	2.60 c±0.55	3.40 b±0.55	1.00 a±0.71	1.60b±0.55	1.60b±0.55	3.0cd±0.707	0.40 b±0.55	3.40c±0.547	15.00c±1.225
1mg/kg chlorpyrifos ± lettuce	3.6 ab±0.55	3.00bc±0.71	4.000b±0.71	1.40 a±0.55	2.60a±0.55	2.60a±0.55	4.40b±0.547	1.00 a±0.00	5.40b±0.547	20.00 b±1.87
2 mg/kg chlorpyrifos ± lettuce	4.40a±0.547	4.40 a±0.5477	5.00a±0.707	1.60a±0.547	2.60a±0.548	2.60 a±0.55	5.20a±0.447	1.40a±0.55	6.60a±0.55	24.60 a±0.89

Values with different superscript letters within columns represent significant statistical differences (P<0.05).

Table 2. Mean values of different chromosomal aberrations induced by chlorpyrifos without and with lettuce leaves in spermatocytes of mice.

Experimental Groups	Structure aberrations				Numerical aberrations			
	x-y univalent	Autosomal univalent	chain	Total structure aberrations	hypoploidy	polyploidy	Total numerical aberrations	Total chromosomal aberrations
Control	1.80b±0.45	0.00 d±0.00	0.00 b±0.00	1.80 e±045	1.40 d±0.89	0.00c±0.00	1.40 d±0.89	3.20 e±0.84
Lettuce	1.80b±045	0.00d±0.00	0.00 b±0.00	1.80 e±.45	1.20d±0.84	0.00 c±0.00	1.20 d±0.84	3.00 e±0.71
cyclophospamide	2.60b±0.55	1.40 c±0.55	0.00 b±0.00	4.00 d±0.00	2.40c±0.55	0.60b± 0.55	3.00 bc±0.71	7.00 d±0.71
0.5 mg/kg chlorpyrifos	4.20a± 0.84	2.60 b±0.55	0.00 b±0.00	6.80 c±.45	2.80 bc±0.45	0.00c±0.00	2.80 c±0.45	9.60 c±0.55
1 mg/kg chlorpyrifos	4.20a±0.84	3.60 a±0.55	0.40 b±0.547	8.20 b±0.84	3.80a±0.84	0.00 c±0.00	3.80 b±0.84	12.00 b±0.71
2mg/kg chlorpyrifos	4.60a±0.55	3.80 a± 0.84	1.60a±0.55	10.00 a±0.71	3.80a±0.45	1.60a±0.55	5.40 a±0.55	15.40 a±0.89
0.5mg/kg chlorpyrifos + Lettuce	3.80a±0.85	2.60b±0.55	0.00 b±0.00	6.40 c±1.14	2.80 bc±0.45	.00 c±0.00	2.800 c±0.45	9.20 c±0.84
1 mg/kg chlorpyrifos + Lettuce	4.00a±0.71	3.40 a±0.55	0.40 b±0.55	7.80 b±0.55	3.60 ab± 0.55	0.00c±0.00	3.60 bc±0.54	11.40 b±1.14
2 mg/kg chlorpyrifos + Lettuce	4.600a±0.547	3.60a±0.55	1.20 a±0.84	9.40 a±1.14	3.80a±0.84	1.20a±0.84	5.00 a±0.71	14.60 a±1.14

Values with different superscript letters within columns represent significant statistical differences (P<0.05).

lymphocytes of workers occupationally exposed to a mixture of pesticides. Also, Rahman et al. (2002) confirmed the ability of the organophosphorus pesticide chloropyrifos to induce *in vivo* genotoxic effect in leucocytes of Swiss albino mice using the single cell gel electrophoresis.

Recently, several studies have suggested that human semen quality has declined over the past decades and some of them have associated it with occupational exposure to pesticides, what has increased the focus on male reproductive health (Jensen. et al., 2006). Recio-Vega et al. (2008) evaluated the effect of organophosphate pesticides at three occupational exposure levels on semen quality. The results showed a significant decrease in total sperm count among subjects with the highest exposure to organophosphate pesticides, check figure 3. This result was confirmed by Joshi et al. (2007) who reported that male rat oral administration of chlorpyrifos at the dose levels of 7.5, 12.5 and 17.5 mg/kg b. wt. /day for 30 days brought about marked reduction in epididymal and testicular sperm counts in exposed males and a decrease in serum testosterone concentration. Moreover, Golec et al. (2003) showed that exposure of employment to organophosphate pesticides in agriculture increases the risk of specific morphological abnormalities in sperm as well as decreases the sperm count and the percentage of viable sperm. Besides, regarding sperm morphology analysis the observed abnormal types in sperm head were amorphous, without hock, small head, while the coiled tail was the only type observed in sperm tail. The previous results are in agreement with the present findings, since the results of sperm count analysis in mice after 90 days of chlorpyrifos administration showed that chlorpyrifos produced significant decrease in the mean value of sperm count (180.6 ± 13.2) at level (p≤0.0 1) when compared to negative control (572.20 ± 50.03). There was no-significant decrease in the mean value of sperm count when compared to positive control (197±5.79) as shown in Figure 3. At the same time, chlorpyrifos induced a marked significant increase in the frequencies of total sperm abnormal head (68 ± 4.47) and coiled tail sperms (329.8 ± 5.26) when compared to negative (26.4 ± 2.88 and 0.0 ± 0.0) and positive controls (105±5.47 and 1.4 ± 1.14), respectively, as shown in figure 4 & 5.

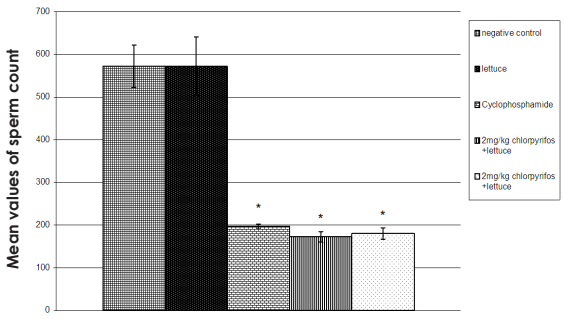

Figure 3. Mean values of sperm count in all experimental groups.

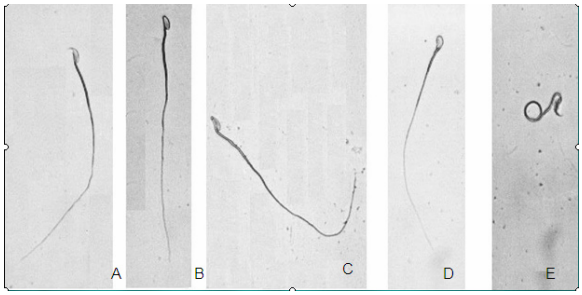

Figure 4. Different types of sperm abnormalities: (A) normal (B) head without hock (C) & and (D) amorphous heads (E) coiled tail

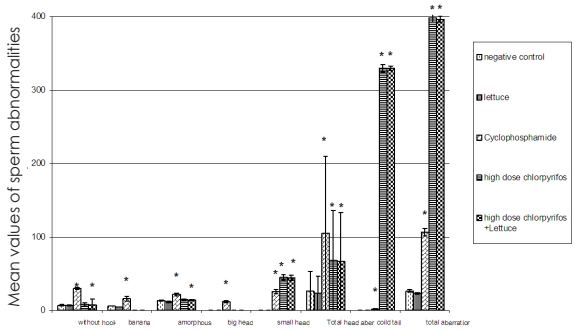

Figure 5. Mean values of sperm abnormalities induced in all experimental groups.

Since chlorpyrifos produced abnormalities in mitotic and meiotic chromosomes of mice as mentioned earlier, the sperm morphological abnormalities observed here might be due to induced alterations in testicular DNA and sperm chromatin structure (Evenson et al., 1986). This concept of pesticide-inflicted sperm toxicity may be supported by the high positive correlation observed between mutagenicity and spermatotoxic effects in mice (Topham, 1980).

Limited animal studies have explored relationships between chlorpyrifos exposure and semen quality. Decreased sperm production and motility was observed in Holstein bulls 6 months after dermal lice treatment with an unknown amount of chlorpyrifos (ATSDR, 1997; Everett, 1982). Pant et al. (1995, 1996) found statistically significant dose-related declines in epididymal sperm count and percent motile sperm, as well as increased sperm with abnormal morphology in chlorpyrifos treated rats. Other studies held with animals have presented no associations between chlorpyrifos exposure and altered male reproductive health (ATSDR, 1997; Breslin et al. 1996). A number of pesticides have also been reported to adverse affect the male reproductive potentiality by causing decreases in sperm count (Whorton et al, 1977) and increases in the frequency of sperm head abnormalities (Bhunya & Behera, 1987; Pandey et al, 1990). Further, it has been observed that almost all germ cell mutagens induce sperm toxic effects in mice (Topham, 1980).

Fruit and vegetable intake is inversely correlated to risks of several chronic diseases in humans. Phytochemicals, and, in particular, phenolic compounds, present in plant foods may be partly responsible for these health benefits through a variety of mechanisms (Young et al., 2005). Higher plants that are extensively used in traditional medicines are being increasingly screened for their role in modulating the activity of environmental genotoxicants. The property of preventing carcinogenesis has been reported for many plant extracts. The observation of a close association between carcinogenesis and mutagenesis has extended the survey to include plant extracts and plant products which are able to modify the process of mutagenesis that involves alteration in genetic material. Natural plant products may modify the action of other known mutagens on the living organisms by activating the existing mutagens within the cell, inhibiting the production of mutagens in the cell, synergizing the activity of existing mutagens or by activating the promutagens within the cell into mutagens (Sarkar et al., 1998).

Many studies have suggested that a high consumption of cereals, fruits and vegetables reduces the risk of cancers at different organ sites. The ability of a diet rich in flavonoids (onions, lettuce, red wine) to reduce the levels of DNA adducts could explain the lower incidence of cancer associated with vegetables and fruits (Hill, 1997; Talaska et al., 2006; Rohrmann et al., 2007). Sarkar et al., (1998); Sangwan et al. (1998) and Pèrez & Gago (2006) demonstrated the ability of natural antimutagen including lettuce extracts to reduce the mutagenic activity of potassium dichromate and Benz[a]pyrene in male Balb/C mice. Souri et al. (2004) found that some vegetable extracts including savory, radish leaf, garden-cress, spearmint, leek, chive, lettuce and dill has shown an antioxidant activity comparable with those of dl-alpha-tocopherol and quercetin. Also, Proteggente et al. (2002) and Tarwadi & Agte (2003) reported that some green leafy vegetables, including lettuce and fruits, are considered rich sources of a number of micronutrients and other phytochemicals, having antioxidant properties. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition.

As for our study, the results do not agree with these findings. Since, the results showed that lettuce leaves were not able to prevent either mitotic, meiotic or sperm abnormalities induced by all the tested doses of chlorpyrifos. Tables 1 and 2 demonstrated that lettuce leaves feeding showed non-significant decrease in the mean values of total chromosomal aberrations induced by chlorpyrifos in both bone marrow cells and spermatocytes (15.20 ± 1.22, 20.00 ± 1.87, 24.60 ±.0.89 and 9.20±0.83, 11.40±1.14, 14.60±1.14 respectively) when compared to those of chlorpyrifos groups (16.00 ± 0.71, 20.80 ± 1.30, 25.00 ±1.41 and 9.60 ± 0.55, 12.00 ±0.71, 15.40 ± 0.89 respectively). Also, lettuce leaves feeding did not induce any significant differences in the mean values of the sperm count (180.60 ± 13.22) or sperm abnormalities (396.60± 3.91) when compared to chlorpyrifos (172.40 ±12.52 and 398.60 ± 6.06 respectively) as shown in figures 3 and 5. Sarkar et al. (2006) supported these results, since they indicated that the Chlorophyll was not able to reduce the clastogenicity of the chromium oxide to a significant level. Khan & Sinha(1994) indicated that vitamin C induced dose-dependent amelioration in the Phosphamidon-treated group whereas, in Endosulfan- and Mancozeb-treated groups, no further amelioration was achieved beyond the double dose of vitamin C and the damage frequency did not come down to the control level.

Conclusion

We can conclude that chloropyrifos has harmful effects on both somatic and germ cells. This leads us to put strict limitations on its use. Although, several studies indicated that some vegetable extracts including lettuce showed an antioxidant activity due to its richness in antioxidants such as vitamins C and E and carotenoids. We have found no statistically significant evidence of the protective role of the tested doses of lettuce leaves against the genotoxicity of Chlorpyrifos.

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