Original Article

The Effects of Acute Toxicity of Dieldrin on *HeLa* Cell Line: An *In Vitro* Assessment

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Abstract

Aim: Among environmental pollutants, there is a great concern about organochlorine pesticides (OCPs) due to their environmental persistence, accumulation in the food chain, detection in breast milk, and their ability to accumulate in adipose tissues. Due to the toxicity of OCPs and its relationship with human health, this study aimed to investigate the effects of dieldrin pesticides on morphological changes in the *HeLa* cell line. **Materials and Methods:** Standard concentrations of dieldrin (0.1-20 ppm) were prepared and cells were cultured in 1640 Roswell Park Memorial Institute (RPMI) medium containing 10% bovine serum albumin and Pen-Strep antibiotic. Subsequently, the morphological effects of dieldrin on *HeLa* cells in a cell culture medium were investigated. **Results:** Morphological and cytopathic changes were not observed in *HeLa* cells treated with concentrations of 0.1, 0.5, and 1 ppm of dieldrin. However, significant changes including cell rounding and cytopathic effects were observed in the cells treated with 5 ppm of dieldrin. Moreover, at concentrations of 15 and 20 ppm of dieldrin, the cells were completely destroyed and could not be examined. **Conclusion:** The effects of dieldrin on *HeLa* cell morphology were observed in the form of cell rounding and cytopathic effects. These morphological changes suggest that dieldrin may induce the process of apoptosis in cells. According to the results, the identification of different factors that aggravate the cytotoxic effects of this pesticide needs further research.

Keywords: Environment, morphological changes, organic pollutants, pesticides, toxicology

Introduction

Persistent organic pollutants (POPs) are toxic chemicals with bioaccumulation potential which are stable in the environment for a long time and accumulate in the food chain.[1] The main reasons for the environmental pollution of POPs that have caused side effects on human health and the environment include extensive production, uncontrolled application, incorrect disposal, and their persistence in the environment.[2,3] High lipophilic properties and slow degradation of these compounds cause their accumulation in the adipose tissues of fish, birds, mammals, and even the human body through food, air, and contaminated aquatic ecosystems.[4-6]

Pesticides are a group of these organic pollutants that are widely used in agriculture to protect seeds and crops. Pesticides' stability and their degradation products in the geosphere cause environmental problems. The pesticides' movement from soil to groundwater contaminates drinking water supplies and then absorbed by humans. The toxicity of pesticides is categorized by their lethal concentrations according to the WHO classification. Therefore, the maximum permissible level in drinking water that has been determined by the European Union for a pesticide is only 0.1 μg/L (0.1 ppb) and for pesticides and decomposition products is 0.5 μg/L.[7]

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Among environmental contaminants, there is a great concern about organochlorine pesticides (OCPs). These pesticides are divided into three main groups: dichlorodiphenyltrichloroethane (DDT), cyclodiene insecticides (aldrin, dieldrin, endrin, heptachlor, and endosulfan), and hexachlorocyclohexane.[8] Dieldrin is an OCPs used in the United States to protect crops from the 1950s to the mid-1970s. It is used as a termite killer for cracks, crevices, and substructures and was continued until it was banned by the US EPA in 1987.[9] Due to their lipophilic properties, OCPs are accumulated in lipid-rich tissues. These chemicals accumulate in fat-rich products such as butter and cream and expose dairy consumers to these residues.[10] It has also been shown that some of the most persistent organochlorines have a half-life of several decades in human tissues.[11] Studies have shown that OCPs increase the risk of breast cancer.[12,13] In a study, Snedeker[14] reviewed the effects of OCPs on the incidence and mortality of breast cancer. The relationship between dieldrin blood levels and the risk of breast cancer in Danish women has also been noted by Roswall *et al*.[11]

All OCPs have been forbidden in Iran since the late 1990s, however, due to their low price, traditional efficacy, lack of comprehensive supervision, and OCPs such as dieldrin are still used in Iran.[4] Although some researches have been performed on the effects of dieldrin on DNA, RNA, protein synthesis, and *HeLa* cell growth,[15-20] however, there is no study on the morphological changes of this pesticide on the *HeLa* cell line. Moreover, cytotoxicity is a biomarker for pesticide exposure and risk assessment. As a consequence, due to the increasing use of dieldrin in agriculture by Iranian farmers and considering the toxicity of OCPs and its relationship with human health, this study was performed to investigate the morphological changes caused by dieldrin pesticides in *HeLa* cell lines.

Materials and Methods

Dieldrin pesticide and *HeLa* cell line prepared by Sigma Company (Sigma-Aldrich, Germany) and Pasteur Institute, Iran, respectively. Then, standard concentrations (0.1-20 ppm) of dieldrin pesticide were prepared based on molecular weight and chemical formula according to previous studies.[13,21,22] All pesticide solutions were pesticide analytical grade and free of interfering residues (99% pure). Cells were also cultured in 1640 RPMI medium containing bovine serum albumin and Pen-Strep antibiotics at 37°C and 5% CO_2.[23] To control cell growth, the flasks containing the cells were examined daily under an inverted microscope for cell morphology, culture medium color, and adhesion to the flask surface. The cells were usually monolayers attached to the bottom of the flask and spindle-shaped.

After the cells developed and multiplied well, they were collected and prepared for the next steps. *HeLa* cells were treated with different concentrations of dieldrin to study the effects of dieldrin pesticide on *HeLa* cell morphology. Concentrations of 0.1, 0.5, 1, 5, 10, 15, and 20 ppm of dieldrin pesticide were applied to seven flasks each containing 10^6 *HeLa* cells. In addition to these seven flasks, seven flasks were placed as controls for each pesticide concentration. Trypan blue staining technique was used to determine the number of living cells. In this method, living cells become colorless and dead cells turn blue.

After 24 h of *HeLa* cells exposure to dieldrin pesticide, an inverted microscope (OPTIKA, Italy) was used to observe the morphological and cytopathic changes in comparison to the control group (without exposure to dieldrin). The cytopathic effect, or cytopathogenic effect, is associated with structural changes in host cells. Due to this effect, the contaminating agent causes the host cell to lyse, or when the cell does not lyse, it dies due to its reproduction failure. Cell disintegration, cell necrosis, formation of intracellular bodies, formation of giant cells, and formation of cytoplasmic cavities are examples of cytopathic effects.

In this study, the desired morphological and cytopathic changes are inhibition of cell growth, change in cells' connection to the flask surface and other cells, shrinkage, and rounding of cells, and reduction of cytoplasm, granulation, and membrane protrusion. This test was performed on about 200 cells from each flask (randomly) and the experiment (above steps) was conducted on triplicate samples. To analyze the data, SPSS software version 20 (IBM Corp., Armonk, NY) was used. Comparison between the changes in different concentrations was considered based on Chi-square or Fisher's exact test. $P < 0.05$ were considered statistically significant differences.

Results

The morphological changes of *HeLa* cells in the control group and after 24 h of exposure to 5 ppm of dieldrin pesticide in a culture medium are presented in Figure 1a and b, respectively. The effects of dieldrin pesticide on *HeLa* cell morphology showed changes in cell roundness and cytopathic effect. These

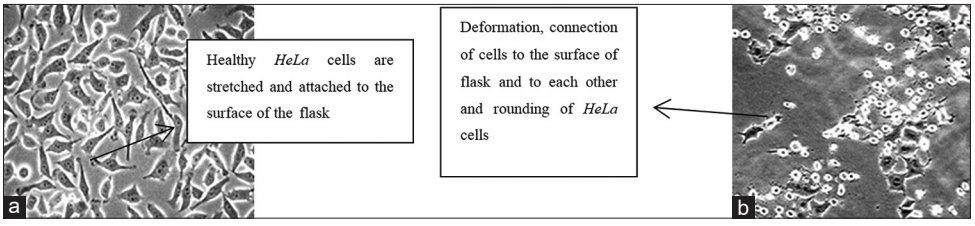

Figure 1: Morphological changes of *HeLa* cells: (a) control group and (b) after 24 h of exposure to 5 ppm of dieldrin pesticide in culture medium

morphological changes indicate that dieldrin may induce the apoptosis process in cells.

Table 1 showed the number of cells with morphological and cytopathic changes in the presence of 5 ppm of dieldrin compared to the number of healthy cells.

Discussion

The results of the effects of different concentrations of dieldrin pesticide on the morphology of *HeLa* cells, under an inverted microscope, showed that morphological and cytopathic changes do not occur at concentrations lower than 1 ppm. However, the 5 ppm of dieldrin not permitted the cells to connect on the flask surface. Moreover, cell rounding and cytopathic effects of the dieldrin pesticide were clearly detected in Figure 1. Furthermore, the cells were completely damaged and could not be examined at a concentration of 15 and 20 ppm of dieldrin pesticide. This is in agreement with the results of Chuah *et al*.[24] who showed that malignant *HeLa* cells revealed a similar sensitivity when exposed to pesticides. According to the results and Chi-square test/Fisher's exact test, there was a statistically significant difference between the number of cells with morphological and cytopathic changes compared to the number of healthy cells (control group) which indicates the effect of dieldrin (5 ppm) on the morphology of *HeLa* cells [Table 1].

The effect of type 1 Vero toxin on the Michigan Cancer Foundation-7 (MCF-7) cell line was investigated by Hossein *et al*.[25] Microscopic observations have shown that Vero toxin prevents the attachment of MCF-7 cells to the surface of culture flasks or microplate wells and creates maximum cytotoxicity on them. Litterst and Lichtenstein[26] investigated the effects of environmental chemicals on human cells using *HeLa* cells and human skin fibroblasts. The results revealed that aspirin and caffeine were 820 times less toxic than the other chemicals used in the study. Ghisari *et al*.[27] in Denmark evaluated the endocrine-disrupting potential of using 13 pesticides in cell culture. Only some of the pesticides were cytotoxic at high concentrations. In addition, malathion, prothioconazole, tau-fluvalinate, cypermethrin, mancozeb, and terbuthylazine significantly encouraged and propiconazole and bitertanol slightly reduced the proliferation of GH3 cells. Moazamian *et al*.[28] investigated the effect of the crystalline toxin on the CCRF-CEM cell line. In this study, the hemolytic activity of the toxin on human erythrocytes as well as the MTT assay and the cytopathic effects of this toxin were tested. The results of this study revealed different toxicity from different concentrations of this toxin on the cell line. In the other study, the effect of podophyllotoxin on bladder carcinoma cell line 5637 was studied by Sadeghi *et al*.[29] Examination with a light microscope showed that the cells underwent changes after treatment with this toxin, including shrinkage, roundness, and protrusion of the membrane. *In vitro* studies have also shown that exposure to OCPs not only proliferates, migrates, and invades human breast cancer cells[30] but also changes the gene expression patterns of these cells.[31] In addition, tumor growth has also been shown in animal studies.[22,32]

Chung *et al*.[17] studied the synthesis of DNA, RNA, and protein in *HeLa* cells exposed to DDT and dieldrin. They reported that the rate of cell changes by dieldrin was lower than DDT; however, dieldrin had a greater effect on the changes in RNA synthesis. In another study,[22] dieldrin was reported to induce oxidative stress and affect the expression of mouse liver cell genes. Thus, dieldrin acts as a nongenetic promoter/accelerator of liver tumor formation in mice. The results of this study are consistent with previous studies on the association of dieldrin with destructive cellular changes. Therefore, it seems that dieldrin pesticide has a morphologically destructive effect on the *HeLa* cell line.

Different studies reported the effects of this pesticide on humans. In a study on the survival of breast cancer patients, Roswall *et al*.[11] reported an inverse correlation between survival and dieldrin serum levels in the blood. Moreover, they reported that the accumulation of organochlorine in human breast tissue, due to its known estrogenic and antiestrogenic

Table 1: The number of cells with morphological and cytopathic changes in the presence of 5 ppm of dieldrin compared to the number of healthy cells

	Number of healthy cells	Number of cells with morphological and cytopathic changes	*P*
The first experiment			
Case group	43	157	<0.001
Control group	194	6	
The second experiment (repeat)			
Case group	32	168	<0.001
Control group	198	2	
The third experiment (repeat)			
Case group	35	165	<0.001
Control group	197	3	
Mean (SD)			
Case group	36.66 (5.68)	163.33 (4.64)	<0.001
Control group	196.33 (1.70)	3.66 (2.08)	

SD: Standard deviation

properties, suggests that they may affect the onset and development of breast cancer. Louis *et al*. (2007)[33] reported a positive and significant relationship between dieldrin exposure with lung cancer and a significant inverse correlation between aldrin exposure with colon cancer. In a case–control study,[34] not only there is no relationship between breast cancer and exposure to OCPs, but also there is no correlation between breast cancer and dieldrin concentrations in serum. Finally, in another study,[35] the cause of death (especially carcinogenic effects) was examined in employees who were exposed to dieldrin and aldrin pesticides occupationally, and the results of this study and other epidemiological and animal studies support the conclusion that dieldrin and aldrin are not possible to be human carcinogens. This reveals the significance of extrapolating *in vitro* studies to the human corresponding exposures.

Conclusion

The results of this study revealed that dieldrin induces morphological and cytopathic changes at concentrations above 5 ppm. However, concentrations of 0.1, 0.5, and 1 ppm of dieldrin were found not to cause morphological and cytopathic changes which provide significant guidance for the selection of dieldrin concentration. Furthermore, determining and identifying different factors that intensify the cytotoxic effect of this pesticide requires more research.

Ethics code

Taken from ethical committee of Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1397.211).

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Conflicts of interest

There are no conflicts of interest.

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