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AN ASSESSMENT OF *IN VITRO* CYTOTOXICITY OF CYPERMETHRIN ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES USING MTT ASSAY

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ABSTRACT

Cypermethrin, an analogue of pyrethrins, is a pesticide used in pest control. This study dealt with the investigation of *in vitro* cytotoxicity of Cypermethrin on human peripheral blood lymphocytes (PBL) at 2 hrs and 18 hrs exposure. Cytotoxicity of Cypermethrin was assessed on human peripheral blood lymphocytes using MTT assay. Cells were treated with 1µg/ml, 2.5µg/ml, 5µg/ml, 7.5µg/ml and 10µg/ml of pesticide. The cell viability at 1µg/ml on 2hrs exposure was found to be 67.09±0.07% while it was 87.10±0.25 % on 18hrs exposure group. A dose related drop in viability in the survival of lymphocytes was found when they were treated with different concentrations of Cypermethrin. Cell viability% on 18hrs exposure was found to be higher than that of 2 hrs exposure group. Less mortality in 18hrs exposed cells might be due to self-recovery process of cells.

KEYWORDS: Cytotoxicity, Human peripheral blood lymphocytes (PBL), MTT assay, Cell viability.

INTRODUCTION

Pyrethrins/Pyrethroids are a family of compounds either directly derived from a species of Chrysanthemum flowers (pyrethrins) or manufactured to resemble these chemicals (pyrethroids). In general, these compounds are considered relatively safe to humans because of their low-toxicity compared with other classes of pesticides. The pyrethroids are considered to be less toxic than the pyrethrins, and are being increasingly used for agricultural purposes and crop protection to control many pests, including pests of cotton,

fruit and vegetable crops (Meister, 1992). However the pyrethroids are highly toxic to aquatic organisms and fish as well as to bees (WHO, Geneva, 1994-95).

Pyrethriods are synthetic analogues of pyrethrins, the active substances in the flowers of Chrysanthemum, *Cineraria folium*. Pyrethroids can be classified into two large groups. Type I pyrethroids do not contain a cyano group in their molecules and include allethrin, tetramethrin, permethrin, and phenothrin. Type II pyrethroids contain a cyano group at the α-carbon position and include newer compounds, such as deltamethrin, cyphenothrin, Cypermethrin and fenvalerate. The two types of pyrethroids cause somewhat different symptoms of mammalian poisoning. Poisoning with type I pyrethroids is characterized by hyper excitation, ataxia, convulsions, and eventual paralysis; poisoning with type II pyrethroids, by hypersensitivity, choreoathetosis, tremors, and paralysis. Despite differences in the symptoms, both types of pyrethroids have the same major target site; the sodium channel of nerve membrane, i.e., the channel directly responsible for generating action potentials (National Academies Press, 1992). Cypermethrin has become one of the most important insecticides in wide scale use. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry.

Cypermethrin is classified by the World Health Organization (WHO) as moderately hazardous (class II). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds compared to the normal period of a few milliseconds, after a signal has been transmitted (Clark J M & M W Brooks, 1989). Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long lasting trains of repetitive impulses in sense organ (Abbassy M A *et al,* 1983).

Human beings especially those who handle cypermethrin and spray it, they are exposed to it by inhalation and cutaneous absorption. Hence this study was designed to study *in vitro* cytotoxicity of Cypermethrin on human PBL Using MTT Assay at 2hrs & 18 hrs exposures.

MATERIALS AND METHODS

Cypermethrin [Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate, used in this study was 25% EC (effective concentration), which was purchased from the market. To prepare desired dilutions, pesticide was dissolved in triple distilled water.

Isolation of lymphocytes from whole blood

Isolation of lymphocytes was performed as per Dayashankar Gautam *et al* (2016) with some modifications. Blood (3 ml) from healthy female young volunteer donor was collected in sterile EDTA vacutainer. This was diluted with double volume of PBS (1X). Three milliliter of HiSepTM Lymphocyte Separation Medium (LSM) 1077 (Hi Media) was transferred aseptically into a centrifuge tube. This was then carefully overlaid with 9 ml of diluted blood. It was centrifuged at 400 × g at room temperature (RT) for 30 min. Erythrocytes were sedimented and the lymphocytes formed a layer above the Hi Sep layer. Most of the supernatant was aspirated out and then the lymphocyte layer along with half of the Hi Sep layer was carefully aspirated into a separate centrifuge tube. It was then given two washes with isotonic PBS. The cells were counted in a haemocytometer. The cells were appropriately diluted in TC 199 medium (Hi Media) supplemented with fetal bovine serum to give a final concentration of 10.8×10^5cells/ml.

MTT assay

MTT assay was performed according to Mosmann (1983) with some modifications. Aliquots (180µl) of the prepared lymphocyte suspension (10.8×10^5 cells/ml) were seeded into a 96‑well polystyrene tissue culture plate in five replicates.

One row containing only medium and cells served as a control. Cypermethrin dilutions were made in such a way that the pesticide was added to cell suspensions in the concentration of 1µg/ml, 2.5µg/ml, µg/ml, 7.5µg/ml and 10µg/ml, of the culture medium. Each concentration of the Cypermethrin was tested in five replicates for 2hrs hrs and 18hrs exposures.

The plate was incubated for 2 hrs at 37°C with cypermethrin at 5% CO_2. After incubation, 20µl aliquots of MTT solution (5 mg/ml in PBS) were added to each well and reincubated for 2 h at 37°C. Then 100µl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals followed by overnight incubation at 37°C. The culture plates were then placed in an enzyme-linked immunosorbent assay (ELISA) microplate reader and absorbance was read at 600 nm. The same procedure as mentioned above was performed for assessing cytotoxicity of same concentrations of cypermethrin (1µg/ml, 2.5µg/ml, 5µg/ml, 7.5µg/ml and 10µg/ml) after 18 hrs exposure to human PBL.

The amount of color produced was directly proportional to the number of viable cells. OD of various concentrations of Cypermethrin was noted and the final OD was calculated after

making the due adjustment for these two factors. Cell viability of controls was taken as 100%.

Cell viability rate was calculated as the % of MTT absorption as follows:

% survival = (Mean experimental absorbance/Mean control absorbance) × 100.

RESULTS

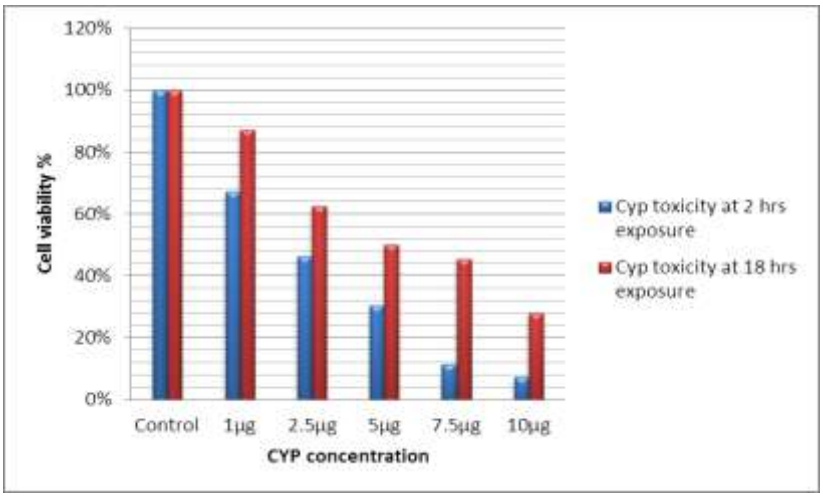

Figure 1: Comparative study of CYP (Cypermethrin) toxicity at 2hrs & 18 hrs exposure to human PBL

Table 1: Comparative study of CYP (Cypermethrin) toxicity at 2hrs & 18 hrs exposure to human PBL

Concentration of CYP	Cell Viability %± SE	
	Cyp toxicity at 2 hrs exposure	Cyp toxicity at 18 hrs exposure
Control	100	100
1µg/ml	67.09±0.07	87.10±0.25
2.5µg/ml	46.40±0.12	62.64±0.23
5µg/ml	30.30±0.12	50.20±0.17
7.5µg/ml	11.40±0.13	45.30±0.19
10µg/ml	7.30±0.09	28.00±0.20

The results (Figue1, Table 1) indicated drop in cell viability % when the cells were treated with different concentrations of Cypermethrin. Cells treated with different concentrations of Cypermethrin for two hrs exposure showed range from 67.09±0.07% to 7.30±0.09% cell viability on 2hrs exposure to human PBL, while it ranged from 87.10±0.25% to 28.0±0.20% cell viability on 18 hrs exposure to human PBL. The drop in cell viability increased when the concentration of Cypermethrin increased. Cell viability% on 18 hrs exposure was found to be higher than that of 2 hrs exposure group.

DISCUSSION

In vitro studies on human blood samples can give information of the toxicological effects of the cypermethrin in human blood samples. So far there are few reports of the impact of cypermethrin on lymphocyte cultures of human. (Amer S M, 1993).

The indiscriminate use of pesticides and herbicides to increase crop productivity has aroused a great concern among the environmental and health scientists due to their adverse effects in both targets as well as non-target species. Although substantial information is available regarding their environmental and ecological impact, not much is known in regard to its toxicity in the mammalian system (Sushila Patel *et al*, 2007).

They studied *In vitro* induction of cytotoxicity and DNA strand breaks in Chinese hamster ovary (CHO) cells exposed to cypermethrin, pendimethalin and dichlorvos. They exposed CHO cells to 1µM, 10µM, 100µM, 1000µM, and 10,000µM, cypermethrin, pendimethalin and dichlorvos for 3h and cytotoxicity was assessed by MTT assay. They found Cypermethrin induced a significant ($p < 0.05$) DNA damage only at higher concentrations (1000 and 5000µM) while dichlorvos and pendimethalin exhibited higher extent of cytotoxicity as compared to cypermethrin.

Assessment of cytotoxicity and response to external factors like pesticides were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) or MTT assay by G Suman *et al* (2006), which measures mitochondrial metabolism in the entire cell culture and provides information about the percentage of cell survival. Utilizing the MTT assay, the cytotoxicity of cypermethrin was determined on lymphocyte cultures from human peripheral blood samples, the short-term lymphocyte cultures were incubated with various aliquots of the cypermethrin and the LC50 was found to be 33.6µM.

In our study Cells treated with 1µg/ml, 2.5 µg/ml, 5µg/ml, 7.5µg/ml and 10µg/ml for two hrs exposure showed range from 67.09±0.07% at 1µg/ml to 7.30±0.09% cell viability% at 10µg/ml) on 2hrs exposure to human PBL, While it ranged from 87.10±0.25 % at 1µg/ml to 28.0±0.20% cell viability at 10µg/ml concentration) on 18 hrs exposure to human PBL. The drop in cell viability increased when the concentration of Cypermethrin increased.

We also found that the drop in cell viability increased when the concentration of Cypermethrin increased**.**

CONCLUSION

The results indicated drop in cell viability % when the cells were treated with different concentrations of Cypermethrin. The drop in cell viability % increased when the concentration of Cypermethrin increased. Less mortality in 18hrs exposed cells was found as compared to 2hrs exposed cells which might be due to self-recovery process of cells.

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