

APOPTOSIS INDUCED BY COMMERCIAL FORMULATION OF CHLORPYRIFOS ON AVIAN LYMPHOCYTES

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SUMMARY

The present work was aimed at evaluating the toxic effects of the commercial formulation of chlorpyrifos (Radar 20% EC) using avian lymphocyte model. Avian lymphocytes were treated with various dilutions of pesticide for 60 and 120 minutes. At the end of respective incubation periods, cells were harvested and analysed for lymphocyte proliferation and apoptosis assays. Results showed, the significant reduction of in both B and T cell counts suggesting the toxic effect of the pesticide. Apoptosis assays demonstrated the DNA fragmentation, phosphatidylserine translocation and plasma membrane blebbing in the pesticide treated cells. Based on the above findings it can be concluded that low dose of commercial formulation of chlorpyrifos is toxic that is mediated through the induction of apoptosis.

Keywords: chlorpyrifos, lymphocytes, apoptosis

INTRODUCTION

Farming community routinely uses various commercial formulations of chlorpyrifos for the control of crop pests. During the time of pesticide application, farmers might get exposed to very low levels of that pesticide either due to unsystematic use or spillage etc. The toxic effects resulting from the exposure to chlorpyrifos depends upon the duration and dose to which animal, plant or humans get exposed. Although the very low dose of chlorpyrifos doesn't cause the apparent toxic signs to humans but appears that it might cause the invisible toxic effects and its residues could become potential environmental pollutants. The present investigation was aimed at examining the toxic effect of commercial formulation of chlorpyrifos using avian lymphocyte model.

MATERIALS AND METHODS

Radar 20% EC (RPG Life Sciences Ltd, Mumbai, India); a commercial formulation of chlorpyrifos was procured from the local market and dissolved in ethanol to give 1% stock solution. In this study the pure form of chlorpyrifos was not used as this study sought to determine the toxic impact from exposures to the formulation of this agent that is routinely encountered by farmers and their livestock. The stock solution was serially diluted to ten folds using RPMI (Sigma, USA) maintenance medium. Avian (White leghorn) lymphocytes isolated from the blood were (final count adjusted to 1×10^7 cells per ml) treated with 1:1000 dilution of chlorpyrifos and

incubated for 60 and 120 min at 37°C. At the end of each incubation period, cells were aliquoted into various groups for lymphocyte proliferation assay and apoptosis studies.

Lymphocyte proliferation assay was carried out as per the method of Rai-el-balhaa *et al.*, (1987). The reduction of the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) dye to formazan was used as an indicator of cell proliferation (Altman, 1976; Mosmann, 1983). The assay was carried out in the wells of 96-well plate. LPS (lipopolysaccharide) and Con-A (concanavalin-A) each were used at 5 µg/ml final concentration. The optical density (OD) was recorded at 570 nm using microscan ELISA reader (ECIL, India). The results were reported as mean change (Δ) in optical density (mean Δ OD = mean OD of mitogen-stimulated wells – mean OD of unstimulated wells). DNA fragmentation assay was carried out as per the method described by Hermann *et al.*, (1994). The pellet was lysed using the lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, pH 7.5), treated with sodium dodecyl sulphate (SDS), 10 M ammonium acetate, precipitated using ethanol and analysed upon agarose gel electrophoresis. Annexin-V binding assay was performed by the method of Chauhan and Tripathi (2002). Smears of cells quenched in 10% H₂O₂ were stained with annexin-V-biotin and avidin-peroxidase conjugates. Slides were examined for cells displaying brown colour on their surface. Electron microscopy was done by the method of Malorni *et al.*, (1998) and ultra-structural changes were recorded.

RESULTS AND DISCUSSION

Commercial formulations of chlorpyrifos are commonly used as organophosphate pesticides for the control of insects of paddy, cotton crops etc. Although they cause no apparent toxic signs following exposure to a very low dose, the adverse impact on the health of humans and animals cannot be ruled out. The present work was aimed at evaluating the toxic effects of very low dose (in this study 1:1000) of commercial formulation of chlorpyrifos using avian lymphocyte model. The mean Δ in OD for con-A stimulated cells was 0.151±0.050 and 0.138±0.027 respectively at 60 and 120 min of pesticide exposure while for the control cells was 0.240±0.007 and 0.210±0.040 for the respective exposure times. The mean Δ OD for LPS stimulated cells was 0.177±0.020 and 0.129±0.072 while for the control cells were 0.302±0.037 and 0.280±0.22 respectively after 60 and 120 min of exposure. The above result indicated the significant ($p \leq 0.05$) reduction in mean Δ in OD in chlorpyrifos treated cells when compared to the control group. The reduction in mean Δ in OD was more at 120 min of exposure when compared to 60 min exposure. Among the treated group, the reduction in mean Δ OD was more in con-A stimulated group in comparison to the LPS stimulated group. The reduction in mean Δ in OD indicated the loss of viability of lymphocytes at that dilution. In addition, the data also suggested the T- cell specific toxicity of the chlorpyrifos. The reduction in mean Δ in OD upon exposure to subsequent dilutions was not significant in comparison to the control group. The above result suggested that 1:1000 dilution of Radar 20% EC is toxic to lymphocytes. DNA fragmentation is the hall mark of the cells undergoing apoptosis. In the present study, to know chlorpyrifos caused toxicity on lymphocytes was due to apoptosis, DNA fragmentation assay was performed and the result revealed that chlorpyrifos induced the DNA fragmentation in avian lymphocytes. DNA fragmentation in lymphocytes appeared as DNA ladder on agarose gel. The intensity of DNA laddering was increased with the time as well as with the dose of exposure. To verify the apoptosis induced by chlorpyrifos, treated cells were assayed for another marker of apoptosis; phosphatidylserine exposure from the inner leaflet to the outer leaflet of plasma membrane (Vermes *et al.*, 1995).

Results demonstrated the translocation of phosphatidylserine to the outer leaflet as detected by the appearance of brown colour on the plasma membrane of the treated cells. This confirmed that the toxicity of chlorpyrifos on lymphocytes was due to apoptosis. In addition, the electron microscopy results also demonstrated the cells with condensed chromatin and plasma membrane blebbing.

Based on the appearance of DNA ladder, phosphatidylserine translocation to the outer leaflet, and the condensed chromatin in cells treated with chlorpyrifos, it can be concluded that commercial formulation chlorpyrifos (Radar 20% EC), even at very low dose cause toxicity in lymphocytes through the induction of apoptosis. The present study emphasizes the safe handling and systematic use of pesticides and also suggests that in-vitro techniques can be useful in toxicological evaluations of pesticides.

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