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EVALUATION OF *IN VITRO* CYTOTOXICITY OF DIMETHOATE ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES USING MTT ASSAY

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ABSTRACT

Dimethoate is an insecticide used to kill a wide range of insects to protect agricultural crops. Farmers are exposed to it when they spread it on their crops. Hence to evaluate *in vitro* cytotoxicity of Dimethoate on human peripheral blood lymphocytes using MTT assay, this study was performed. Cells were treated with 3µg/ml, 6µg/ml, 9µg/ml, 12µg/ml and 15µg/ml of ethanolic solution of the pesticide. Cells treated with different concentrations of ethanolic Dimethoate for two hours (hrs) exposure showed cell viability range from 96.88% to 85.75%. The cytotoxicity was also assessed at 48 hrs exposure. Cell viability % ranged from 67.28% to 57.90%. LC_{50}seemed to beapproximately 45µg/ml and 15 µg/ml at 2 hrs and 48 hrs exposure respectively. Cytotoxicity of the pesticide was found to be directly proportional to the exposure time. Farmers are suggested to wear masks and gloves while applying this pesticide in the fields to be safe from its toxicity.

KEYWORDS: Dimethoate Cytotoxicity, Human peripheral blood lymphocytes (PBL), MTT assay, Cellviability.

INTRODUCTION

Dimethoate is an insecticide used to kill mites and insects systemically and on contact. It is used against a wide range of insects, including aphids, thrips, plant hoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. Dimethoate has been administered to livestock for control of botflies. Dimethoate is

available in aerosol spray, dust, emulsifiable concentrate, and ULV concentrate formulations. (Hayes et al, 1990 and Meister 1992).

Dimethoate is moderately toxic by ingestion, inhalation and dermal absorption. As with all organophosphates, dimethoate is readily absorbed through the skin. Skin which has come in contact with this material should be washed immediately with soap and water and all contaminated clothing should be removed. Organophosphates are easily absorbed through the lungs. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or with liver malfunction may be at increased risk from exposure to dimethoate. High environmental temperatures or exposure of dimethoate to visible or UV light may enhance its toxicity (Occupational Health Services, Inc. 1991).

The organophosphate insecticides are cholinesterase inhibitors. They are highly toxic by all routes of exposure. When inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing, chest discomfort, difficult or short breath, and wheezing due to constriction or excess fluid in the bronchial tubes. Skin contact with organophosphates may cause localized sweating and involuntary muscle contractions. Eye contact will cause pain, bleeding, tears, pupil constriction, and blurred vision. Following exposure by any route, other systemic effects may begin within a few minutes or be delayed for up to 12 hours. These may include pallor, nausea, vomiting, diarrhea, abdominal cramps, headache, dizziness, eye pain, blurred vision, constriction or dilation of the eye pupils, tears, salivation, sweating, and confusion. Severe poisoning will affect the central nervous system, producing incoordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contractions, twitching, tremors of the tongue or eyelids, and eventually paralysis of the body extremities and the respiratory muscles. In severe cases there may also be involuntary defecation or urination, psychosis, irregular heartbeats, unconsciousness, convulsions and coma. Death may be caused by respiratory failure or cardiac arrest (Occupational Health Services, Inc. 1991).

Plants have been a source of medicinal products since a long time. To date, approximately 100 species of plants have been examined and some active constituents isolated and identified, for instance several of the current chemotherapeutic drugs like vinblastine, methotrexate, taxol, and so forth, were first identified in plants. *Oroxylum indicum* (L.) Vent. tree has been used for the preparation of ayurvedic medicines since a long time. Every part of this tree contains metabolites that possess medicinal value with anticancer potential.

Ameliorative effect of root extract of *O. indicum* was reported earlier against damage induced through tobacco extract in human lymphocytes (Richa Soni *et al*, 2016).

Dimethoate has been used in Jabalpur for the protection of crops from pests for last many years. Its aqueous solution is sprayed by the farmers in the fields. They come to its exposure while applying it. Hence this study was conducted to evaluate its *in vitro* toxicity on human peripheral blood lymphocytes using MTT assay on 2 Hours exposure and 48 Hours exposure.

MATERIALS AND METHODS

Dimethoate used in this study was 30% EC (effective concentration), which was purchased from the market which is sold with the name of Dimethoate 30% EC Rogor Insecticide. It formed milky white solution when mixed with water as a solvent. Hence to prepare desired dilutions, pesticide was dissolved in absolute ethanol to obtain clear solution.

Isolation of lymphocytes from whole blood

Isolation of lymphocytes was performed as per Dayashankar Gautam *et al* (2017) with some modifications. Blood (3 ml) from healthy female young volunteer donor was collected insterile 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 Seplayer was carefully aspirated into a separate centrifuge tube. It was then given two washeswith 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 7.6×10^5cells/ml.

MTT assay

MTT assay was performed according to Mosmann (1983) with some modifications. Aliquots (180µl) of the prepared lymphocyte suspension (7.6×10^5cells/ml) were seeded into a96‑well polystyrene tissue culture plate in five replicates. One row containing only medium and cells served as a control. Dimethoate dilutions were made in such a way that the pesticide was added to cell suspensions in the concentration of3µg/ml, 6µg/ml, 9µg/ml, 12µg/ml and 15µg/ml of the culture medium. Each concentration of the Dimethoate was tested in six

replicates for 2hrs and 48hrs exposures. The plate was incubated for 2 hrs at 37°C with dimethoate at 5% CO2. After incubation,20µl aliquots of MTT solution (5 mg/ml in PBS) were added to each well and re-incubated for2 h at 37°C. Then 100µl of dimethyl sulfoxide (DMSO) was added to each well to dissolvethe formazan crystals for 44 hrs at 37°C. The culture plates were thenplaced in an enzyme-linked immunosorbent assay (ELISA) microplate reader and absorbancewas read at 600nm. The same procedure as mentioned above was performed for assessing cytotoxicity of same concentrations of Dimethoate (3µg/ml, 6µg/ml, 9µg/ml, 12µg/mland 15µg/ml) after 48 hrs exposure to human PBL. The amount of color produced was directly proportional to the number of viable cells. OD of various concentrations of Dimethoate was noted and the final OD was calculated after making the due adjustment 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

Drop in cell viability % was observed (Fig1,2,3,4, Table 1) when the cells were treated with different concentrations of Dimethoate. Cells treated with different concentrations of ethanolic Dimethoate for two hrs exposure showed cell viability range from 83.37 % to 72.25%, while for at 48 hrs exposure, it ranged from 38.41 % to 29.03%. Since the clear solution of pesticide was obtained using ethanol as a solvent for the study, the toxicity of ethanol was also found which is expressed in fig1 and 3. To find the cytotoxicity of pesticide only, the cytotoxicity caused by ethanol was reduced (fig 2 and 4). The drop in cell viability increased when the concentration of Dimethoate increased. Cell viability% on 2 hrs exposure was found to be higher than that of 48 hrs exposure group. Cytotoxicity of the pesticide was found to be directly proportional to the exposure time.

Table 1: - Cytotoxicity of Dimethoate at different exposures time.

Concentration of Dimethoate	Cell viability % at 2 hrs Exposure	Cell viability % at 2 hrs exposure after adjusting ethanol toxicity	Cell viability% at 48 hrs Exposure	Cell viability % at 48 hrs exposure after adjusting ethanol toxicity
Ctrl	100	100	100	100
3 µg/ml	83.37	96.88	38.41	67.28
6 µg/ml	82.21	95.72	35.56	64.44
9 µg/ml	80.85	94.35	33.68	62.55
12 µg/ml	72.66	86.16	29.03	57.90
15 µg/ml	72.25	85.75	39.07	67.94
100% ethanol	86.53	100	70.79	100

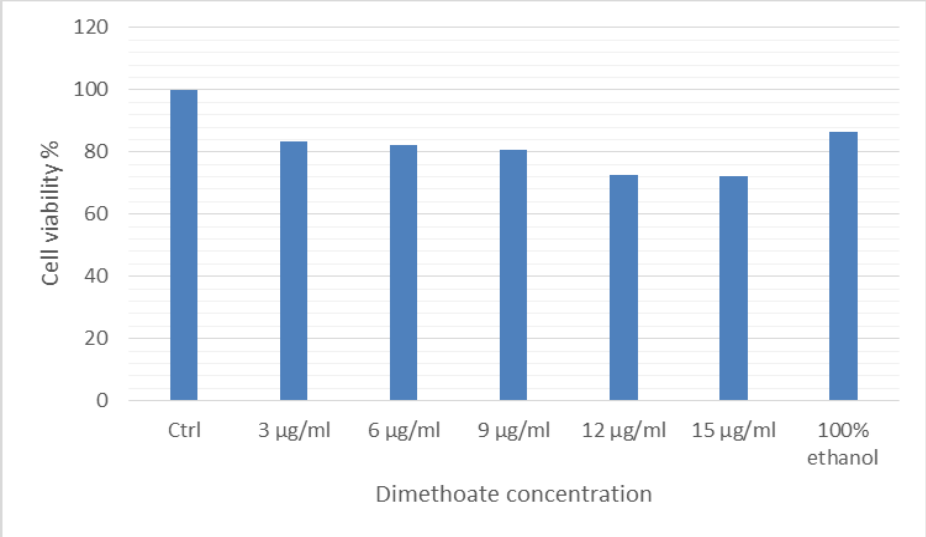

Fig 1: - Cytotoxicity of Dimethoate at 2 hrs exposures.

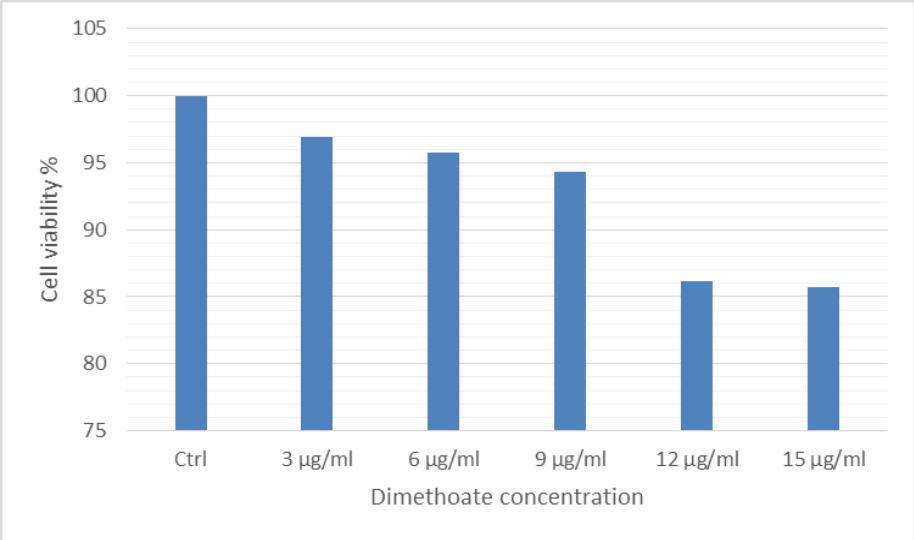

Fig 2: - Cell viability % at 2 hrs exposure after adjusting ethanol toxicity.

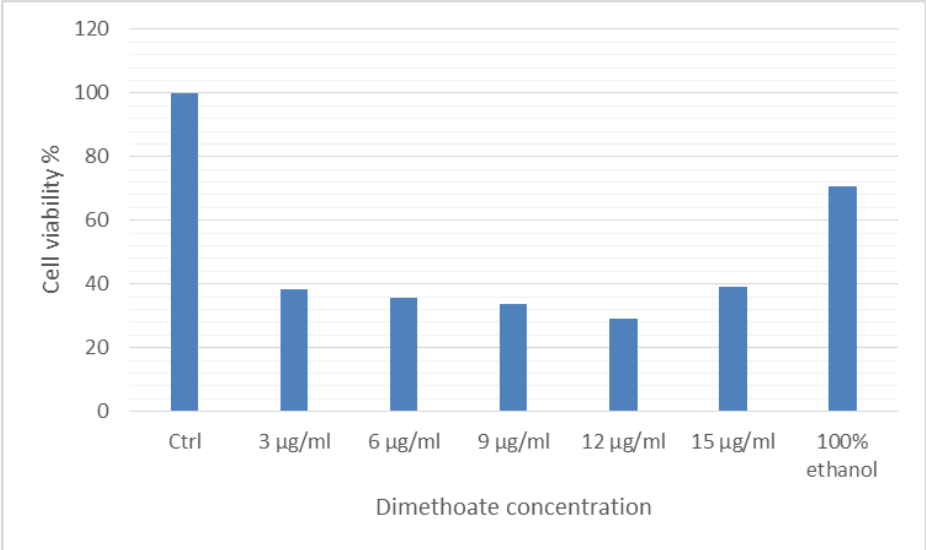

Fig 3: - Cytotoxicity of Dimethoate at 48 hrs exposures.

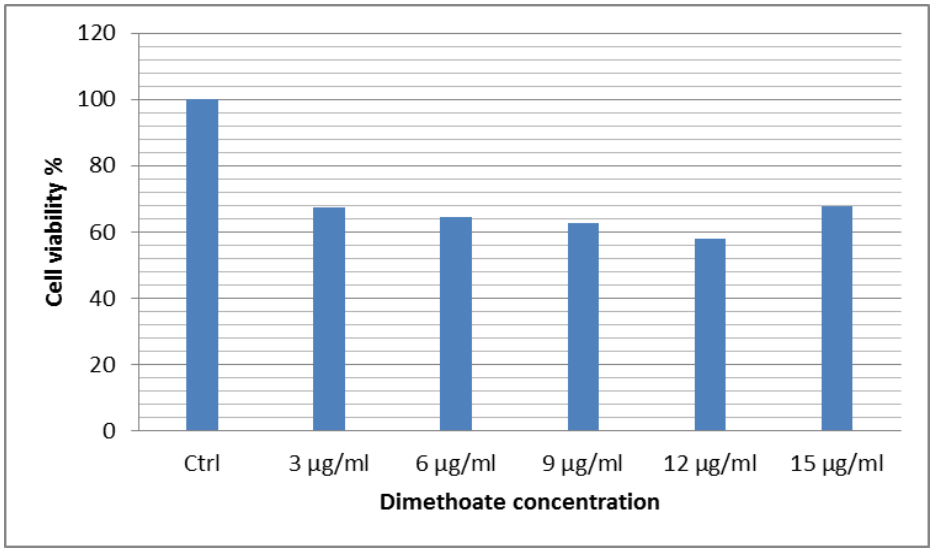

Fig 4: - Cell viability % at 48 hrs exposure after adjusting ethanol toxicity.

DISCUSSION

A little work has been performed on the assessment of Dimethoate toxicity using MTT assay. However, the cytotoxic response of endosulfan and chlorpyrifos was studied in poultry lymphocytes cultures by Mehta *et al* 2008.They found that the median inhibitory concentration of chlorpyrifos of chlorpyrifos for growth inhibition for cultured poultry lymphocytes. In our study LC_{50} seemed to be approximately 45µg/ml and 15 µg/ml at 2 hrs and 48 hrs exposure respectively.

The indiscriminate use of pesticides and herbicides to increase crop productivity has aroused 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).

The toxic effect of the 20, 40, 60 and 100 mM concentrations of Dimethoate was studied after 2 hours exposure by Richa *et al* 2016. The viability ranged from 96.08% at 20 mM to 85.08% at 100 mM.

Assessment of cytotoxicity and response to external factors like pesticides were evaluated by3-(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 andprovides information about the percentage of cell survival. Utilizing the MTT assay, the cytotoxicity of cypermethrin was determined on lymphocyte cultures from human

peripheralblood samples, the short-term lymphocyte cultures were incubated with various aliquots of the cypermethrin and the LC_{50} was found to be 33.6µM.

In our study, Cells treated with different concentrations of Dimethoate for two hrs exposure showed cell viability range from 96.88% to 85.75, while at 48 hrs exposure, it ranged from 67.28% to 57.90%.

The effect of dimethoate on lymphocytes was studied by Gargouri *et al* (2011). They divided the lymphocytes into two groups. The first group was incubated for 4 hrs at 37^0C with different concentrations of dimethoate (0, 40, 60 and 100 mM). The second group was pre incubated for 4 hrs at 37^0C with quercitin. They found that dimethoate caused a significant increase in malondialdehyde levels and increase in superoxide dismutase and catalase activities in lymphocytes at different concentrations. Quercetin preincubated lymphocytes showed a significant protection against the cytotoxic effect induced by dimethoate. We also found that the drop in cell viability % increased when theconcentration of the pesticide increased.

CONCLUSION

The results indicated drop in cell viability % when the cells were treated with different concentrations of Dimethoate. The drop in cell viability % increased when the concentration of the pesticide increased. Cytotoxicity of the pesticide was found to be directly proportional to the exposure time. Dose dependent relationship was found. LC_{50} seemed to be approximately 45µg/ml and 15 µg/ml at 2 hrs and 48 hrs exposure respectively. Farmers are suggested to wear masks and gloves while applying this pesticide in the fields to be safe from its toxicity.

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REFERENCE

1. Dayashankar Gautam, Reena Chauhan, Archana Patel, Nandini Lodhi An assessment of *in vitro* cytotoxicity of cypermethrin on human peripheral blood lymphocytes using MTT assay. World Journal of Pharmaceutical Research, 2017; 6(8): 1373-1378, ISSN 2277– 7105.
2. Gargouri B, Mansour R, Abdallah F, Elfekih A, Lassoued S, Khaled H Protective effect of quercetin against oxidative stress caused by dimethoate in human peripheral blood lymphocytes. Lipids in Health and Disease, 2011; 10: 149.
3. Hayes WJ and ER Laws (ed.). Handbook of Pesticide Toxicology, Classes of Pesticides. Academic Press, Inc., NY., 1990; 3.
4. Mehta G, Singh S P, Pandey S K and Sharma L D Cytotoxic response of endosulfan and chlorpyrifos pesticides in poultry lymphocyte culture. Toxicol Int, 2008; 15(2): 97-101.
5. Meister RT (ed.). Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH, 1992.
6. Mosmann T Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J lmmun Methods, 1983; 65: 55-63.
7. Occupational Health Services, Inc. (Sept. 16). MSDS for Dimethoate. OHS Inc., Secaucus, NJ, 1991.
8. Patel Sushila, Bajpayee Mahima, Pandey Alok Kumar, Parmar Devendra, Dhawan Alok *In vitro* introduction of cytotoxicity and DNA strand breaks in CHO cells exposed to cypermethrin, pendimethalin and dichlorvos. Toxicology *in Vitro*, 2007; 21(8): 1409- 1418.
9. Richa Soni, Asha Khanna, Mamta Gokhale, Dayashankar Gautam A study of the toxicity of dimethoate on human lymphocytes and the ameliorative effect of root extract of Oroxylum indicum (L.) Vent. Pharmaceutical and biological evaluations, 2016; 3(4): 424-430.
10. Suman G, Rambabu Naravaneni & Kaiser Jamil *In vitro* cytogenetic studies of cypermethrin on human lymphocytes. Indian Journal of Experimental Biology, 2006; 44: 233-239.