RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 4: 416-419 (April 2023)

Predicted and *In vitro* Effect of Deltamethrin on Bone Marrow Progenitors

A. Mangala Gowri[1], Abhiroopa Ashokkumar[1]

10.18805/IJAR.B-4328

ABSTRACT

Background: Deltamethrin, a type 2 pyrethroid, reported toxicity said to have atoxic effect upon chronic exposure on the immune system. Since bone marrow is the source of immune cells, it is appropriate to test impact of the widely used Deltamethrin on BMSCs (Bone marrow stem cells). It was found that long term exposure to these toxic pesticides used for domestic and agricultural purposes showed to cause severe hematotoxicity and degenerative marrow diseases, precisely hypoplastic bone marrow failure condition in mammals. The present study analysed the less explored predicted effects of Deltamethrin on blood stem cells through CD45 receptor and virtual effects on cultured cells.

Methods: The effect of deltamethrin on bone marrow-derived stem cells at different concentrations and time periods was analysed. To further understand the molecular basis of how these chemicals affect the protein-ligand binding, molecular docking studies were conducted.

Result: Our results on iGEMDOCK 2.1 software used to dock CD45 receptors with DLM and the binding mode indicated that the bound ligand and the active site of CD45 had a good binding score of -68.04 and formed the five H bonds with the amino acids residues of CD45. It is found from the current study that there is an increase in cytotoxicity with increased exposure to deltamethrin. Further studies can be done to note the precise target of such agents to quickly underst and and control the effect of such drugs on a safer lifestyle.

Key words: Analysis, Bone marrow stem cells, Culture, Effect of deltamethrin, Prediction.

INTRODUCTION

Pesticides show deleterious effect on various tissue metabolism during long time exposure to more than just the pests at which they are targeted. They are toxic and exposure to them causes severe health hazards, they are linked to several severe illnesses and diseases in humans and animals (Aktar *et al*., 2009). Exposure can occur in agriculture, through the treatment of crops, plants and grain stores, through the treatment of livestock with anti-parasitic preparations. Inorganic fertilizers effect on soil chemical properties was discussed by (Almaz *et al*., 2017) and stated that the inorganic nitrogen fertilizer can be replaced with soybean residues. (Haripriya *et al*., 2019) stated that the evaluation of biocontrol agents and biopesticides showed the need for biointensive management. Besides, pesticide residues can be found on and in the food, crops also put the animals and us at risk (Nicolopoulou *et al*., 2016). Long term exposure to toxic pesticides used for domestic and industrial purposes has been shown to cause moderate to severe hematotoxicity and increased incidence of several marrow degenerative diseases, precisely hypoplastic bone marrow failure condition in humans. The exact underlying mechanisms of pesticide-induced hematotoxicity that play a significant role in reducing normal hematopoiesis are not quite well explained (Massin and Thouvenot 1993; Chatterjee *et al.,* 2014). The decreased hematopoietic progenitor cell colony formation indicated the toxicity induced inhibition of cellular proliferation and functional maturation of hematopoietic stem/progenitor cells in pesticide exposed marrow (Thomas *et al*., 1990). Exposure to these agents, especially bone marrow with its rapidly renewing cell population, is one of the most sensitive tissues to these toxic agents, represents a risk for the immune system leading to the onset of different pathologies (Chatterjee *et al*., 2013). Insecticide resistance studies of Cypermenthrin 25EC and Chlorpyriphos 20EC (RupaSule and Dollykumar, 2019) indicated that there is onset of resistance in Cypermethrin 25EC, which may cause adverse effects, if not used in proper rotation with other insecticides. Deltamethrin (DLM) is a type 2 pyrethroid insecticide used in pest control, agriculture, disease vector control (Yousef *et al*., 2006). Deltamethrin is a neurotoxin to many insects and many other animals. It has the tendency to accumulate in different tissues resulting in histological and biochemical changes,

[1]Centralised Instrumentation Laboratory, Madras Veterinary College, Chennai-600 051, Tamil Nadu, India.

Corresponding Author: A. Mangala Gowri, Centralised Instrumentation Laboratory, Madras Veterinary College, Chennai-600 051, Tamil Nadu, India. Email: gowrivalavan1@gmail.com

How to cite this article: Gowri, A.M. and Ashokkumar, A. (2023). Predicted and *In vitro* Effect of Deltamethrin on Bone Marrow Progenitors. Indian Journal of Animal Research. 57(4): 416-419. doi: 10.18805/IJAR.B-4328.

Submitted: 05-10-2020 **Accepted:** 09-03-2021 **Online:** 22-04-2021

including the toxic effect on the immune system (Antonchuk *et al*., 2002). Only few reports regarding the effect of deltamethrin on immune functions is known. Rarely are there molecular docking studies carried out to address the affinity of deltamethrin towards T cell receptors. Bone marrow found in the center of the bone is one of the sources of these types of cells (Jeyalakshmi *et al*., 2012). Red marrow of the bone marrow contains blood stem cells that can become red blood cells, white blood cells, or platelets. Bone marrow-derived stem and immunecells such as B-cells, T-cells plays critical roles in maintaining the health, regeneration, and repair of many tissues (Zhao *et al*., 2013).

Therefore, the present study is taken up with the objective to study the pathways leading to DLM-induced blood stem cell apoptosis by docking studies" and thus to predict the binding affinity of DLM towards cell receptor CD45 and also to study the pathways leading to DLM-induced blood stem cell apoptosis. To do this, docking studies were carried out. DLM has been targeted to the CD45 receptor using iGEMDOCK 2.1 software.

MATERIALS AND METHODS

Ethical committee permission

The research work has been carried out as per the approval of the Institutional Ethical Committee for Stem Cell Research and Therapy N0.05/ICSCRT/2017 MVC, Chennai-7.

Molecular docking tools employed

The CD45 structures were downloaded from the protein data bank server PDB:http://www.rcsb.org/pdb). The generated structures for CD45 was 5fmv which was the structure for human. Easy modeller and Python were used to modify the structure for *Capra aegagrus hircus*. The protein sequence for this was downloaded from NCBI. The DLM structure was constructed using ACDChemSketch12.01 software. iGEMDOCK 2.1 software was used for docking. It detected the cavities in the CD45 structure. Deltamethrin and reference ligand structures were added to the software along with the CD45 structure. The software calculated the DOC score, number of H bonds, bond length and amino acid residue. This was visuvalized using PyMol 2.3 software. (Table 1 and Fig 1).

Isolation, enrichment of cd45+ progenitor cells and culture with mesenchymal cells

Bone marrow collection and processing from apparently healthy goats slaughtered for food purpose were collected and processed for isolation of progenitor cells as per manufactures instruction of Easy Sep™(Stem Cell Technologies) with minor modifications to suit our needs. Isolated enriched cells were cultured in T25 culture flask at cell concentration of 1×10^6cells/ml in maintenance medium. The culture flasks were allowed to incubate at 37°C at 5% CO_2 level without any disturbance for 1-2 days. Then the suspension cells were transferred in to 6 well plate with fresh growth medium containing a monolayer of bone marrow mesenchymal cells (Gowri *et al*., 2013) to simulate bone marrow matrix *in vitro.*

The cultures were allowed to grow for 7-14 days with change of medium at two days intervals. The confluent monolayer cells were subcultured, and third passage cells were used for proliferation analysis for a period of 24, 72,120 and 168 hrs. The cells are also treated with different concentrations of Deltamethrin (1.25% solution, type Veterinary raw material). Commercially available form of the drug was obtained and different concentration were prepared with DMSO as a solvent (10 mM stock) and the proliferative ability was measured as the number of viable cells over a period of 7 days. Concentrations such as 0.2 mM, 0.3 mM, 0.5 mM and 1 mM were used to test the system along with the control and specifically, the number of viable cells was taken into consideration. To confirm that there is a reduction in overall cell survival, performed MTT and cell count assay.

Cell count assay

The cells were cultured in a 6-well plate with a growth medium. After 24 hrs of seeding, an equal volume of cells (5 µL) and tryphan blue dye (5 µL) were mixed well and

Table 1: Docking of deltamethrin (DLM) with the CD45 receptor.

Ligand name	Docking score	Number of H bond	Amino acid residue	H bond length
NAG	-68.04	5	HIS 418	2.7
			ASN 419	3.5
			ASP 436	2.7
			LYS 437	2.8
			ASN 438	3.1
DLM	-78.78	2	ASN 419	5.1
			ASP 436	3.6

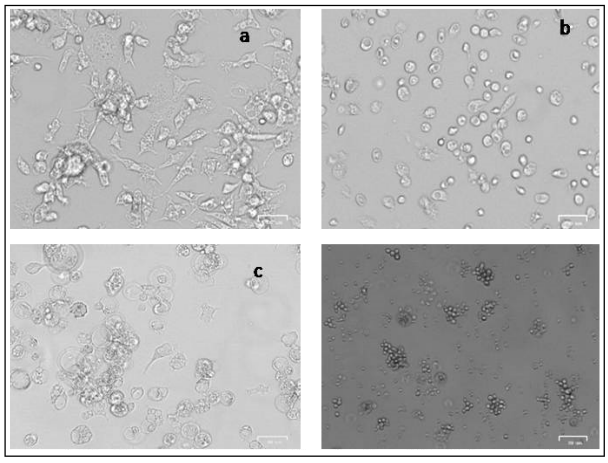

Fig 1: Effect of DLM on co cultured HSC and MSC bone marrow cells.
a) Representative image ofcell without drud treatment (scale bar inum).
b) Data metherin effect on cacultured goat BMSc Day 3 showing change in morphology rounding of spindle cells (scale bar).
c) Aggrecation of cells leading to clumping day 5.
d) Apoptotic changes shows in cells day 7.

used for viable cell count at 24, 72, 120 and 168 hrs were plotted for proliferation analysis.

Dye-based proliferation assay

The cells were cultured in a 96-well plate at a density of 1×10^6 cells/mL. 20 µL of MTT (5 mg/ ml) solution was added to each well and incubated for four hours. To each well, 200 µL of DMSO working solution was added and the OD of the reaction product was evaluated in an ELISA reader at a 570-nm wavelength. At least three independent experiments from different sample were performed to examine proliferation.

RESULTS AND DISCUSSION

Synthetic pyrethroid insecticide-Deltamethrin is widely used throughout the world as pesticide in agriculture, animal husbandry, to control pests at home, to protect food items and in vector control as disease prophylaxis measure. Its mechanism of dermal toxicity, neurotoxicity, pulmonary toxicity and carcinogenic properties is well known. Apart from causing multiorgan toxicity, it is also toxic to the immune system (Kumar *et al*., 2014). In this study bone marrow stem cells, from the higher animal model *caprus* was isolated and expanded *in vitro*. To test the difference in the effect of deltamethrin on CD 45+ cells at different concentrations of the pesticide, *in vitro* stem cell culture system from bone marrow with HSC and MSC was used. Progenitor cells proliferation was checked on alternate days. Changes in the morphology of the cells were recorded. Fig 1 represents the growth of the SCs without the treatment of deltamethrin; the growth of cells is apparently normal and healthy. The control cells spread and grow in the media with normal morphology. While under the treatment of deltamethrin (from 200 µM or 0.2 mM to 1 M), there was an abnormality in the morphology of the BMSCs and cells showed apoptotic changes at 1 mM concentration. There was a decrease in the number of viable cells with an increase in the concentration of deltamethrin. This may be because more concentration of deltamethrin means number of active sites it binds to is higher and quicker the apoptosis. Liu *et al*., (2014) showed that deltamethrin induced concentration-dependent influx of Ca 2+ into the cell this affects the cellular response and causes apoptosis (Fig 3 and 4).

There was also a significant difference in the proliferative ability between the cells grown at different time points. There was a decrease in the number of viable cells with increasing time of exposure to deltamethrin. This signifies that the longer the time of exposure, the more toxic the compound is to the system, this is more prominent at higher concentrations of the pesticide. While at the lower concentration of 0.2 mM concentration, there is an increase of cell death (toxicity) by 8% from day 1 to day 7, at a higher concentration of 1 mM, there is an increase of cell death (toxicity) by 19% from day 1 to day 7. (Kumar *et al*., 2014) study indicated that the cells go through apoptosis because deltamethrin occupies and binds on the binding domains of the immune receptors present on the surface of these cells, causing the normal immune pathways to become dysfunctional. Therefore the cells undergo apoptosis. (Hossain and Richardson, 2011), pyrethroid pesticide deltamethrin has been demonstrated the ability of pyrethroid pesticide deltamethrin to cause apoptosis both *in vitro* and *in vivo*. However, the molecular pathways leading to deltamethrin-induced apoptosis has not been established. To identify these pathways, SK-N-AS neuroblastoma cells

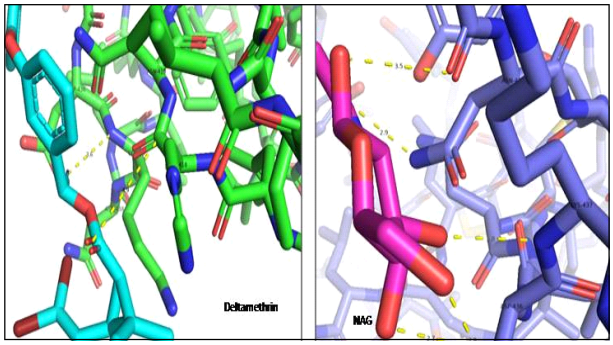

Fig 2: Molecular Docking deltamethrin (DLM) and NAG with the CD45 receptors. Deltamethrin (light blue on Fig 2a docked with CD45 (green). Fig 2b with NAG (pink) docked with CD45 (dark).

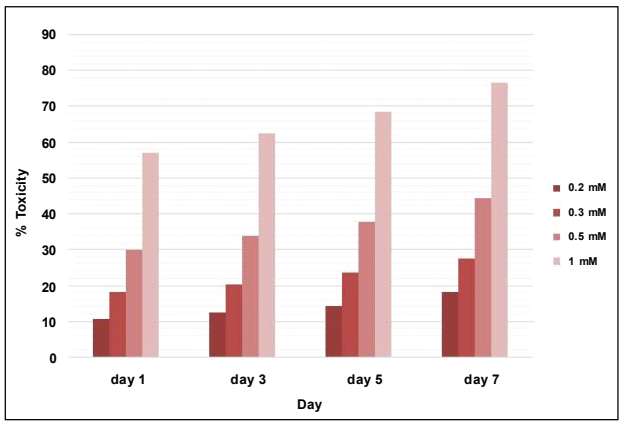

Fig 3: Toxicity analysis by MTT assay of BMSCs different time points *in vivo.*

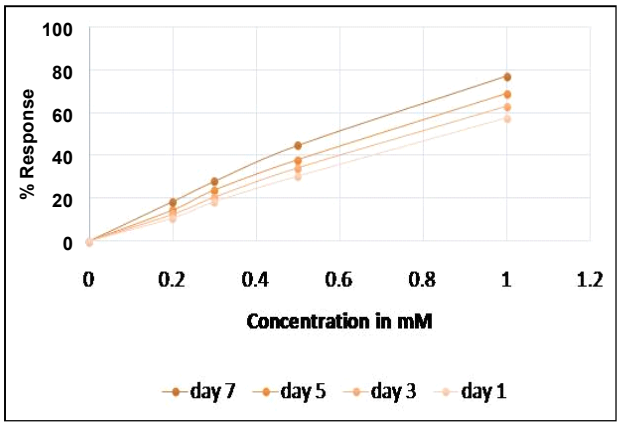

Fig 4: Dode response curve.

were exposed to deltamethrin (100 nM-5 µM) for 24-48 hrs. Deltamethrin produced a time and dose-dependent increase (21-121%) in DNA fragmentation, an indicator of apoptosis. Data demonstrated that the initiation of DNA fragmentation resulted from interaction of deltamethrin with Na+ channels and consequent calcium influx, as tetrodotoxin and the intracellular Ca2+ chelator BAPTA. The present study results correlated to the apoptosis effect and analysed the less explored predicted effects of deltamethrin on blood stem cells through CD45 receptor and virtual effects on cultured cells. iGEMDOCK 2.1 software was used to dock CD45 receptors with DLM. The binding mode indicates that the bound ligand and the active site of CD45 had a good binding score of -68.04 and formed the five H bonds with the amino acids residues (HIS 418, ASN 419, ASP 436, LYS 437, ASN 438) of CD45 (Fig 2). Whereas, DLM showed a docking score of 78.78 and formed hydrogen bond with ASN 419, ASP 436 amino acid residues of CD45 (Table 1). Deltamethrin has a higher docking score as compared to the reference ligand, this might mean that deltamethrin might take the place of a molecule or a ligand that normally binds to CD45 towards immune pathway. NAG (N-acetyl glucosamine) enhances N-glycan branching promotes CTLA-4 surface expression and inhibits adoptive transfer EAE (Grigorian *et al*., 2007). In this pathway NAG interacts with T cell receptor (TCR) and CD45 (Grigorian *et al*., 2011). This might affect the overall immune response of the cell.

CONCLUSION

Synthetic pyrethroid insecticide-Deltamethrin mechanism of toxicity to the immune system is not well explored. The present research reported on the less explored predicted effects of Deltamethrin on blood stem cells through CD45 receptor and virtual effects on cultured cells using stem cell culture system from bone marrow in DLM treatment showed apoptotic changes, iGEMDOCK 2.1 software used to dock CD45 receptors indicated the bound ligand (NAG) and the active site of CD45 had a good binding score of -68.04 and formed the five H bonds with the amino acids residues of CD45 cells.

REFERENCES

Aktar, M.W., Sengupta, D., Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. Inter Disciplinary Oxicology. 2: 1-12.

Antonchuk, J., Sauvageau, G., Humohries, R.K. (2002). HOXB4-induced expansion of adult hematopoietic stem cells. *Ex vivo*. Cell. 109: 39-45.

Chatterjee, S., Basak, P., Chaklader, M., Das, P., Pereira, J.A., Chaudhuri, S., Law, S. (2013). Pesticide induced marrow toxicity and effects on marrow cell population and on hematopoietic stroma. Experimental Toxicology Pathology. 65: 287-295.

Chatterjee, S., Basak, P., Chaklader, M., Das, P., Pereira, J.A., Chaudhuri, S., Law, S. (2014). Pesticide induced alterations in marrow physiology and depletion of stem and stromal progenitor population: An experimental model to study the toxic effects of pesticide. Environ. Toxicol. 29: 84-97.

Grigorian, A., Lee, S.U., Tian, W., Chen, I.J., Gao, G., Mendelsohn, R., Dennis J. W., Demetriou M. J. (2007). Control of T Cell-mediated autoimmunity by metabolite flux to N-glycan biosynthesis. The Journal of Biological Chemistry. 282: 20027-20035.

Grigorian, A., Araujo, L., Naidu, N.N., Place, D.J., Choudhury, B. and Demetriou, M. (2011). N- acetylglucosamine inhibits T-helper 1 (Th1)/T-helper17(Th17) cell responses and treats experimental autoimmune encephalomyelitis. Journal of Biological Chemistry. 286(46): 40133-40141.

Haripriya, K.S., Jeyarani, S., Mohan kumar and Soundararajan, R.P. (2019). Field evaluation of biocontrol agents and biopesticides against spotted pod borer, *Maruca vitrata* (Geyer) on lablab. Indian Journal of Agricultural Research. 53: 599-603.

Jeyalakshmi, T. and Shanmugasundaran, R. (2012). Evaluation of certain insecticides on nesting for their efficacy and wash resistance against mosquito species. Indian Journal of Experimental Biology. 50: 489-493.

Kumar, A., Sasmal, D., Sharma, N. (2014). Deltamethrin induced an apoptogenic signalling pathway in murine thymocytes: Exploring the molecular mechanism. Journal of Applied Toxicology. 34: 1303-10.

Mangala Gowri, A., Kavitha, G., Rajasundari, M., Mubeen Fathima, S., Senthil Kumar, T.M.A. and Dhinakar Raj, G. (2013). Fetal stem cell derivation and characterization for osteogenic lineage. Indian Journal of Medical Research. 137: 129-136.

Massin and Thouvenot (1993). *In vitro* study of pesticide hematotoxicity in human and rat progenitors. Journal of Pharmacololgy and Toxicology Methods. 30: 203-207.

Nicolopoulou, S.P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Frontiers in Public Health. 4: 148. https://doi.org/10.3389/fpubh.2016.00148.

Rupa Sule and Dollykumar, (2019). Insecticide resistance studies of Cypermenthrin 25EC and Chlorpyriphos 20EC against *Spodoptera lituraabricius*, 1775 (Lepidoptera:Noctuidae) Indian Journal of Agricultural Research. 53: 453-457.

Thomas, P.T., Busse, W.W., Kerkvliet, N.I., Luster, M.I., Munson, A.E., Murray, M., Roberts, D., Robinson, M., Silkworth, J., Sjoblad R., Smialowicz, R. (1990). Immunologic Effects of Pesticides. In: The Effects of Pesticides on Human Health. Advances in Modern Environmental Toxicology. Princeton, N.J. Princeton Scientific Publishing Co. 18: 261-295.

Wang, J., Ding, F., Gu, Y. (2009). Bone marrow mesenchymal stem cells promote cell proliferation neurotrophic function of Schwann cells *in vitro* and *in vivo*. Brain Research. 1262: 7-15.

Yousef, M., Awad, T., Mohamed, E. (2001). DLM-induced oxidative damage and bio chemical alterations in rat and its attenuation by vitamin E. Toxicology. 227: 240-247.

Zhao, Y., Bower, A.J., Graf, B.W., Boppart, M.D., Boppart, S.A. (2013). Imaging and Tracking of Bone Marrow- Derived Immune and Stem Cells. Methods in Molecular Biology. 1052: 57-76.