

REVIEW

Mechanism of Deltamethrin induced Immunotoxicity: Current and Future Perspectives

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The immune system is the most vulnerable system regarding toxicity of pesticides. Any alteration in the immune functions makes an individual immunocompromised and more susceptible to cancer, infections, autoimmunity and allergies. Deltamethrin is the most popular type 2 pyrethroid insecticide which is widely use in agriculture and home due to restriction on the organophosphate insecticides. Due to their extensive use, it becomes an increasingly serious source of chemical pollution. We all are exposed to deltamethrin through inhalation, ingestion and dermal contact. It has been demonstrated that deltamethrin alters the immune response signalling pathways, but its mechanism of immunotoxicity is still an open question for researchers to be explored. Thus, herein we tried to understand the mechanism of deltamethrin induced immunotoxicity. Possibilities of deltamethrin induced other immunotoxic signalling pathways have also been discussed and should be considered in future studies. Further, current challenges and future perspectives have been also discussed.

Keywords: Deltamethrin; Apoptosis; Immunostimulation

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Introduction

Deltamethrin (DLM) is a type 2 synthetic pyrethroid insecticide which contain alpha cyano group as shown in figure 1. It was first synthesized in 1974 and has been widely applied in agriculture to increase yields by controlling pests and diseases [1-2]. DLM is known to remain for longer periods in the water, soil, air and food. Farm workers are chronically exposed to the DLM [4]. The most common exposure of the general population to DLM occurs within home [5]. DLM containing products is widely available in the market and easy to purchase. They are useful in homes and garden in the form of fly sprays, pest strips, wood preservatives, to kill cockroaches, rodenticides, in shampoo to treat head lice [6]. It is also used in the control of malaria and other insecticides borne diseases [2, 7]. Due to restrictions on the sales of

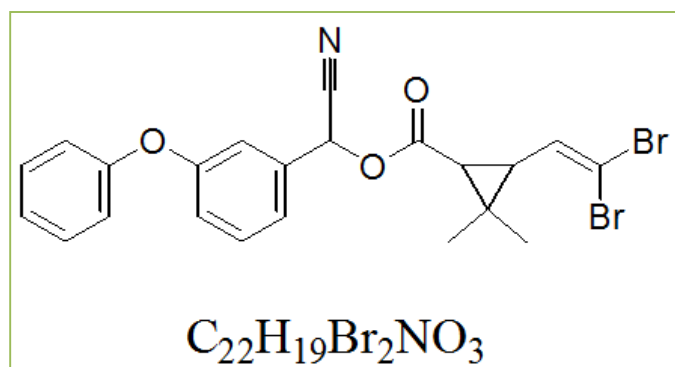


Figure 1. Structure of DLM.

organophosphorus insecticides, its use has markedly increased as insecticides and antiparasitic formulations [8]. We are all exposed to them on a daily basis, whether we

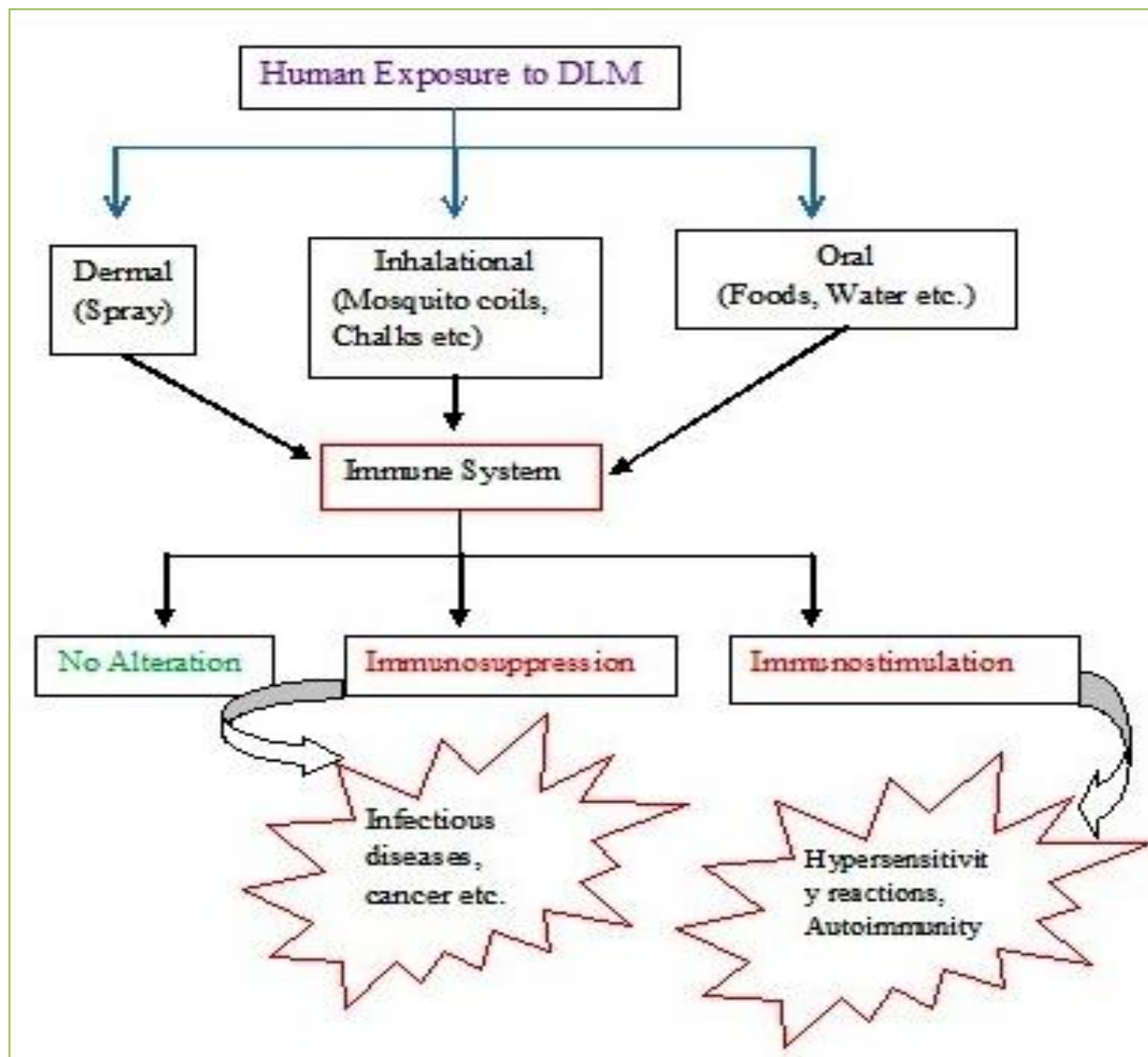


Figure 2. Human exposure to DLM and consequences of interaction of DLM with the immune system.

work with them directly or not. The different routes of exposure such as inhalation, ingestion and dermal contact are shown in figure 2 [3].

Pesticides have established to be very beneficial to peoples, increasing crop yields and preventing the spread of disease. Unluckily, due to over application and misapplications, there can also be unwanted consequences. The maximum number of population exposure occurs at relatively low doses. Generally, at low doses, DLM do not cause any severe harm to humans. However, agricultural workers and children are two groups of individuals which are at a greater risk. Agricultural workers are mainly susceptible due to quantity of DLM they are exposed to and the extent of exposure. Children are also more susceptible due to an immature immune system.

The immune system is the most delicate system regarding toxicity of environmental toxins [9]. Some environmental toxicants such as heavy metals (mercury, copper, manganese, cobalt and cadmium), alkylating agents, and halogenated organic compounds (dioxins, furans, PCBs, PBBs, organochlorine insecticides) have been found to be immunosuppressive in experimental models [10]. An immunotoxic compound can modify one or more immune function resulting in an adverse effect for the host. Two core adverse effects can be identified: 1) decreased in the immunity (immunosuppression), which may consequence in more severe or extended infections as well as the development of cancer. 2) In-appropriate immunostimulation, which may result in the hypersensitivity reactions and autoimmune diseases. A third common effect is the inflammation, which contributes to tissue and organ damage [11, 12]. Cells respond and adjust through

Table 1. Effect of DLM on immune system

S.no	Dose (DLM)	Time	Species	Conditions	Cells/cell line	Outcomes	References
1	25mg/kg	24hrs	Mice	In vivo	Thymocytes	DNA fragmentation	[22]
2	50µM	24hrs	Mice	In vitro	Thymocytes	Apoptosis	[22]
3	10,25 and 50µM	18hrs	Mice	In vitro	Thymocytes	Apoptosis	[15]
4	10,25 and 50µM	18hrs	Mice	In vitro	Splenocytes	Apoptosis	[16]
5	3mg/kg/day/ orally	30days	Rat	In vivo	Lymphoid organs	Oxidative stress	[21]
6	2mg/kg	4weeks	Rat	In vivo	Thymus	DNA damage	[17]
7	5 and 10mg/kg	14days	Mice	In vivo	NA	Decrease in total white cell and lymphocyte count	[18]
8	1.28mg/kg	4weeks	Rat	In vivo	NA	Decrease in white blood cells	[20]
9	1µg µl ⁻¹	14days	Fish species	In vitro	NA	IgM level decreased	[19]

numerous mechanisms that involve communication pathways or signal transduction processes against environmental signals [13]. A number of cell receptors recognize the foreign compounds and induce a cascade of events that is required for the neutralization and excretion of these compounds. Various different and complex mechanisms are responsible for immunotoxicity. More frequently, toxicants can interfere with several immune specific signaling pathways, resulting in alterations in the cytokine production, surface marker expression, cell differentiation and activation. On the other point, small molecular weight chemicals (<1000 Da) can form protein adducts, thus acting as antigen, leading to allergy or autoimmune complaints. Lastly, immunosuppressive compounds can break down the central tolerance of auto reactive B or T cells, especially during in utero or early life exposure, which has been connected later in life to autoimmunity and abnormal hypersensitivity [14].

Deltamethrin is well known neurotoxin, but regarding immunotoxicity very few reports are available. Thus, in this review, we try to describe the mechanism of DLM induced immunotoxicity based on our own studies [15, 16] as well as those reported in the recent literature.

Deltamethrin induced Immunotoxicity

Immunotoxicity is a challenging area of toxicology because the immune system is regulated by many external factors and feedback mechanisms [12]. Xenobiotics, such as pesticides, can be lethal to the cells of the immune system. There are four possible outcomes when DLM interact with the immune system as shown in figure 2. The first outcome is no alteration of the immune system as an effect of DLM exposure. The second possible outcome is an enhancement of the immune response, results into autoimmune diseases. A third potential outcome of DLM exposure is a decrease in the immune response (immunosuppression). The final possible

outcome is the progress of hypersensitivity reactions. In the literature, both *in vitro* and *in vivo* data demonstrate that DLM can induce alteration of the immune system. Hamid *et al.* (2013) [17] has been observed that DLM at 2 mg/kg (*in vivo*, albino rats) for 4 weeks induced thyroid and DNA damage. Suwanchaichinda *et al.* (2005) [18] observed that DLM at 5 and 10mg/kg for 14 days caused significant decrease in total white cell and lymphocyte count in Swiss albino mice. IgM level is decreased in fish species after deltamethrin exposure (1µg µl⁻¹) for 14 days [19]. Saudi *et al.* (2011) [20] has been observed that DLM at 1.28mg/kg (*in vivo*) for 4 weeks leads to decrease in white blood cells (WBC) in male Wistar rats. Aydin reported that thiacloprid and deltamethrin, both individually and in combination, could cause immunosuppression in immune organs of rats [21]. From these reports, it has been concluded that DLM interfere with the immune system.

Deltamethrin induced Immunosuppression

Deltamethrin induce immunosuppression by activating various apoptogenic signalling pathways. Under normal physiological conditions, apoptosis plays a vital role, but when it is out of normal control, it can contribute to several diseases such as immunodeficiency, autoimmunity and cancer. Emerging evidences indicate that DLM induces apoptosis in various cells [8, 15-16, 22-23]. DLM induced apoptosis in various cells is well accepted phenomenon, but the mechanism underlying DLM induced apoptotic signalling pathways in immune cells has not been completely understood. From literature, it has been observed that DLM may induce intrinsic or oxidative stress pathways in immune cells, which leads to apoptosis.

Deltamethrin induced intrinsic signalling pathways

The intrinsic pathway of apoptosis includes oxidative

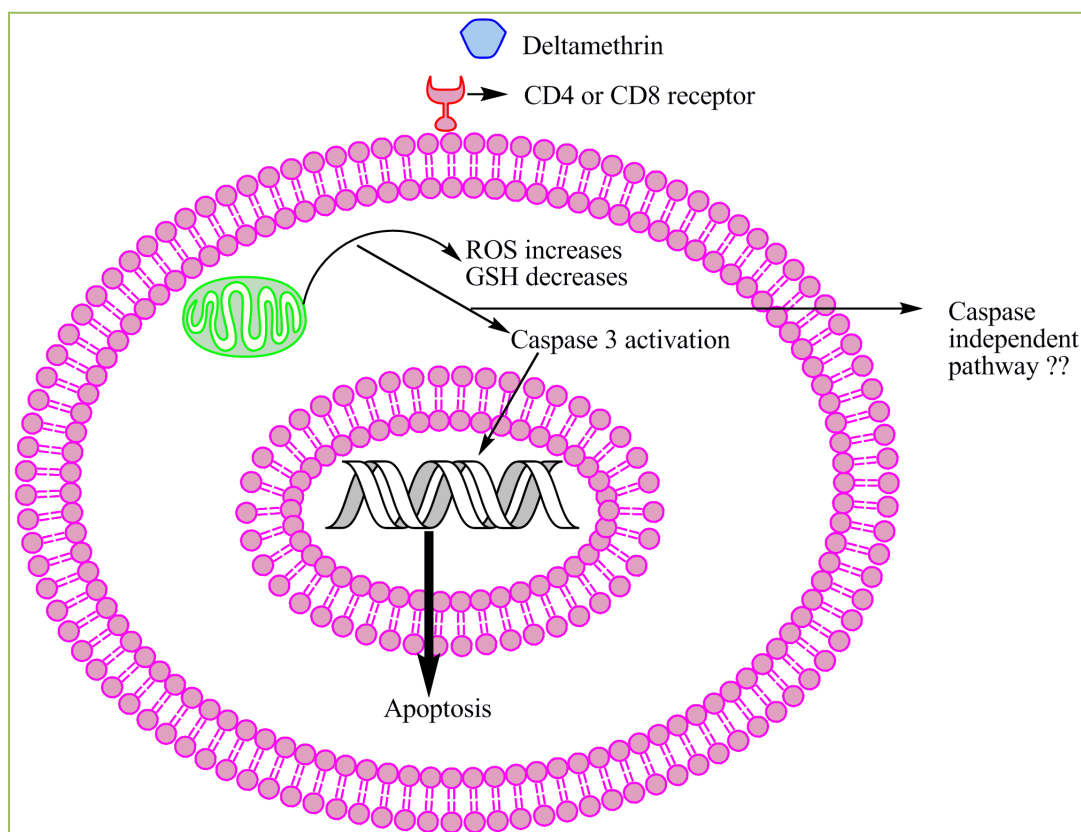


Figure 3. Mechanism of Immunotoxicity of DLM in murine thymocytes. DLM shows a strong binding affinity towards the CD4 and CD8 receptors. DLM induced thymocytes apoptosis by activating oxidative stress and caspase dependent signaling pathways.

stress, caspase dependent as well as caspase independent pathways which are activated by variety of environmental toxicants.

Mechanism of Deltamethrin induced oxidative stress

The imbalance between reactive oxygen species (ROS) production and antioxidant enzyme system is termed as oxidative stress. It plays a major role in deltamethrin induced toxicity in mammals [24]. Deltamethrin 3mg/kg (*in vivo*) treatment decreased the level of antioxidant enzyme (catalase, glutathione peroxidase) and increased the lipid peroxidation in rats [21]. Recently, we have also observed that DLM induced ROS may activate apoptogenic signalling pathways in both murine thymocytes and splenocytes as shown in figure 3 & 4. Further our study have shown that antioxidant (NAC) has effectively reduced and GSH Depletor (BSO) has significantly increased the percentage of apoptotic cells, verify the role of oxidative stress in the DLM induced splenic and thymic apoptosis. The oxidative stress induced by DLM may result in opening of caspase dependent and caspase independent pathways of apoptosis.

Mechanism of Deltamethrin induced caspase dependent

pathways

Caspases are key mediators of programmed cell death [25]. Oxidative stress leads to up regulation of pro apoptotic (Bad, Bax) and down regulation of anti-apoptotic (bcl-2) proteins. Deltamethrin activated oxidative stress also results into the up regulation of Bax and down regulation of Bcl-2 as shown in figure 4 [26]. The relative ratios of the various bcl-2 and bax proteins, determine how much cellular trauma is required to induce apoptosis [27]. Bax and Bak are critical proteins for inducing permeabilization of the outer mitochondrial membrane and the discharge of cytochrome *c* which results in the loss of the mitochondrial membrane potential (MMP). This leads to the formation of apoptosome and activation of caspase-9 that in turn stimulates the activation of execution caspases [27-29]. Enan *et al.*, (1996) [22] have been shown that DLM 50µM (*in vitro*) and 25mg/kg (*in vivo*) induced an intrinsic apoptogenic signalling pathways in the murine thymocytes. Recently, we have also observed that DLM induced thymic and splenic apoptosis by activating mitochondrial caspase dependent signalling pathways of apoptosis as summarized in figure 3 & 4 [15, 16].

Effect of Deltamethrin on immune functions

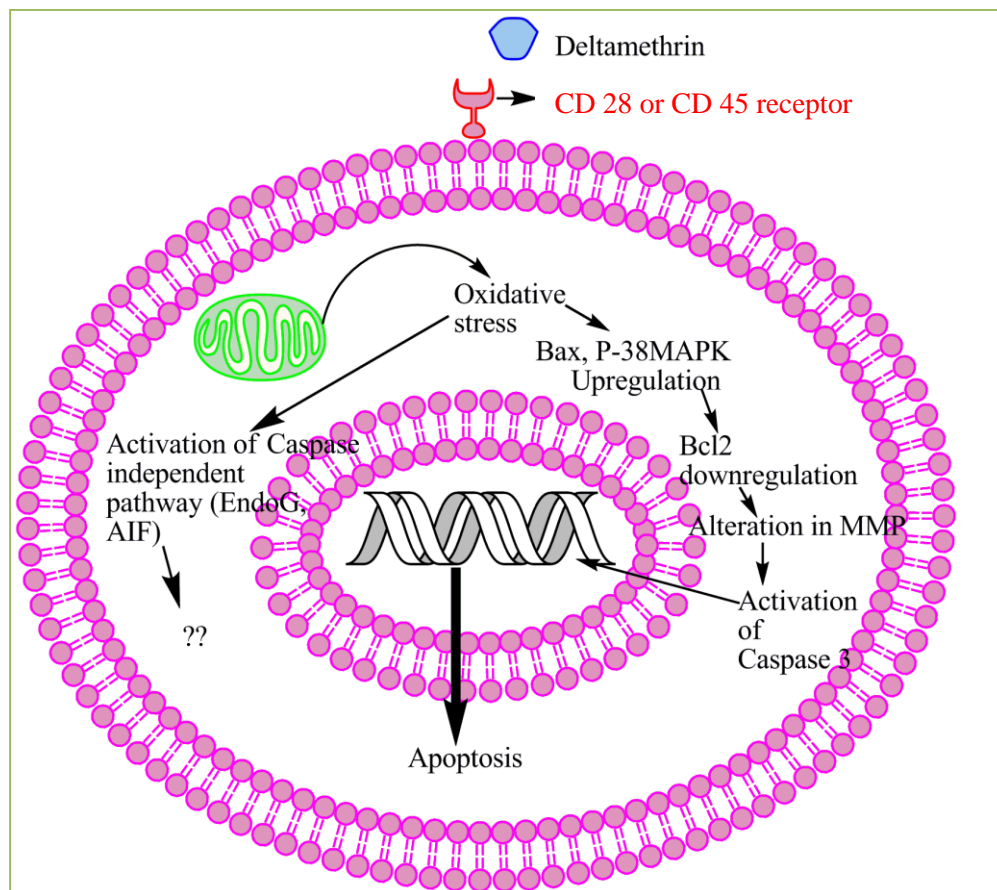


Figure 4. Mechanism of Immunotoxicity of DLM in murine splenocytes. DLM shows a strong binding affinity toward the CD45 and CD28 receptors. The apoptogenic activity of DLM in murine splenocytes occurs through ROS production followed by alteration in p38 MAP Kinase, Bcl and Bax ratio, decrease in mitochondrial membrane potential (MMP), caspase-3 activation, GSH depletion which results in alteration of immune functions.

Phenotyping and cytokine assays are important indexes for assessing the immune response. Recently, we have observed that DLM treatment leads to decline in splenic T and B cell population in a concentration dependent manner [16]. In the literature various studies point out the deficient production of cytokines induced by the toxicants. We have also observed that DLM suppressed the cytokines (IFN γ , IL-2 and IL-4) and alter the immune functions [15-16].

Possibilities of DLM induced other Immunotoxic signaling pathways

DLM induced mitochondrial caspase dependent pathways of apoptosis but there is also possibility of activation of the other immunotoxic signalling pathways. As we observed in our study, the percentage of apoptotic cells has been partially prevented in the presence of a caspase inhibitor (Z-DEVD-fmk). Therefore, the role of caspase independent pathways (AIF, Endo G) in DLM induced immunotoxicity need to be further investigated. We have only explored the DLM induce intrinsic pathways of apoptosis. There also may

be a possibility of deltamethrin induced extrinsic pathways.

Pesticides can induce immunostimulation by increasing secretions of various cytokines, but in case of DLM, no report is available yet. Some environmental toxicants bind and modify, host cells or proteins to such a point that they are no longer recognized by the immune cells as self. Pesticide exposure may be associated with the exacerbation of autoimmune disease in the experimental animal. The injection of malathion (organophosphate insecticide) increased the levels of auto antibodies and rheumatoid factor as well as the number of inflamed glomeruli in the kidney [30]. In case of DLM, no report is available yet. These all DLM induced immunotoxic possible signalling pathways should be investigated in future studies for better understanding of the mechanism by which DLM induce immunotoxicity.

Current challenges

Few studies have investigated the effect of DLM on the

immune system, because such research is extremely difficult to plan and execute. Although most people are exposed to chronic low concentrations rather than the high doses used in laboratory tests but there is no basis exists for assuming that humans are free from the risk. Recently, we have observed that DLM affect the immune system of mice. Thus, particular attention for immunotoxicity studies in humans has to be addressed to these compounds. The most important question arising is whether environmental toxicants showing toxicity to the immune system in the laboratory animals can affect human immune system in conditions of low dose and prolonged exposure. This is a very important matter because the extrapolation from the high doses of the laboratory animals to the low doses of the environmental and occupational exposures can be very complex task.

Conclusion and Future Perspectives

DLM induced immunotoxicity by altering various immune signaling pathways which results in immunosuppression. Immunosuppression can be occurred by activating caspase dependent and oxidative stress signaling pathways which results in apoptosis.

Most of the experimental animal immunotoxicity studies, including pesticide studies, have been conducted by the dosing of a single chemical. In reality, human are exposed to a mixture of chemicals, which may have different effect as that of a single chemical. So, In future studies, effect of mixtures of chemicals as well as single chemical on immune system should be considered. Research on DLM activated immunotoxic signaling pathways is ongoing, but the effects of DLM on developing immune system, very few reports are available. The developing immune system is very much sensitive to chemicals. So, the future studies should plan to check the early life exposures to DLM.

There is no clinical antidote available for deltamethrin poisoning and the symptomatic treatment is the only choice. Herbs have traditionally been used for centuries for the strengthening of the immune system^[31]. Recently, we have also reported the immunoprotective role of piperine in the DLM induced thymic apoptosis and altered immune functions^[32]. Thus, role of herbals should be investigated in the future for attenuation of DLM induced immunotoxicity.

A computational method predicts the binding affinity of ligand towards particular receptor which provides toxicologists a valuable information that augments, enriches and complements *in vitro* and *in vivo* efforts. Docking is one of the computational method which predict the interaction of drugs, toxins towards particular receptors along with its ADME properties. Recently, we have also demonstrated the

interaction of DLM towards various immune cell receptors^[15-16]. Thus, in the future, researchers should use computational approaches for prediction of toxicity.

ICH (S8) guidelines provide testing approaches to recognize compounds which have the potential to be Immunotoxic^[33]. In these guidelines approaches to find out the mechanism of Immunotoxicity of Environmental toxins is still lacking, should be considered in future guidelines.

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Conflicting interests

The authors have declared that no competing interests exist.

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