ABSTRACT

Interactions with cytochrome P450 are known to occur ubiquitously as well as evolutionarily conserved in human and fishes and further the human population as well due to the inability of detoxification of these pesticides. Particularly, these pesticides are harmful and toxic to the aquatic biota on neurolemma. Thus, under both acute and chronic exposures, the pesticide deltamethrin showed the highest interaction with cytochrome P450 for coding cytochrome P450 functions. Among a number of cytochrome P450 families, the mitochondrial cytochrome P450 is of the highest importance as it is primarily responsible for detoxifying drugs, drug metabolites, alcohol and others. In human, there are about 57 genes reported for coding cytochrome P450. These proteins are different in human and fishes and further the human population as well due to the inability of detoxification of these pesticides.

INTRODUCTION

Synthetic pesticides of any origin can exert varied effects on different target as well as non-target organisms which further may also affect humans by biomagnification [1]. The organophosphate group of pesticides (like triazophos and chlorpyrifos) can directly affect the synapses present between neuronal and neuromotor junctions by affecting acetylcholinesterase (AChE) activity [2]. The pyrethroids (like deltamethrin and cypermethrin) also exert a similar effect by altering the voltage gated sodium channels present on neurotransmitters [3]. Thus, under both acute and chronic exposures, these pesticides are harmful and toxic to the aquatic biota particularly fish species [4] and further the human population as well due to the inability of detoxification of these pesticides [1].

The main detoxifying enzyme which is present in almost all cells ubiquitously as well as evolutionarily conserved one is cytochrome P450 (CYP) family proteins which use haem as a cofactor for their functions [5]. Among a number of cytochrome P450 proteins, the mitochondrial cytochrome P450 is of the highest importance as it is primarily responsible for detoxifying drugs, drug metabolites, alcohol and others [6]. In human, there are about 57 genes reported for coding cytochrome P450. There are few reports, delineating the interactions of chlorpyrifos with cytochrome P450 in human, rat and mouse [8] but till date reports are lacking in the field of homology alignment of cytochrome P450 in human and fishes and further the comparative interactions of cytochrome P450 with different pesticides.

The previous studies lacked the information on the structural homology of cytochrome P450 in human and a key representative of fish (Zebra fish; Danio rerio), therefore the present study was undertaken to note their similarities in functional activities. Further, the study was elaborated to note the interactions of pesticides (organophosphates and pyrethroids) with cytochrome P450 to get a speculative analogy of effects as exerted by these pesticides in fish similar in human or not.

MATERIALS AND METHODS

Homology alignment of cytochrome P450 between human and zebra fish

We compared the sequence of cytochrome P450 of Zebra fish and Homo sapiens using Basic Local Alignment Search Tool (BLAST).

Molecular docking

For in silico study, the protein structure of Cytochrome P450 with PDB ID: 4R21 were retrieved from RCSB protein database [9]. Further, the geometry optimisation and active site prediction of this compound were done by using Discovery studio 3.0 [10]. The 2D structure of selected pesticides namely triazophos, deltamethrin, chlorpyrifos and cypermethrin were retrieved from Pubchem compound database [11] and converted into 3D format using Discovery studio 3.0. Then the best-docked compound was taken for interactive 2D-3D visualization using Discovery studio 3.0. Further molecular docking calculation was done by using YASARA software [12]. Using YASARA, receptors and ligands files were set and macro was run. The result log files were prepared for all the ligands. Binding energy and dissociation constant were used for sorting the docking result. The compound with more positive binding energy shows more interaction with the receptor.

RESULTS

Homology alignment of cytochrome P450 between human and zebra fish

We found 99% query cover with 32% identity showing homology between human and zebra fish upon BLAST (fig. 1).
Fig. 1: Showing sequence homology alignment between human and zebra fish

Molecular docking

Molecular docking result showed that pesticide, deltamethrin exerted the best interaction with cytochrome $P_{450}$. All other pesticides namely triazophos, chlorpyrifos and cypermethrin also shows interaction but lesser than deltamethrin (table 1). The binding energy and dissociation constant for deltamethrin were found to be $8.233 \text{ kcal/mol}$ and $922849.687 \text{ pM}$ respectively.

Further, the best-docked compound deltamethrin was taken for interactive 2D-3D visualization using Discovery studio 3.0. The active site amino acid residues, ArgA$^{103}$ IleA$^{119}$ AlaA$^{120}$ TrpA$^{128}$ ArgA$^{132}$ AlaA$^{201}$ ThrA$^{206}$ ThrA$^{316}$ MetA$^{361}$ ValA$^{366}$ SerA$^{367}$ LeuA$^{370}$ IleA$^{371}$ HisA$^{373}$ ProA$^{375}$ PheA$^{433}$ GlyA$^{434}$ ArgA$^{438}$ ValA$^{439}$ CysA$^{440}$ ValA$^{441}$ GlyA$^{442}$ and AlaA$^{446}$ of cytochrome $P_{450}$ were involved in interaction with deltamethrin.

The pink colour residues show electrostatic interaction while green colour residues show Vander Waals interaction. The residue ArgA$^{103}$ shows direct interaction with deltamethrin which shows its inhibition activity (fig. 2A and fig. 2B).

Table 1: Binding energy and dissociation constant for all selected pesticides with cytochrome $P_{450}$

| Pesticides      | Cytochrome P$_{450}$ | Binding energy [kcal/mol] | Dissociation constant [pM] |
|-----------------|----------------------|---------------------------|-----------------------------|
| Deltamethrin    |                      | 8.233                     | 922849.687                  |
| Cypermethrin    |                      | 7.533                     | 300772.75                   |
| Triazophos      |                      | 6.497                     | 17283666                   |
| Chlorpyrifos    |                      | 5.433                     | 104125760                   |

Fig. 2A: 2D interaction of deltamethrin with cytochrome $P_{450}$. The pink colour residues show electrostatic interaction while green colour residues show Vander Waals interactions. The residues ArgA$^{103}$ shows direct interaction with deltamethrin.
CONCLUSION
From the present in silico study, it may be concluded that the potential toxicity in the terms of reaction kinetics and binding efficiency of two groups of pesticides namely organophosphates and pyrethroids are variable being higher in deltamethrin followed by cypermethrin, triazophos and chlorpyrifos respectively. Further, we can also speculate that the mode of action of these pesticides are almost similar even in human and fishes due to the structural homology of cytochrome P<sub>450</sub> in both of the organisms.

AUTHORS CONTRIBUTION
Conceptualization of the study was suggested by RSP. The relevant literature survey and structural analysis of pesticides were done by RKT and SS (contributed equally). The analysis of results, writing of paper was done by SG. The docking analysis was done by SKR and PS.

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CONFLICT OF INTERESTS
All the authors declare that they do not have any conflict of interest.

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