



IN SILICO PREDICTION OF THE MITIGATIVE POTENTIAL OF CURCUMIN AGAINST DELTAMETHRIN TOXICITY

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ABSTRACT

Deltamethrin (DLM) is a widely used pyrethroid, pest control insecticide due to restriction on the sale of organophosphate. Deltamethrin (DLM) being a potent hepatotoxic compound, that affects physiological functions of liver; however, the implications of the hepatotoxic effects of DLM on the mammalian system and its influence on the hepatic enzymatic activity are still unclear. Curcumin (CUR), a major polyphenol component of Curcuma longa Linn, is a potent antioxidant often used in traditional medicine as an ameliorative agent. However, its underlying mechanism as an anti-oxidant against DLM-toxicity remains unknown. Hence, the present study has been designed to determine the binding affinity of DLM to a hepatic enzyme marker such as Aspartate aminotransferase (AST). Moreover, the study is aimed to delineate the role of CUR on DLM-induced hepatotoxic effects through a computational approach. The molecular docking carried out demonstrated that CUR manifests a stronger binding affinity towards the liver marker enzyme, AST as compared to deltamethrin. This could explain the possible mechanism for inhibition of the toxic influence of DLM-induced reactive oxygen species on liver enzyme activity. Thus, this computational evidence validates the tenet that CUR displays anti-oxidative properties for attenuation of DLM induced hepatotoxicity, alteration in the mechanism of liver marker enzyme and its subsequent impact on hepatic tissue.

Keywords: Deltamethrin (DLM), Curcumin (CUR), Hepatotoxicity, Molecular docking.

INTRODUCTION

It is well established that pesticides exhibit exceedingly harmful effects on sensitive non-target human tissues and consequently they cause varied diseases (Le Couteur et al., 1999). In this respect, the use of various pesticides can result into wide range of human diseases and adversely impact the health and quality of life of humans (Nasterlack, 2001). Recent reports have revealed that pesticides may cause liver, pancreas, brain, prostate and other cancers due to their inherent toxicity in human (Koutros et al., 2013; Boada et al., 2012; Liu, 2012; Giannandrea, 2012; Govett et al., 2011).

Due to many advantages over other insecticides such as low cost, safety and duration of residual action, pyrethroid insecticides are especially important and widely applied in modern agriculture in many countries at present (World Health Organization, 2013). The pyrethroids, synthetic analogues of the naturally occurring esters of chrysanthemic acid, contain broad-spectrum insecticidal activity combined with low mammalian toxicity (Crombie and Elliott, 1961). Uncontrolled use of pyrethroid insecticides led to the wide spread of pesticides into the environment ranging from soil to foodstuff (Rutsaert et al., 2013). Although acute toxicity of pyrethroids to mammals was thought to be minimal, their potential detrimental effect on human health has now become a matter of public concern. Long-term insecticide exposure has also been reported to pose potential risk to non-target tissues mainly via the interactions of proteins with insecticides (Tan and Song, 2014), it is very much important to investigate the interaction between protein and insecticide at molecular level. Computational tools have provided the resources to adequately focus on the precise interactions of the molecular structure of various insecticides with proteins, validated by molecular modelling.

The use of pyrethroid as insecticidal and anti-parasitic formulations has markedly increased in the last 2 decades due to restrictions on the sales of organophosphorus insecticides (IPCS, 1990; Mestres and Mestres., 1992; Muhammad et al., 2011). Due to this widespread use, pyrethroid contamination has become a potential problem. Pyrethroids are classified into two subclasses,

Type 1 and type 2, based on the production of syndromes. Type 1 pyrethroids are characterized by the induction of whole-body tremor, whereas type 2 pyrethroids produce sinusoidal writhing convulsions (chorea) accompanied by salivation (Lawrence and Casida, 1982; Verschoyle and Aldridge, 1980).

Deltamethrin (DLM), an alpha cyano type 2 synthetic pyrethroid, first synthesized in 1974, has been widely used to protect crops, fruits, and vegetables from pests such as mites, ants, weevils, and beetles (Suwanchaichinda et al., 2005; Chandra et al., 2013). Synthetic pyrethroids have become a main class of active insecticides due to their high insecticidal potency, photostability, and low mammalian toxicity to birds and mammals (Bradburry and Coast, 1989; Rehman et al., 2006). Deltamethrin (DLM) is globally used in agriculture, home pest control, the protection of food stuff and for disease vector control. (Yousef et al., 2001). Due to the restrictions on the sales of organophosphorus insecticides, the chances of human exposure to pyrethroid insecticide have been enhanced (Hossain and Richardson, 2011). Deltamethrin is considered to be the safest class of insecticide with a relatively low mammalian toxicity (Patro et al., 1997). Although initially thought to be less toxic, a number of reports have also indicated its toxicity in mammalian, non-mammalian laboratory and wild animals. Humans are exposed to pyrethroid like DLM by polluted food and water and it is readily absorbed by the oral route (FAO/WHO, 1999; Barlow et al., 2001). DLM is reported to cause toxicity through oxidative stress under *in vivo* conditions, i.e. induced thiobarbituric acid-reactive substances (TBARS-marker of lipid peroxidation) in plasma, reactive oxygen species (ROS) generation, glutathione (GSH) depletion and suppressions of glutathione peroxidase, glutathione S-transferase and catalase (Aydin, 2011; Rehman et al., 2006; Yousef et al., 2001). Humans are exposed to DLM through various exposure routes like inhalation, dermal and oral. Later, DLM is absorbed from all these routes and finally enter into the systemic circulation.

Curcumin, 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3, 5-dione, is a naturally occurring pigment and component of the spice which is present in the rhizome of the plant

Curcuma longa. Curcumin contains a wide range of biological properties including antioxidant (Tuba and Gulcin, 2008), anticarcinogenic (Xia et al., 2007) and anti-inflammatory activity (Sandur et al., 2007). Curcumin is a potent antioxidant, but its ameliorative efficacy against DLM induced alteration in hepatic enzyme activity and subsequent hepatotoxicity is still unexplored. Therefore, the main objective of the present study is to investigate the role of curcumin in the modulation of oxidative and hepatotoxic effects of DLM using computational approach. A docking study has been used to predict the binding affinity of curcumin towards one of the liver marker enzyme, AST.

AST is a tissue specific intracellular enzyme which catalyzes the transfer of α -amino group from amino acid to α -keto acid which occurs in liver, heart and striated muscles. This transaminase is an important liver enzyme responsible for detoxification processes (Abbassy and Mossa, 2012) and useful for the diagnosis of liver functions. They are abundantly present in hepatocytes and considered as important markers of hepatotoxicity. Measurement of serum AST activity plays an important role to evaluate the hepatotoxic effects of pesticides (Bradberry et al., 2005; Eraslan et al., 2007). In view of the function of AST, pyrethroid insecticide such as DLM (Figure 1), and mitigative agent, CUR (Figure 2) were selected as the investigation model to decipher the interactions of DLM and CUR with AST in this study. The conformation of AST and the binding mechanism of DLM and CUR with AST were investigated by means of molecular modeling in order to elucidate the effect of pyrethroid insecticides in the alteration of enzymatic action of AST and its reversal

by CUR.

In the present investigation, an *in silico* study was performed to predict the binding affinity of DLM and CUR toward AST which is a marker enzyme of hepatic tissue. Such prediction of binding affinity of ligand toward a particular receptor using computational docking method is of great importance in the field of structure-based drug designing.

MATERIALS AND METHODS

Curcumin, one of the active components of turmeric has been used as an antidote in the present study to attenuate deltamethrin induced toxicity. In this study, the interaction profiles of curcumin and deltamethrin with hepatic marker enzyme such as Aspartate transaminase (AST) have been evaluated through computational approaches like molecular docking to assess the potential strength of curcumin against deltamethrin toxicity.

The details of the target proteins showing PDB ID/ UniProt ID, resolution and number of amino acids present in the protein structure are as following:

Table 1: Details of target Proteins

Protein Name	PDB ID/ UniprotID	Resolution	No. of Aminoacids	Reference
Aspartate aminotransferase(AST)	3II0	2.05 Å	422	Ugochukwu et al., 2009

Molecular files preparation

In the present study, three-dimensional crystal structure of selected protein i.e., Aspartate aminotransferase (AST) (PDB ID: 3II0) was obtained using X-ray diffraction from the RCSB (Research Collaboratory for Structural Bio-informatics) protein data bank with the PDB code (Table 1).

Molecular Docking

Molecular docking is a method for the prediction of the arrangement of one molecule with another molecule when they bound with each other for the formation of a stable complex. Further, the information of the favourable alignment in turn can be used for the calculation regarding the strength of binding affinity between two molecules by using scoring functions (Engel et al., 2008). In the present study, Aspartate aminotransferase (AST) (PDB ID: 3II0) was blind docked with deltamethrin and curcumin to assess the plausible interaction profiles. The structural depth of resolution and number of amino acid residues present in the selected protein are as mentioned in the Table 1. Protein preparation and ligand preparation for the study have been performed using YASARA (Yet Another Scientific Artificial Reality Application) software version 13.1.25 commercial package (Krieger et al., 2002). Removal of water molecules and energy minimization (Kumar et al., 2015; Modi et al., 2016) were performed by using the standard steepest decent conjugate method in YASARA (Krieger et al., 2004; Patel et al., 2017). Interaction profiles of various ligands of protein has been demonstrated by molecular docking and binding free energy was calculated by using the following equation:

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

Where,

ΔG_{vdW} = van der Waals term for docking energy

ΔG_{Hbond} = H bonding term for docking energy

ΔG_{elec} = electrostatic term for docking energy

ΔG_{tor} = torsional free energy term for ligand when the ligand transits from unbounded to bounded state

ΔG_{desolv} = desolvation term for docking energy

After finishing the molecular docking process, interaction profiles have been assessed to recognize the mechanism of better binding affection (Borad et al., 2018). Further, determination of van der Waals, Conventional hydrogen bonds, Alkyl, Pi-alkyl, Unfavourable donor-donor, Unfavourable acceptor-acceptor, Pi-Pi T-shaped, Carbon hydrogen Bond, Pi-Anion, Pi-Sigma was carried out to anticipate the interacting atoms with respective selected receptors (Li et al., 2017).

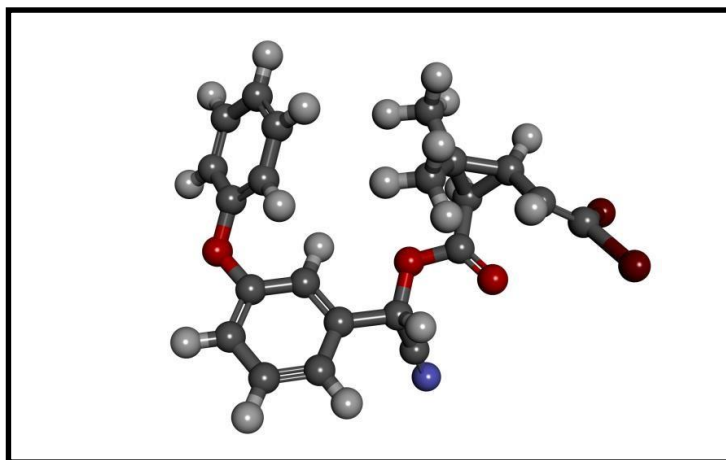


Figure 1 – 3D Structure of Deltamethrin (CID – 40585)

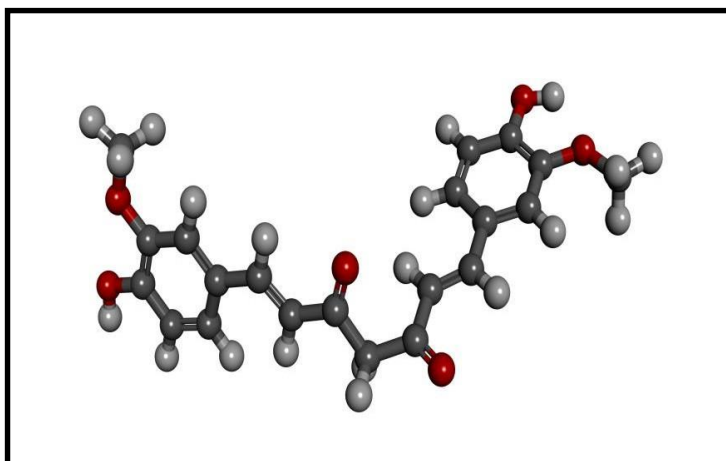


Figure 2 – 3D Structure of Curcumin (CID – 2889)

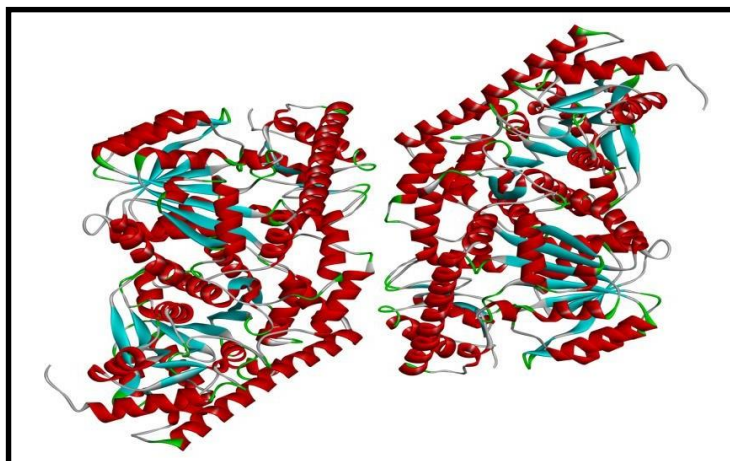


Figure 3 – Crystal structure of Aspartate aminotransferase (AST) (PDB ID – 3II0) Results Multiple protein docking strategy

To check dynamic properties of selected protein i.e. AST, ensemble-based approaches were used to dock two ligands against dynamic protein captures. For multiple targets, collective pocket mobility of the crystal structures can be accounted and employed for docking process. Two ligands from library were utilized for multiple target docking to determine promiscuous ligand by using computational approach. In the present study, Aspartate transaminase (AST) was used to assess interaction profile with CUR and DEL (Table 2). Autodock vina algorithm enumerated ligand poses and its energetic evaluations were made by (Y) AMBER03 force field calculations.

Molecular docking of Aspartate Transaminase (AST)

Docking analysis of Aspartate transaminase with two selected ligands i.e., DLM and CUR exhibited different kinds of interactions including traditional hydrogen bonding interactions, hydrophobic interactions, Van der Waals interactions and pi-pi interactions. Results of the docking

analysis revealed 9.423 Kcal/mol and 8.838 Kcal/mol binding energy for CUR and DLM respectively. The docking score of CUR was found to be superior for binding with aspartate transaminase compared to DLM. Figure 4(A) and 4(B) shows 3D and 2D pose of docked complex (AST – CUR) with the interaction of 2 conventional hydrogen bonds, 1 carbon hydrogen bond, 4 pi-alkyl bonds and 1 pi-pi stacked bond while the interactions of AST –DLM were as depicted in Figure 5(A) and 5(B) with the interaction of 4 conventional hydrogen bonds, 2 pi-alkyl bonds and 1 pi-pi stacked bond (Table 2).

Table 2: Showing Binding energy, Dissociation constant, Number of hydrogen bonds and Contacting receptor residues of selected protein

Protein (PDBID)	Ligand	Binding energy (Kcal/ mol)	Dissociation constant (pM)	No. of Hydrogen bonds	Contacting receptor residues
AST	Curcumin (CUR)	9.423	123836.7	2	Val 18 Phe 19 Val 38 Gly 39 Ala 40 Arg 42 Gly 108 Gly 109 Thr 110 Leu 113 Trp 141 Asn 195 Asp 223 Ala 225 Tyr 226 Ser 256 Ser 258 Lys

(3II0)					259 Tyr 264 Asn 265 Arg 267 Phe 361 Arg 387
	Deltamethrin	8.838	332399.3	4	Val 16 Val 18 Phe 19 Gly 108 Gly 109 Thr

	(DLM)				110 Leu 113 Trp 141 Asn 143 His 144 His 190 Asn 195 Asp
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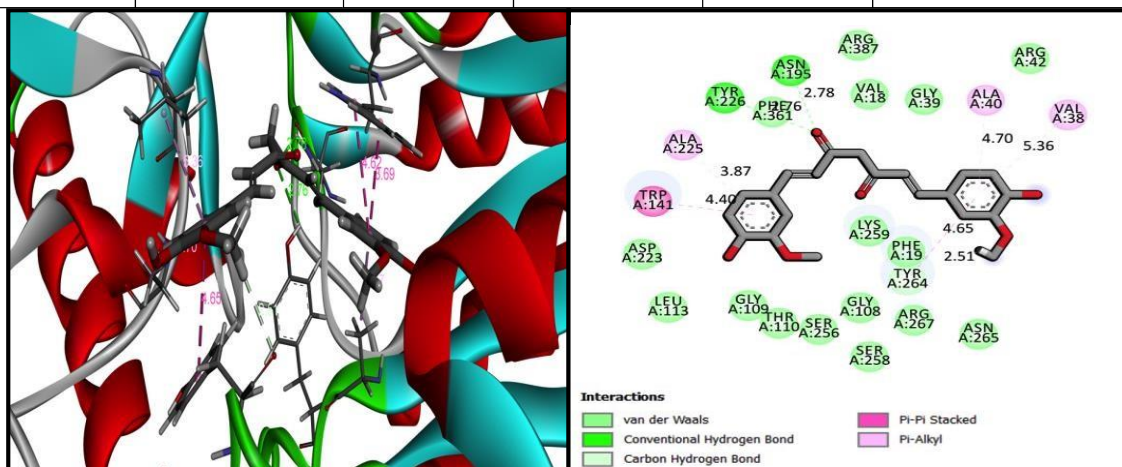


Figure 4(A) - Binding modes of Curcumin (CUR) with the Crystal structure of human glutamate oxaloacetate transaminase (GOT)/Aspartate transaminase (AST) (PDB ID: 3II0) (B) 2D representation of Receptor-ligand interactions

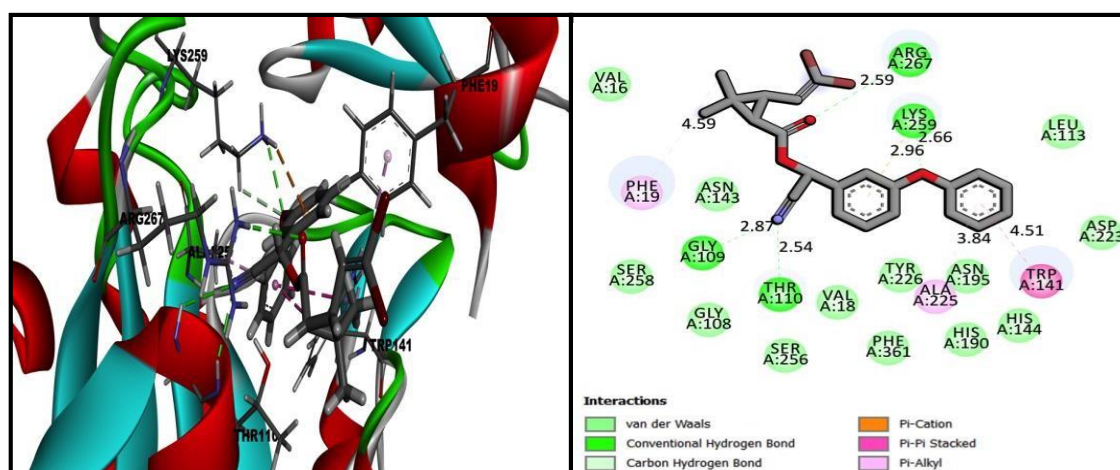


Figure 5(A) - Binding modes of Deltamethrin (DLM) with the crystal structure of human glutamate oxaloacetate transaminase (GOT)/Aspartate transaminase (AST) (PDB ID: 3II0) (B) 2D representation of Receptor-ligand interactions

DISCUSSION

Deltamethrin (DLM) is an α -cyano type 2 synthetic pyrethroid, widely used as an insecticide because of its short biodegradation period and low tendency to accumulate in organisms (Yonar and Sakin, 2011; Laskowski, 2002). In the literature, various reports have shown its toxicity on various organs and tissues in the mammalian system (Gohary et al., 1999, Muhammad and Richardson, 2011; Enan et al., 1996; Wu and Liu, 2000). Thus, the management of its toxicity is important for the health of general populations.

Various herbal products around the world are used as antioxidants. Curcumin is a much researched herbal medicine and acts as an antioxidant but the exact mechanism of action of curcumin is still unclear. In the present investigation, we have predicted the binding affinity of curcumin and deltamethrin towards liver marker enzyme, AST. Docking is frequently used to predict the binding affinity of ligands with its receptors. The molecular docking study indicated that Curcumin has greater binding affinity for AST as compared to deltamethrin. These results demonstrate that curcumin could play a vital role in the attenuation of DLM induced toxicity by effectively displacing its binding to liver enzymes. In support of this, Verma et al. (2016) have demonstrated a significant recovery in AST and ALT activity was observed when curcumin was administered simultaneously with β -cyfluthrin in Swiss albino mice. Further, curcumin has also been proved to work as a nutraceutical agent by helping to stabilize the activity of AST, ALT and ALPase in propanil treated rats (Otuochere et al., 2014) and nicotine intoxicated rats (Kalpana et al., 2005).

ROS are formed continuously in cells as a consequence of both oxidative biochemical reactions and external factors. However, they prove to be detrimental when generated in excess in certain conditions such as exposure to certain environmental toxicants such as pesticides. Under such exposure conditions, the endogenous antioxidant system may be unable to combat ROS formation and require supplementation by an exogenous antioxidant agent such as curcumin. Sankar et al. (2010) have demonstrated that concurrent curcumin treatment has a beneficial role in mitigating the immunotoxicological effects of cypermethrin in rats. Recent reports also suggest the protective effect of curcumin against arsenic-induced apoptosis in murine splenocytes (Khan et al., 2012). The *in silico* binding energy data obtained in this investigation validates the premise that curcumin effectively protects target liver enzymes from the inhibitory binding interaction of deltamethrin.

CONCLUSION

In the present study interaction profiles of liver marker, AST and its binding affinity with curcumin was analyzed through molecular docking. Results of the present study indicate that molecular docking of curcumin and deltamethrin with the AST exhibited stronger binding potential of curcumin as compared to deltamethrin. Based on the present investigation the ameliorative potential of curcumin is clearly attributed to its multi-faceted antioxidative, anti-apoptotic and restorative abilities against the DLM induced alterations in the action of the hepatic marker enzyme, AST. Molecular docking of this study reveals the plausible mechanism of interactions between DLM and AST as well as CUR and AST. Thus, based on this *in silico* study, it can be suggested that curcumin exerts promising nutraceutical effects against deltamethrin toxicity. Hence, this predicted mitigative action of curcumin, makes it an agent of choice in hepatotoxic conditions.

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