

Evaluation of the efficacy of Herbal antidotes of Viper snakes through In-Silico docking analysis of their bioactive components targeting phospholipase A2

Abstract:

Introduction: Snake bite is common in Rural areas. Many toxic snakes produce neurotoxic, Myotoxic and hemotoxic effects. Snake venom is rich in metalloproteinases and Phospholipases. Phospholipase A2 accounts for most of the toxic effects of Viper snakes. Many herbal antidotes has been in practice in Siddha system which lack scientific evidence.

Aim: This study aims to rule out the Binding pose and affinity of phytochemicals derived from certain herbs with the target by forming hydrogen bond so that the function of Phospholipases A2 with PDB – 2QOG would be hindered.

Materials and methods: 6 such phytochemicals were selected and In siico docking analysis was done with the target 2QOG. Andrographolide (49 ASP, 52 TYR), Aristolochic acid (49 ASP, 52 TYR), Genistein (49 ASP, 52 TYR) and Nimbolide (49 ASP, 52 TYR) revealed maximum of 2 interactions (50%) with the core active amino acid residues present on the target 2QOG. Piperic acid (48 HIS) and Linoleic acid (48 HIS) reveals 1 interaction with the core active amino acid residues present on the target 2QOG.

Conclusion: Based on the results it was concluded that the bio-active compounds Andrographolide, Aristolochic acid and Genistein present in the herbal ingredients possess significant binding against the target enzyme phospholipases A2.

Key Words:

Phospholipase A2, Viper snakes, Docking, Phytocomponents, Andrographolide, Aristolochic acid, Genistein, Piperic acid, Linoleic acid and Nimbolide.

1. INTRODUCTION

1.1 Snake venom:

Snakebite envenomation is an abandoned crisis seen worldwide, especially in rural, tropical, and subtropical zones. In 2009 the World Health Organization (WHO) acknowledged snake bites as a neglected disease in tropical areas. (1) Envenomation results in significant death, disability, and emotional morbidity. Snake venoms are a complex mixture of active molecules including

proteins and non-protein fractions of different sizes. (2) They consist of various group of amines, nucleosides, and carbohydrates. Among all the snake members of Elapidae, hydrophiidae and viperidae have been studied much. Chemicals present in venom affect a variety of cells and tissues. As a result they produce definite responses such as digestion of cell membrane and even the whole cell, interference of procoagulant and anticoagulant activities of blood cells, synthesis of oxidizing agents, collapse of tissue collagen and intercellular substances, damage of nervous tissue etc., Based on the response produced snake venoms are classified as neurotoxins, hemotoxins, cardiotoxins, cytotoxins and myotoxins. The venom of viperid family constitute as much enzymes as 90% and 25% in some elapids. Over 30 enzymes have been identified from snake venom so far (3).

1.2 Phospholipase A2:

The phospholipase A2 presents a high amount of disulphide bonds, low molecular weight of 13.5 k Dalton. Phospholipases are divided into five categories: 1) the secreted phospholipase A2 (sPLA2), 2) the cytosolic phospholipase A2 (cPLA2), 3) the Ca²⁺ independent phospholipase A2 (iPLA2), 4) the platelet-activating factor acetylhydrolase (PAF-AH), and 5) the lysosomal phospholipase A2 (IPA₂). The basic form of PLA₂ possess the most potent pharmacological effect causing tissue damage or toxicity (types of pla₂). In a single snake venom Phospholipase A2 is represented in many isoforms (4). Phospholipase A2 is present in many cell membranes. Thus phospholipase A2 present in venoms possess both exogenous and endogenous enzyme activity. As it is cytolytic in nature It facilitate the lysis of cell membrane of several cells including erythrocytes, which results in haemorrhage. Cell damage is caused as phospholipase A2 modifies the structure and function of cell membrane. It forms pores on the lipid bilayer of the cell membranes as a result of oligomerization. More than 523 plant species from 122 families were reported to have their neutralizing capacity against toxic snake venom. (5)

1.3 Herbal substitutes:

The secondary metabolites extracted from herbs play a vital role in neutralizing the toxic effects of the venom. Several studies have been done to prove the inhibitory potential of the herbal plants against the enzymes of snake venom. Therefore, there is a requirement for more studies on plant metabolites and the development of an antidote for the better treatment of snakebite. In Siddha Literature, plants such as *Indigofera tinctoria*, *Piper nigrum*, *Aristolochia bracteolata*, *Azadirachta indica*, *Andrographis paniculata*, *Cyanodon dactylon*, *Leucas aspera* etc., have been mentioned as antidotes against snake poisons. We selected 6 bioactive components from the above mentioned plants. This article focuses on the documentation of the binding pose and binding affinity of some herbal plants which were mentioned in Siddha literature as antidotes for snake bite through *In silico* Molecular docking study of some selected bioactive components of those herbs with Phospholipase A2 as a common target from the results of which the potency of those antidotes could be established further.

2.AIM & OBJECTIVE:

To rule out the Binding pose and affinity of Bioactive components from the selected plants with the core amino acids (His48, Lys49, Tyr52, and Asp99) of the target by forming hydrogen bond so that the function of Phospholipases A2 with PDB – 2QOG would be hindered.

3.Materials and Methods:

3.1 Preparation of Target:

Crystalline structure of the target protein phospholipase A2 was retrieved from protein data bank and protein clean-up process was completed(Table. 1)(Fig.1).Different orientation of the lead molecules with respect to the target protein was evaluated by the Autodock program and the best dock pose was selected based on the interaction study analysis.

Table. 1 : Preparation of Target

PDB	Name of the Target
2QOG	Phospholipases A2

Fig.1 3D- Structure of Phospholipases A2 (PDB) - 2QOG



3.2 Preparation of Ligand:

The 3 D structure of Andrographolide, Aristolochic acid , Genistein, Piperic acid, Linoleic acid and Nimbolide were downloaded. Hydrogen was added to the ligands. (Table .2)

Table .2 List of Bioactive components selected

Andrographis paniculata	Andrographolide(6)
Aristolochia bracteolata	Aristolochic acid(7)
Indigofera tinctoria	Genistein(8)
Piper nigrum	Piperic acid(9)
Cyanodon dactylon	Linoleic acid(10)
Azadirachta indica	Nimbolide(11)

Total of 6 bioactive lead compounds were retrieved from the herbal ingredients.

3.3 Docking:

Docking calculations were carried out for retrieved phytochemicals against target enzyme Phospholipases A2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (12). Affinity (grid) maps of $\times\times$ Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (13). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

4. Results:

Fig.2 Docking Pose

Fig 2.1- Andrographolide with Phospholipases A2 (PDB) - 2QOG

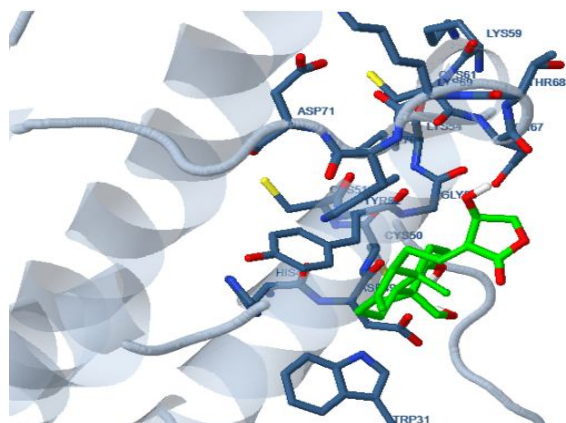


Fig 2.2 - Aristolochic acid with Phospholipases A2 (PDB) - 2QOG

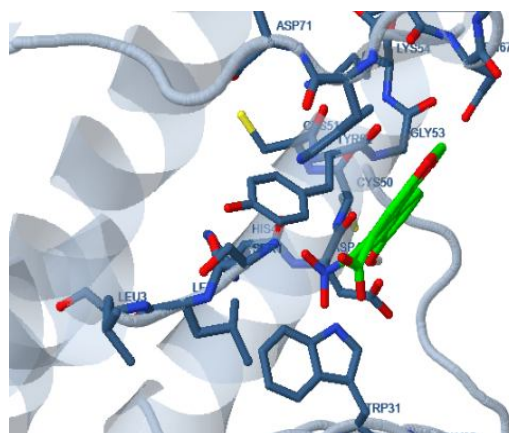


Fig 2.3 - Genistein with Phospholipases A2 (PDB) - 2QOG

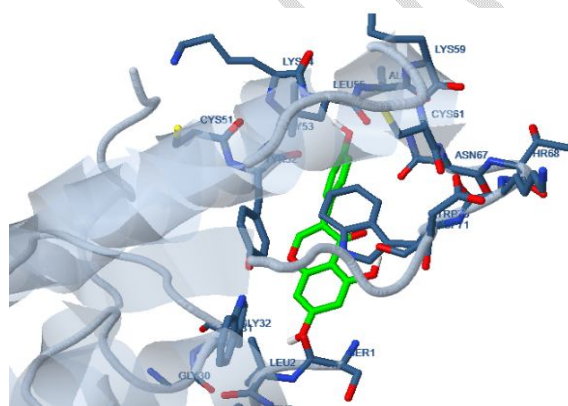


Fig 2.4- Piperic acid with Phospholipases A2 (PDB) - 2QOG

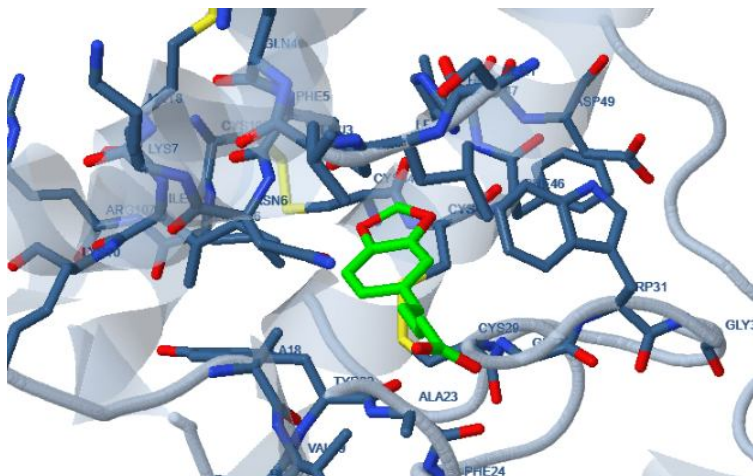


Fig 2.5- Linoleic acid with Phospholipases A2 (PDB) - 2QOG

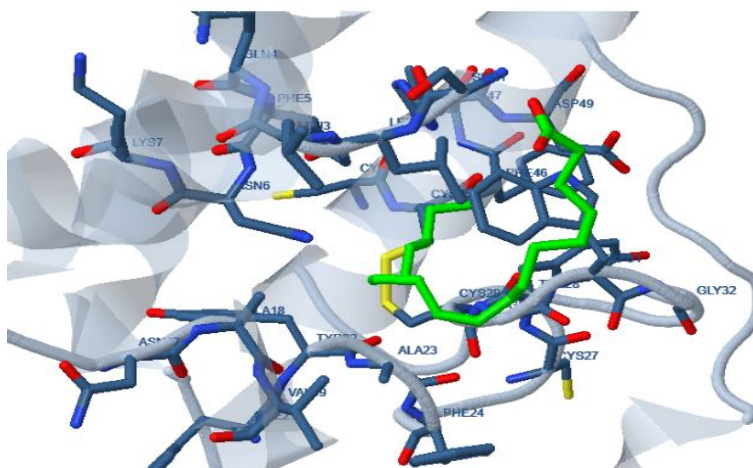


Fig 2.6- Nimbolide with Phospholipases A2 (PDB) - 2QOG

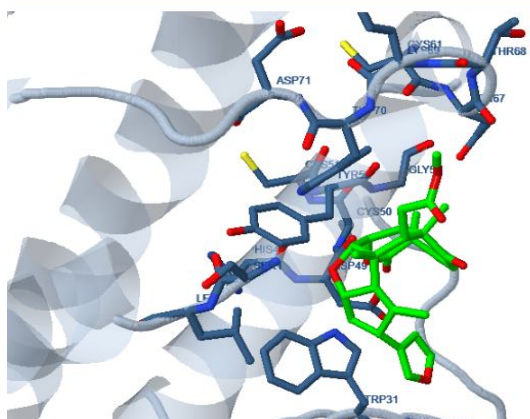


Table 3: Summary of the molecular docking studies of compounds against Phospholipases A2 (PDB) - 2QOG

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Andrographolide	-6.74 kcal/mol	11.55 uM	-0.26 kcal/mol	-7.48 kcal/mol	537.587
Aristolochic acid	-7.33 kcal/mol	4.26 uM	-1.42 kcal/mol	-8.29 kcal/mol	514.032
Genistein	-5.48 kcal/mol	96.33 uM	-0.19 kcal/mol	-5.97 kcal/mol	500.395
Piperic acid	-5.38 kcal/mol	112.96 uM	-0.26 kcal/mol	-6.27 kcal/mol	520.522
Linoleic acid	-6.45 kcal/mol	18.73 uM	-1.09 kcal/mol	-9.82 kcal/mol	703.306
Nimbolide	-6.21 kcal/mol	27.85 uM	-0.06 kcal/mol	-7.20 kcal/mol	611.343

Docking poses of various bioactive components of the selected herbal plants against Phospholipases A2 (PDB) - 2QOG in Fig.2. The docking results of the above mentioned bioactive components against the core amino acids of phospholipase A2 generated negative values for free energy -6.74 kcal/mol for Andrographolide -7.33 kcal/mol Aristolochic acid -5.48 kcal/mol for Genistein -5.38 kcal/mol for Piperic acid -6.45 kcal/mol for Linoleic acid and -6.21 kcal/mol for Nimbolide in the grid box, suggestive of high affinity for the binding pocket, with Aristolochic acid with the highest affinity.(Table .3) From reported data of the herbs, Andrographolide(49 ASP, 52 TYR), Aristolochic acid(49 ASP, 52 TYR) ,Genistein(49 ASP, 52 TYR) and Nimbolide (49 ASP, 52 TYR) present in the herbal ingredients reveals maximum of 2 interactions (50%) with the core active amino acid residues present on the target Phospholipases A2. Piperic acid (48 HIS) and Linoleic acid(48 HIS) reveals 1 interaction with the core active amino acid residues present on the target Phospholipases A2.

5. Discussion

Cytotoxins, phospholipases A₂, and metalloproteinases represent major classes of proteins found in snake venoms. Proteins and polypeptides make up approximately 95% of the

dry weight of the venom. (14). Many of these proteins are responsible for producing severe pathophysiological events following envenomation and therefore represent a significant hazard for snakebite victims. Crotoxin is one such major toxic constituent present in the venom of *Crotalus durissus terrificus*, a viper species. It is a complex protein having the basic subunit phospholipase A2, and the acid subunit crotoxin. (15), (16) phospholipase A2 (2QOG), component of crotoxin causes neurotoxicity. (17) Hence the crystal structure of Phospholipase A2 available with the PDB ID: 2 QOG is used for the current investigation.

As phospholipase A2 is cytolytic in nature it facilitates the lysis of cell membrane. It modifies the structure and function of cell membrane. It forms pores on the lipid bilayer of the cell membranes as a result of oligomerization that involves a His48–Asp49 pair in the catalytic site, which activates a structurally conserved water molecule, thereby initiating the nucleophilic attack on the sn-2 position of the substrate. (18)(19). The cells with lipid bilayers made up of only phosphatidylcholine (PC) are extremely prone to lipid hydrolysis by phospholipase A2, while lipid bilayers containing a more phosphatidylcholine and a less amount of acidic phospholipids were somewhat vulnerable to phospholipase A2-mediated lipid hydrolysis. Thus it ruptures the erythrocytes. (20)

Hydrogen bonds play an important part in the determination of the specificity of protein-ligand binding. (21) Hydrogen (H) bonds enhance various cellular functions by facilitating intermolecular interactions. The affinity between ligand and protein depends on the number of hydrogen bond acceptors than donors and also dependent on rotatable bonds. (22) Andrographolide, Aristolochic acid and Genistein possess a total number of 8 Hydrogen bonds each, whereas, Piperic acid has 5 hydrogen bonds, Linoleic acid has 3 hydrogen bonds and Nimbolide 7 hydrogen bonds.

His48, Lys49, Tyr52, and Asp99 are the core amino acids of the protein phospholipase A2. These amino acid residues are functionally responsible for activation of Neurotoxicity, myotoxicity and oedema. Thereby phytochemicals which inhibit the target enzyme Phospholipase A2 may occupy these active amino acid residues and be able to block the hydrophobic channel and prevent the binding of the fatty acid necessary for the toxin allosteric activation during snake envenomation and act as a potential therapeutic agent for management of snake bite.

Clinically, snake envenomation is very prevalent and contributes to a high mortality rate in developing countries. Moreover, it is disappointing that the lack of sufficient anti-venom supplies and clinics has not been addressed in developing countries. Hence, it is imperative that more funding and research is dedicated towards finding more efficient anti-venom and alternative treatments in order to significantly decrease the number of deaths due to snakebites. (23)

Siddha system has mentioned many herbal plants and compound medicines for various types of animal poisons. A vast information is given regarding the types of snakes, the symptoms of poisoning and their treatment plants. Many herbal plants have been mentioned as antidotes for various snake poisons which remain cost effective and easy available. *Andrographis paniculata* is traditionally used for venomous bites in various parts of Asia(24). In a previous study *Andrographis paniculata* along with immunotherapy was used against *Naja naja* snake venom. It markedly increased the average time of survival and mortality rate when compared with control mice which received only immune therapy.(25) Aqueous extract of *Aristolochia bracteolata* was found to have 80% inhibition of Toxic effect caused by Russell's viper and cobra venom. From the same study it was proven that Aristolochic acid actively inhibits phospholipase A2 (26). Extract of *Indigofera tinctoria* was found effective in treating skin lesions caused by viperine snake bite.(27). Leaf paste of *Azadirachta indica* was used against viper snake venom.(28) *Piper nigrum* was an effective antidote in cobra poisoning (29). *Cyanodon dactylon* is being used in the treatment of snake bite in Chengalpet district. (30).

This work is to repurpose those ideas of using herbal antidotes which are more even safer. The need to bring equally or more potent alternatives in the form of plant derived drug is very essential as they are much safer. Among the chosen ligands Andrographolide, Aristolochic acid and Genistein present in the *Andrographis paniculata*, *Aristolochia bracteolata* and *Indigofera tinctoria* respectively, reveals maximum of 2 interactions (50%) with the core active amino acid residues present on the target Phospholipases A2. Although these Antidote properties are appreciable *in silico*, further studies of *in vitro* and clinical studies dealing with phospholipase A2 of snake venom should be considered for further studies.

6. Conclusion:.

Based on the results of the computational analysis it was concluded that the phytochemicals Andrographolide, Aristolochic acid and Genistein present in the herbal ingredients possess significant binding against the target enzyme phospholipases A2. Thereby phytochemicals which inhibit the target enzyme Phospholipases A2 may occupy these active amino acids and could be able to block the hydrophobic channel and prevention of the binding of the fatty acid necessary for the toxin allosteric activation during snake envenomation and act as a potential therapeutic agent for management of snake bite. Thus *Andrographis paniculata*, *Aristolochia bracteolata* and *Indigofera tinctoria* could be considered to possess effective antidote property in viper snake envenomation. However further studies involving docking of all the phytochemicals in these herbs would help in establishing complete documentation.

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