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Herbal antidotes: A possible replacement for serum antivenom with effective lead compound using bioinformatics tools

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ABSTRACT

Anti snake venom serum is the only rescue method in health centerswhen there is a fatal snake bite condition. And these anti venoms derived from animal tissues are not properly designed so they have a lot of side effects like anaphylaxis and even a high dose can be fatal. So instead of serum anti venom, various plant products can be used which have less side effects. These plant extracts must be tested in silico with their structure for effective proper binding with the target venom protein. These herbal antidotes must be designed in such a way that it can target the venom protein. To know the exact lead compound which can act upon that protein, Bioinformatics tools are used as a starting point for in silico lead compound development. Toxic protein are downloaded from PDB site and are docked with probable lead compound and based on the docking respective lead compounds are screened. This is an attempt to show the probable lead compounds extracted from plants which can act upon various venom proteins and can be a probable cure for wide ranges of snake bites.

1. Introduction

More than 2,000,000 snake bites are reported in the India, and it is estimated that >50000 people die of snakebite each year. Still it is a surprise that snake bite poisoning is seldom mentioned as a priority for health research in the developing country like India (Barma *et al*., 2014) Snake envenomation is an important global health issue. Snakebite is declared as a "Neglected Tropical Disease" by the World Health Organization. As a result, this may be considered as a matter of global health concern for the people in general and the rural communities of the developing countries in particular. It constitutes an occupational hazard especially in field of agriculture for farmers, farm labours, villagers, migrating population and hunters. It is a major health hazard that leads to high mortality and great suffering in victims. Highest incidence and mortality due to snake bites is reported from South and Southeast Asian countries having extensive agricultural practices and diversity in snake specie (Gupta & Peshin, 2014). There are more than 3000 known species of snakes of which around 300 are poisonous. In India out of 216 species, approximately 53 are poisonous. It is estimated that in India alone, there are more than 2, 00,000 venomous bites per year, of which 35,000-50,000 are fatal. The estimates are arbitrary as majority of cases goes unreported. In rural areas, where most of the bites take place, the victims are mostly taken to traditional healers, who neither report them to the authorities nor document the cases, hence paucity of reliable epidemiological data. The factors mainly responsible for high mortality associated with scorpion bite are poor health services, difficult and untimely transportation facilities, wrong traditional beliefs, delay in anti-snake venom administration.

2. Snake venom proteins

Snake venoms are complex mixtures of small molecules and peptides/proteins, and most of them display

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certain kinds of bioactivities. They include neurotoxin, cytotoxic, cardio toxic, myotoxic, and many different enzymatic activities (Chan *et al*., 2016). Snake venom toxicity is due to the cumulative effect of various toxins present in the venom. Although most toxins are active individually, they will exert synergistic effects in combination. For example, neurotoxic components from different families of proteins act in concert and block the neuromuscular transmission. Venoms from Viperidae, Crotalidaecontains serine-proteases, hemorrhagins (fibrinogenases possessing high anti-thrombotic activity), fibrinolytic activators, metalloproteases and group II PLA2 isoenzymes, as well as non-enzymatic proteins (C-type lectins, CRISP and disintegrins) that activate or inhibit coagulant factors or platelets, or disrupt the endothelium.

Phospholipase A2 (PLA2) is probably the most thoroughly investigated toxins both in hemotoxic and presynaptic neurotoxic snake venoms. PLA2 has also been classified as a presynaptic neurotoxin, identified in the venoms of *Crotalidae*, *Elapidae*, *Hydrophiidae*and *Viperidae*snakes. PLA2 are ubiquitous intra- and extra-cellular enzymes hydrolyzing glycerophospholipids at the *sn*-2 position of the glycerol backbone releasing lysophospholipids and fatty acids, in turn arachidonate metabolites control inflammation and pain. PLA2 are responsible for the local inflammation following *viperid* snakebite envenomation. Though PLA2 has happened to be the most important compounds in major snake bite issues, it can be taken as the effective target protein.

3. Phospholipase A2 (Pla2) from *naja naja* (common cobra) venom

Cobra venom is rich in postsynaptic neurotoxins called alpha-bungarotoxin and cobratoxin (Fig. 1). Cardio- toxin content of cobra venom has direct action on skeletal, cardiac, smooth muscles, nerves and neuromuscular junction causes paralysis, circulatory, respiratory failure, cardiac arrhythmias, various heart block and cardiac arrest because the venom releases calcium ions from the surface membrane to the myocardium (Doley & Kini, 2009). Cobra venom is of smaller molecular size and rapidly absorbed into circulation. Cobra venom binds especially to Ach receptors, prevents the interaction between Ach and receptors on postsynaptic membrane result in neuromuscular blockade.

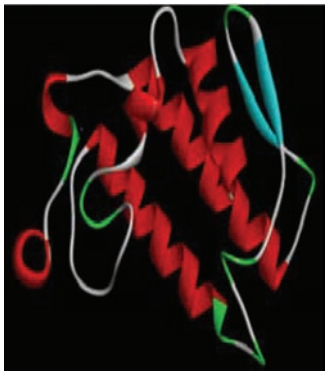

Fig.1: Three dimensional structure (PDB ID-IA3D) of Phospholipase A2 (PLA2) from *Naja naja* (Common Cobra) venom

4. Phospholipase A2 from common indian krait venom (PDB ID-1TC8)

Common Indian krait venom contains both presynaptic beta bungarotoxin and alpha bungarotoxin (Fig. 2). These toxins initially release Ach at the nerve endings, at neuromuscular junction and then damage it subsequently preventing the release of Ach. Irrespective of Krait, its venom is 10 times more lethal than cobra. But unfortunately unlike as in cobra bite, the victim reports too late due to delayed clinical manifestations. It injects the venom into skin or skin deep.

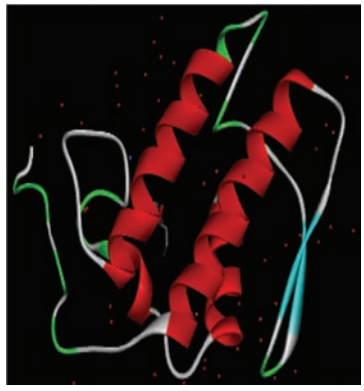

Fig.2: Three dimensional structure (PDB ID-ITC8) of Phospholipase A2 from Common Indian Krait venom

5. Phospholipase A2 from russel viper venom (PDBID-1VIP)

Viper venom interferes with blood clotting. Venoms contain serine proteases, metalloproteinases, C-type lectins, disintegrins, and phospholipases and it exhibits both anticoagulant and procoagulant effects on blood clotting mechanism resulting in defibrination syndrome or disseminated intravascular fibrino-coagulopathy (Bernard *et al*., 2001). Russell's venom is a rich source of enzymes that activates factor X to convert prothrombin to thrombin in presence of calcium factor V and platelets (Fig. 3). Haemorrhagins-1, 2 and metallo-endopeptidase causes acute rapid bleeding in brain, lungs, kidney, heart, and gastrointestinal tract. It causes severe vasoconstriction followed by vasodilatation of the microvessels.

6. Anti snake venom serum

The most effective antidote against snake venom is the anti snake venom. It is usually pepsin refined F (ab) fragments of IgG purified from the serum or plasma of a horse or sheep that has been immunized with the venom of

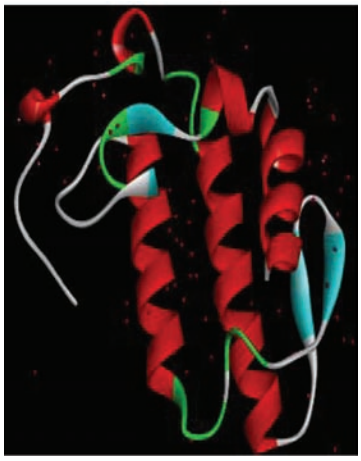

Fig.3: Three dimensional structure (PDB ID-1 VIP) of Phospholipase A2 from Russel Viper venom

one or more species of snakes (Kumarapppan *et al*., 2011). ASV neutralizes the venom of a particular species (monovalent/ monospecific) or various different species (polyvalent/polyspecific). The antibodies against a particular species may also neutralize the venom of a closely related species (paraspecific activity). In India, horses are hyper immunized against the venom of four common poisonous snakes the "Big Four" (Cobra, Krait, Russell's viper and Saw-scaled viper), to produce polyvalent anti snake venom.

7. Antivenom treatment of snake envenomation and its limitation

Antivenomes, in most countries are costly and may be in limited supply. Antivenomes for therapeutic use are often preserved as freeze-dried ampoules, but some are available only in liquid form and must be kept refrigerated. The majority of snake antivenomes are administered intravenously. The intramuscular route has been questioned in some situations as they are not uniformly effective. Antivenome should be given as quickly as possible so that the venom's side effects can be managed. It should be given only if the range of specificity is stated which includes the species known or through to have been responsible for the bite. Liquid antivenome that turned opaque should not be used because precipitation of protein indicates loss of activity which is directly proportional to increased risk of reactions. There are some critical issues with ASV. The main issues with ASV in actual clinical practice are species specificity, difficulty inavailability, affordability and ideal storage conditions. One of the principal drawbacks of the immunotherapy is the issue of specificity. There is a huge species variation with current taxonomy identifying one, four and eight species of Russell's viper, cobras and kraits, respectively. Two subspecies of saw-scaled vipers have also been identified. Russell's viper venom has also shown regional variation. So the variable composition and antigenic reactivity of the venom restricts the use of a particular ASV to a geographical area withrelevant specificity. Moreover, ASV cannot be raised against all species because the literature on distribution and diversity of venomous species is scarce. The concept of "Big Four" restricts the development of an effective ASV. Venom variation, low potency, bites by otherspecies could be responsible for the reported failure of polyvalent ASV in countering the venom effects in India.The liquid form requires cold chain. The production of monovalent ASV is a costly affair. In India the monovalent ASV is not produced. The other drawbacks with ASV therapy are the adverse reactions ranging from early reactions (pruritus, urticaria) to potentially fatal anaphylaxis. Few cases may also develop serum sickness. Endotoxin contamination could also lead to pyrogen reactions.Side effects of anti-venom therapy are anaphylactic reaction (difficulty in breathing and swallowing; hives; itching, especially of feet or hands, reddening of skin, especially around ears, swelling of eyes, face, or inside of nose, unusual tiredness or weakness, sudden and severe), serum sickness (enlargement of the lymph glands, fever, generalized rash and itching; inflammation of joints), pyrogen reaction-probably due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyper-immune anti-venom.

8. Herbal antidote: A possible choice

Thus, plants can be a possible choice as they have fewer side effects and have capacity to respond wide range of venom molecules. plant species were found to possess different herbal compounds (acids, alkaloids, steroids, enzymes, peptides, pigments, glycoproteins and glycosides, phenols, pterocarpanes, tannins, terpenoids, quinonoid xanthenes and other compounds) which are effective against snake envenomation by neutralizing different enzymes and toxins (procoagulent enzymes, haemorrhagins, cytolytic or necrotic toxins, phospholipases A2,B, C, D, hydrolases, phosphatises, proteases, esterases, acetylcholine esterase, transaminase, hyaluronidase, phosphodiesterase, nucleotidase, ATPase and nucleosidases) in venoms. Due to inadequate health care facilities especially in rural areas of India, people largely depend on alternative treatment by traditional healers who have knowledge based on ancient culture, ethnic practices and herbal antidotes (Makhija & Khamar, 2010). The plant kingdom provides an inexhaustible source of various herbal compounds with pharmacological potential which hold the key to antivenin activity. The plant kingdom has tremendous resources which have been thoroughly exploited by ethnic tribes in India.

Various phytochemicals with enzyme inhibiting and protein binding properties, active against snake envenomation include flavonoids, polyphenols, saponins, tannins,

terpenoids, xanthene etc. Phenolic, especially polyphenols, like some tannin bind proteins and act directly on venom components. They could also competitively block the receptors. Flavonoids like myricetin, quercetin, amenthoflavone have antihemorrhagic potential. Ursolic acid commonly found in many medicinal plants has enzyme inhibitory activity. Gallic acid (3,4,5-tri-hydroxy benzoic acid), on testing against the local toxicity of *Daboia russelli* venom and its purified hemorrhagic complex, showed inhibition of in-vitro proteolytic activity of both venom and hemorrhagic complex, without inhibiting phospholipase activity of venom. In-vivo experiments, showed inhibition of hemorrhage, edema forming, dermo- and myonecrotic activities of both the venom and the complex. 2- hydroxy-4-methoxy benzoic acid, salicylic acid and p-anisic acid have shown neutralization of phospholipase A2 activity of banded krait, which was superior to ASV neutralization. The terpenoids from the plant have antiproliferative effects and certain phenolic glycoside derivatives have demonstrated enzyme inhibitory activity against venom. Triterpenoids from the root extract of *Emblica officinalis* and *Vitex negundo* are suggested to significantly neutralize the venom induced effects of *Vipera russelii* and *Naja kauothia*. There is a huge collection of Indian medicinal plants used for treating snake bites.

9. Virtual screening of lead compound

Today *in silico* approaches have gained immense popularity and have become an integral part of the industrial and academic research, directing drug designing and discovery. However, bioinformatics tools have seldom been applied for analysis of plant-based medicines used in traditional systems of health care, particularly to find out anti-venom from phytochemicals (Fig. 4 & 5). Recently, efforts in this line have been made; for example, Pithayanukal *et al*. (2009) made an attempt for *in vitro* and molecular docking studies for revealing the anti-snake venom activity of seed kernel extract of *Mangifera indica* L. cv. 'Fahlum' (Thai mango). Prashar *et al*. (2015) studied anti-snake venom activities of ethanol and aqueous extracts of *Cassia hirsuta* against Indian cobra (*Naja naja*) venom induced toxicity. The species of medicinal plants having inhibitory properties against snake venoms has also been reviewed by Soares *et al*. (2005).

Phospholipase A2, a hemolytic protein which is found in the venom of all the five common poisonous snake species in India was selected as a target protein and plant derived molecules such as 2-dihydroxy 4-methoxy benzoic acid from *Hemidesmus indicus* R. Br. and 5-hydroxy 7,8-dimethoxy flavon from *Andrographis paniculata* (Burm. f.) Wall. ex Nees were selected as the ligand molecules and docking of these molecules were done (Nisha *et al*, 2014). The results indicate that both the plant derived compound have significant inhibitory effect. It is noteworthy to note that the crude root drug of *H. indicus* was reported to have anti-viper venom activity (Alam *et al*., 1994) but the docking results between Phospholipase A2 isolated from the five different snake species [*O. hannah Cantor* (King Cobra) - PDB ID : 1GP7, *N. naja L.* (Indian Cobra) - PDB ID :1A3D, D. *rusellii* Shaw & Nodder (Russell's Viper) - PDB ID : 1Q7A, *B. caeruleus* Schneider (Krait) - PDB ID: 1DPY and *E. carinatus* Schneider (Saw-scaled Viper) - PDB ID : 1OZ6] with 2-hydroxy –4-methoxy benzoic acid, the active compound from *H. indicus* revealed that the compound has more inhibitory effect to Indian Cobra venom. These investigations indicate the possibility of the discovery of novel lead molecules through virtual screening of plant derived drug molecules.

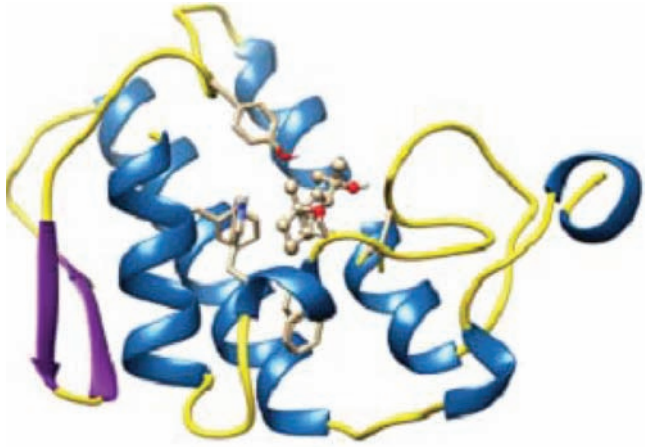

Fig. 4: Proximadiol isolated from *Acorus camalus* L. docked with cobra venome phospholipase A2 (PDB ID IA3D)

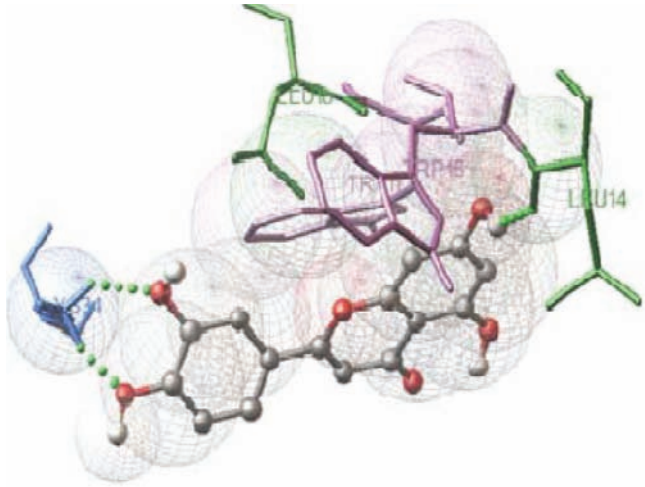

Fig. 5: Molecular interaction of Firamperemophillane reported from *Vitex negundo* L. with the active site residues of Serine protease in Cobra venome; hydrogen bonds are represented in green dots.

10. Conclusion

The use of plant extracts and isolated chemical compounds as antidotes for snake venom is a common practice in places where a prompt access to serum therapy is lacking, and is also used as a supplemental alternative to conventional antivenom serotherapy. Although there are a number of reports on plants from different geographical areas that are able to neutralize snake venoms, only a few chemical compounds have been isolated and identified as active components. In reviewing this area of research, one comes to the conclusion that many plants recorded as anti-snake venoms in popular use may display antidotal properties due to the great number of active compounds they contain. The isolated plant components or their mixtures offer a great potential to complement serotherapy, once their inhibitory effects and action mechanisms are explored and fully characterized. Screening studies of venom inhibition by vegetal extracts and components, which are conveniently performed using *in vitro* or pre-incubation assays, should be complemented by *in vivo* tests that more adequately evaluate their therapeutic potential as alternative or supplemental drugs against snake bites. The active principles of plants structurally resemble mammalian secondary metabolites and this similarity is the basis for their physiological action. The anti-venom activity of plant extracts may be due to the presence of enzymatic inhibitors, chemical inactivators or immune-modulator principles. The efficiency of the vegetal species as inhibitors of the toxic and pharmacological action of snake venoms may be attributed to the presence of multiple factors. The identified active substances are mostly low molecular weight compounds that exhibit more than one biochemical/pharmacological property in addition to antidotal effect. Further studies on the isolation, structural characterization and action mechanism of these natural inhibitors must be carried out in the future. Snake venom inhibitors from plants may become helpful alternative or supplemental tools for the treatment of envenoming, as well as important leads for the synthesis of new drugs of medical interest.

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