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Targeting Mangrove Species as an Alternative for Snake Bite Envenomation Therapy with Special Reference to Phospholipase A_2 Inhibitory Activity: A Mini Review.

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ABSTRACT

Owing to the persistence of a constant annual mortality rate caused by snake bites, the present mini review inspects the prospect of using folklore knowledge involving the use of plants as an alternative to snake bite envenomation treatment. It also tries to magnify the demand of awareness regarding the knowledge of snake venom composition, essentiality of targeted enzyme (PLA_2, Phospholipase A_2) inhibition and the bizarre side effects associated with the use of antivenom serum. Thus, it emphasizes on the need for exploring the biological activities as well as the chemical constituents of the unexplored mangrove species, the abundant availability of which makes them desirable for scientific exploration. Upon revelation can be harnessed for the future development of potent PLA_2 inhibitor hence, a potential antivenom drug.

Keywords: Snake Venom, Phospholipase A_2, PLA_2 Inhibition, Antivenom Serum, Folklore, Mangroves.

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INTRODUCTION

India might be the second largest populated land on earth but it definitely is not the home for the largest number of snakes in the world rather it quixotically shares the burden of the fact that at least 35,000 to 50,000 Indians die each year due to snake bites as estimated by the World Health Organization. This theory was better supported by the path breaking project named "The Million Death Study" [1] where researchers collaborated from different nations including Canada, the UK and India. As a concluding remark a very striking statement was made which says that for every two people who succumb to AIDS in India, there is one snake bite fatality. However, the cause of death due to snake bite can be accounted to variable reasons like lack of awareness among people regarding treatment resulting in overdose of antivenom, unavailability of proper antivenom in remote areas and also the fact that there are geographical variants of the same species of snake making it an extremely vulnerable reality that the potency of anti venom serum is certainly expected to vary likewise [2]. And as such the statement of Dr. David Warrell (University of Oxford, UK) can rightly be utilized to conclude that snakebite is indeed the most neglected of all the neglected tropical diseases of the 21st Century.

Anti-venom therapy and its side effects

Dr. Albert Calmette (1895) first developed what we call today as the anti venom serum [3]. Antivenom is usually generated by hyper immunizing animals like horse or sheep with venom from a single snake species (monovalent) or several different species (polyvalent) at the same time [4, 5]. In response to this the animals undergo an immune response that produces antibodies which is then harvested from the animal's blood and are used to treat envenomation. Use of potent immunoglobulin is definitely the effective method of snake bite envenomation treatment but it is also associated with some fatal side effects [4, 6-8]. These may include early anaphylactic reactions (urticaria, dry cough, tachycardia, bronchospasm, reddening of skin) [9, 10] pyrogenic reactions (arterial hypotension, rigor, vasodilatation, fever, chills) and late reactions or serum sickness (fever, itching and rash, swelling of joints, neurological impairments) [5, 11-13]. And as such the fatal impact of anaphylaxis can be considered as the major hurdle in treatment using antivenom [11] thereby creating an obvious need to look for alternative therapy for treating snake bite envenomation.

Snake Venom Composition

One of the most important factors that govern envenomation treatment is the specificity of inhibition which in turn depends on the constituent of snake venom we are targeting. Snake venom as most assumed is not a single toxin, it is a complicated mixture [14] of various ingredients like: enzymes mostly hydrolytic (Phospholipase A_2: major component found in all snake species), L-amino acid oxidase, metalloproteinases (hemorrhagins) [15, 16], toxin proteins (particularly neurotoxins, polypeptide toxins like cytotoxins), non-toxic proteins, metals, lipids, nucleosides, carbohydrates, amines etc [17]. Each one of these is known to have an individual impact and usually varies from species to species. A quintessential investigation is thus required to be conducted to screen small molecules that are capable of selectively inhibiting the action of any of these components.

The studies can howsoever be initiated with the enzyme Phospholipases A_2 due to its ubiquitous presence in snake venom composition and also since it plays important role in several biological processes [18].

Why Inhibit Phospholipases A_2 (PLA_2)?

PLA_2 is a superfamily of lipolytic enzymes which recognizes the sn-2 ester bond of glycerophospholipids and catalyses the hydrolysis of the ester bond resulting into the release of fatty acid (arachidonic acid) and lysophospholipids [19-21]. This superfamily comprises of a broad range of enzymes consisting of almost 15 groups [22]. However, it basically can be categorized into four major classes or types: secreted PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), Ca^{2+} independent PLA2 ($iPLA_2$) [23] and lipoprotein associated PLA_2 ($lp\text{-}PLA_2$).

The secreted PLA_2s were the first discovered among the PLA_2s. This is the extracellular form of PLA_2 which is commonly found in snake, bee [24], scorpion or wasp venoms, mammalian tissues (pancreas and kidneys) [25, 26], arthritic synovial fluids [27-29] as well as bacteria.

In recent times, $sPLA_2$s are subjected to extensive studies including crystallographic studies [30] owing to the fact that $sPLA_2$s promote inflammation [31] in mammals by following the basic hydrolysing mechanism as that of the PLA_2s resulting into the formation of fatty acids including arachidonic acid. This arachidonic acid liberated helps in the biosynthesis of eicosanoids [32] to form several inflammatory and thrombogenic molecules in mammals. In sum, targeting and inhibiting $sPLA_2$ is an effective therapeutic strategy [33] for combating sPLA2 mediated inflammatory responses caused due to snake bite envenomation.

Folklore medicine as an alternative treatment

Folklore and traditional medicines have been a part of snake envenomation treatment since hundreds of years in India. When the Indian subcontinent was invaded by Alexander the Great in 356 B.C, he was highly impressed by the treatment procedures adapted by the Indian physicians especially for the treatment of snake bites [34]. There are also mentions of the use of different types of plant species in the works of Charaka and Sushrutha [2], the Atharva and the Rig Vedas [35].

Thus, being the cultivar of such vast traditional knowledge attempts should be made to investigate the plants that are so far being cited for use in snake bite treatments. So far, fairly a large number of plant species [36-43] have been identified to be used for envenomation treatment. But in spite of the availability of such vast pool of ethno-pharmacological information only very few of them have been subjected to proper scientific investigations to determine their anti-venom activity and also to isolate and identify the bioactive compounds exhibiting inhibitory actions. However, following are listed very few among the numerous number of Indian plants (**Table 1**) that have been subjected to PLA_2 inhibition studies at molecular level based on ethnomedical approaches.

Why mangroves should be explored?

Mangroves [77] have constantly been a source of amusement to both layman as well as the scientific community. Most among those who are acquainted with the concept of mangrove consider it as a dense and dangerous swamp or wetland forest located along the coast whereas the later half targets it as an excellent avenue of exploration. Firstly, the reason being that these plants grow under extreme environmental conditions like high concentration of moisture and salt, low and high water tides [78], simultaneously they also provide a good platform for studying their ecosystem since they grow in an area where there is a continues flush of saline and fresh water.

The second reason is that various species of mangroves are reported to have been used in folklore medicine and few of them have recently shown activity again various human [79-85] and plant pathogens [86, 87]. This implies that further more investigation might lead us to some important revealing of pharmacological value.

In addition to these, surveys conducted among the locale of people residing in and around the mangrove forest area and few recent investigations highlight the use of extracts from different mangrove species as an astringent, expectorant, haemostat, styptic, tonic etc. Many of them are even locally used for the treatment of diseases and disorders like diarrhoea [88, 89], dysentery, fever, elephantiasis, skin wounds, haemorrhage [90], inflammation, jaundice, kidney stones, lesions, malaria [91], sore throat, toothache, ulcers [92] and snakebites [78].

Their healing properties and vast availability makes them the most justified subject of study for exploration of their chemical compositions and their contribution in several treatment purposes, especially, in snake bite envenomation therapy. Following (**Table 2**) are few reported mangrove species that are being traditionally used for the treatment of snake bites in India.

CONCLUSION

Science has always witnessed the contribution plants had made in providing novel metabolites that are very unique and specific to the environment. Besides, it is also an undeniable fact that natural products or products that are both synthetically or semi-synthetically derived from natural products have played an undaunted role in the process of drug discovery and development. So, it is quite expected to consider carrying out investigation into the knowing of plant phytochemistry especially the mangroves which lies beneath the sheath of mystery. Also, India is the home for the largest and the second largest mangrove forest in the world which is an added source of gratification. Very recently basic level research is being carried out on some mangroves, which are revealing. This clearly manifests the idea that further more veracious and indulging scrutiny of knowing the chemical constituents of these species is not only desirable for coming up with novel therapeutic small molecules but because such findings might guide the way for those who are extremely keen to highlight the importance of folklore remedies for snake bite envenomation treatment.

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Table 1

Botanical Name	Family	Extract	References
Acalypha indica L.	Euphorbiaceae	Leaf	[44]
Aloe vera (L.) Burm. F. *Anacardium occidentale* L.	Xanthorrhoeaceae Anacardiaceae	Leaf Bark	[45] [46]
Andrographis paniculate (Burm. F.) Wall. Ex Nees	Acanthaceae	Whole Plant	[47-49]
Aristolochia sp.	Aristolochiaceae	Purified Compound	[50]
Aristolochia indica L.	Aristolochiaceae	Whole Plant	[48, 49, 51, 52]
Aristolochia radix	Aristolochiaceae	Whole Plant	[53]
Azadirachta indica A. Juss.	Meliaceae	Leaf	[54]
Betula alba L. *Calotropis gigantean* (L.) R. Br.	Betulaceae Apocynaceae	Leaf Latex	[55] [56]
Curcuma longa L.	Zingiberaceae	Legume	[57, 58]
Emblica officinalis Gaertn.	Euphorbiaceae	Root	[39, 59, 60]
Hemidesmus indicus (L.) R. Br.	Apocynaceae	Root	[61-64]
Mangifera indica L.	Anacardiaceae	Stem Bark	[65]
Mimosa pudica L.	Fabaceae	Root	[66, 67]
Morus alba L.	Moraceae	Leaf	[68]
Pluchea indica (Less)	Asteraceae	Root	[69, 70]
Pouzolzia indica (L.) Gaudich	Urticaceae		[71]
Tamarindus indica L.	Fabaceae	Seed	[72]
Vitex negundo L.	Lamiaceae	Root	[59]
Vitisvinifera L.	Vitaceae	Seed	[73, 74]
Withania somnifera (L.) Dunal	Solanaceae		[75, 76]

Indian plants reported to exhibit PLA_2 inhibitory activity.

Table 2

Botanical Name	Family Name
***Acanthus ebracteatus* L.**	Acanthaceae
***Acanthus ilicifolius* L.**	Acanthaceae
Avicennia ebracteatus	Avicenniaceae
***Lumnitzera racemosa* Willd.**	Combretaceae
***Nypa fruiticans* Wurmb.**	Arecaceae

Mangrove species that are traditionally being used for treating snake bite in India
Source: [78]

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