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Review Article

Phyto-Peptides as Anti-Snake Venom Agents

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Abstract: About 5.4 million snakebites occur each year, resulting in 1.8 to 2.7 million cases of poisonous envenomation. Most of these occur in Africa, Asia and Latin America. Application of medicinal plants with anti-snake venom activities might be useful in treating victims of snakebites, which is particularly important in rural areas where antivenins are not readily available. However, antivenins have some disadvantages, thus limiting their efficient use. Therefore, complementary therapeutics needs to be investigated, with plants being considered as major sources. Interest in bioactive peptides (BPs) has considerably increased in the last decade. Apart from the better characterized glycoproteins from snakes and mammals, which exhibit inhibitory properties against toxins from snakes and their toxic properties, there are reports on newly described peptides isolated from medicinal plants reported to be active against snakebite envenomation. The presence review has been focused on some of the isolated, purified and characterized phyto-peptides with anti-snake venom properties. These peptides includes; RW-12, WSG, β-Tumerin, Tumerin, BGS-Haridrin, Snakin-Z and MP-4. However, it is important to note that the presence of antisnake venom proteins and other compounds in plants opens the possibility to search for natural inhibitors of snake venom toxin from plants for therapeutic purposes. It is likely that other plants shown to be effective at neutralizing the toxic properties of various snake venoms, may also serve as sources for antivenoms that could be used in the future as potent alternatives to serum-based antivenins.

Keywords: Snakebite, Envenomation, Medicinal plants, Peptides, Antivenin.

1. INTRODUCTION

Snakebite till date remains a public health hazard, and global health statistics for this incidence and their severity remain unknown or misunderstood. In spite of the lack of data, a global estimation of the number of ophidian accidents reaches over one million cases per year, accounting for more than 20,000 deaths, especially along rural areas in Asia, South America and Africa. In addition to mortality, these envenomations are also a public health concern as a result of the chronic morbidity associated with them (e.g. amputations, deformations and renal failure), which causes significant social and economic impact (Sani, I. *et al.*, 2018). In Nigeria, snakebite remain a common and serious problems especially in rural areas where access to prompt and effective treatment is limited. The tropical climate and the favourable environmental factors within this region are known to provide suitable habitat for snakes. In addition, the society is largely agrarian and the greater part of its population engaged in farming activities, livestock rearing, hunting and collection of firewood. These activities constitute occupational hazard for Snakebites and are responsible for sustaining the high burden of the problem (Sun, M., & Zigma, S. 1978).

The treatment for Snakebite is as variable as the bite itself; the only available treatment is the usage of serum-based antivenom. Antivenom binds to and neutralizes the venom, stopping further damage, but do not reverses the damage already done. Unfortunately, the conventional anti-venoms currently available are not only expensive, but do not effectively neutralize venom-induced toxicity. Some of the anti-venoms cause allergic reaction in patients (Ries, N.L., & Dart, R.C. 2005).

In almost all parts of the world where venomous snakes occur, numerous plant species are used as folk medicine to treat Snakebite. Topical application of the plant or its sap on to the bitten area, chewing leaves or barks or drinking plant extracts or decoctions or injecting the extracts are some procedures intended to counteract snake venom toxicity

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(Saha, A. *et al.,* 2006). Several studies have been reported on isolation, purification and characterization of active biomolecules from plants, which are effective inhibitors in reducing the local tissue damage and toxicity of snake venom (Girish, K.S., & Kemparaju, K. 2005).

Though there is much literature on plant poly-phenolic compounds showing anti-venom activities, only few studies focus on plant peptides with anti-venom activity. Houghton *et al.,*., (1992), have reported a peptide inhibitor of cobra venom from the stem-bark of *Schumanniophyton magnificum*. The peptide showed dose-related inhibitions of the effects of cardiotoxin and total venom of cobra species using the chick biventer cervicis preparation (Houghton, P. J. *et al.,* 1992). A glycoprotein inhibitor of toxic phospholipase isolated from *Withania somnifera* completely neutralised a toxic PLA_2 from *Naja naja* venom and demonstrated physical interaction of both the toxic PLA_2 and the inhibitor (Deepa, M., & Gowda, T.V. 2002). Study has been reported that, Turmerin , a protein from Turmeric (*Curcuma longa* L.) is tested for its ability to quench free radicals and thus acting as potent antioxidant against *Naja naja* venom phospholipase A_2 induced oxidative organ damage (Chethankumar, M. 2010). Kumar *et al.,*., (2016) also reported a peptide (M.P-4) isolated from *Mucuna pruriens* seed which contributes to snake venom neutralization through an indirect antibody mediated mechanism. Anti-snake venom plant peptides could be expected to be antioxidants. In this perspective, the antivenom plant peptides with antioxidant properties exert effective metal ion (Fe^{2+}/Cu^{2+}) chelating activity and lipid peroxidation inhibitory capacity. The most attractive feature of these peptides is their ability to display very few side effects in humans due to their natural sources (Wang, B. *et al.,* 2014). Hence, this review has focused on the isolated and characterized peptides from plants with the antisnake venom properties to serve as stepping stone in the development of phyto-antivenom as alternative to the serum-based antivenom due to its limitations.

Table 1: List of Some Isolated Phyto-peptides used Against Snakebite Envenomation

Peptide	Plant	Part Used	Reference
RW-12	*Schumanniophyton magnificum*	Stem-bark	(Houghton, P.J., *et al*., 1992)
WSG	*Withania somnifera*	Root	(Deepa, M. & Gowda, T.V. 2002)
β-Turmerin	*Curcuma longa* L.	Waste grits	(Smitha, S. *et al*., 2009)
Turmerin	*Curcuma longa* L.	Leaf	(Chethankumar, M. 2010)
BGS-Haridrin	*Curcuma longa* L.	Leaf	(Ramadas, D. & Srinivas, L. 2011)
Snakin-Z	*Ziziphus jujube*	Fruit	(Zare-Zardini, H. *et al*., 2013)
MP-4	*Mucuna pruriens*	Seed	(Kumar, A. *et al.*, 2016)

2. RW-12

This peptide, RW-12 has been isolated from the aqueous extract of the stem-bark of *Schumanniophyton magnificum* (Rubiaceae) by Houghton *et al.,*., (1992) for it anti-venom activity. RW-12 gave a purple spot with ninhydrin reagent for amino acids and it also gave a positive test with biuret reagent for protein. Its relative molecular mass was estimated by size exclusion chromatography to be about 6kDa and this protein is similar in amino acid composition to the cardiotoxins present in snake venom. The amino acid composition data suggest that RW-12 contains 53 amino acids; consisting of 5 aspartame, 2 threonine, 2 serine, 2 glutamic acid, 9 glycine, 17 alanine, 2 valine, 1 leucine, 1 tyrosine, 2 phenylalanine, 1 histidine, and 4 arginine units. This peptide showed dose-related inhibition of the effects of cardiotoxin and total venom of cobra species using the chick biventer cervicis preparation.

It is interesting that RW-12 is not only active against the cardiotoxin component of the venom but also against the total venom. Earlier studies had shown that the crude extracts show no activity against the neurotoxin component of the venom but these results indicate that RW-12 must have some sort of inhibitory effect since the effect of the total venom is inhibited.

Houghton *et al.,*., (1992) reported that, the inhibitory mechanism of RW-12 is brought about by inhibition of snake venom cardiotoxins through formation of complex between the RW-12 and the venom cardiotoxins.

3. *Withania somnifera* Glycoprotein (WSG)

Withania somnifera glycoprotein (WSG) is an inhibitor of snake venom phospholipases. This peptide has been purified by Deepa and Gowda (2002) from *Withania somnifera* root using gel-filtration and ion-exchange chromatography. It is an acidic glycoprotein and its molecular mass was 27kDa. WSG neutralized the enzyme activity and pharmacological properties such as cytotoxicity, edema, and myotoxicity of a multi-toxic Indian cobra venom phospholipase A_2 but failed to neutralize the neurotoxicity due to its weak electrostatic interaction with the toxin. Carbohydrate moieties of WSG did not contribute to the inhibitory activity of the catalytic and the myotoxic effects of the Indian cobra venom phospholipase A_2 (PLA_2). In comparison, 6 phospholipase inhibitors (PLIs) isolated from a range of Australian elapid sera, were able to protect *in vivo* the lethal effects of homologous PLA_2 (HAINS, P. G., & BROADY, K. W. 2000). The PLIs are generally composed of two protein chains, an α-chain and a β-chain. The α-chains are 20 to 30kDa glycoprotein subunits and the β-chains are non-

glycosylated 20 to 25kDa protein subunits. The carbohydrate moiety of these inhibitors is found not to affect the *in vitro* function of the inhibitor (Hains, P. G. & Broady, K. W. 2000). One of the mechanisms by which PLA_2s induced edema is by release of lysophosphotides and fatty acids. However, enzyme activity is not strictly required to induce this effect[15]. Interaction of WSG with multitoxic *N. naja* PLA_2 suggests that WSG appears to confer beneficial effects against snake venom toxicity.

4. β-TURMERIN

β-Turmerin is a peptide from turmeric (*Curcuma longa* L.) waste grits obtained after extraction of curcumin with an apparent molecular mass of 34 kDa (Smitha, S. *et al.*, 2009). It is a hydrophobic glycoprotein as it shows the presence of amino sugars. It has been named as β-Turmerin (34 kDa) to differentiate it from original Turmerin (14kDa). The effective antioxidant activity of β-turmerin has been assessed in different model systems i.e., linolenic acid micelles, erythrocyte membrane system and liposomes. All these three model systems offer unsaturated fatty acids, the most vulnerable targets of reactive oxygen species (ROS) as the substrate for the action of ROS and lipid peroxidation. It was also found that β-Turmerin is effective in preventing lipid peroxidation at very low concentration of 0.125mM. Smitha *et al.*,., (2009) also reported that, Ellmans's test for 'S–S' group proved positive, indicating the presence of cysteine or cysteine residues in this peptides. The presence of these amino acids might be responsible for the antioxidant property of β-Turmerin. It has been reported that SH group act as a free radical scavenger in plants and animal tissues and SH group of cysteine facilitates the antioxidant activity of glutathione (Selvam, R., & Devaraj, S. 1996). Hence presence of SH group in β-Turmerin could be additive along with other unknown functional groups for effective antioxidant activity.

The mechanism of antioxidant activity of β-Turmerin could probably be through quenching of ROS, thereby reducing the potential of pro-oxidants to attack cellular components (lipids or proteins).

5. TURMERIN

Turmerin is a peptide isolated by Chethankumar (2010) from the leaf of Turmeric (*Curcuma longa* L., Zingiberaceae) which is a perennial plant with oblong rhizomes, often branched and brownish yellow in colour. It is extensively used in Ayurveda, Unani and Siddha medicine for treating various diseases (Ammon, H.P.T., & Wahl, M.A. 1991). It has a relative molecular mass of 14kDa and has been investigated for its ability to prevent oxidative organ damage against *Naja naja* venom phospholipase A_2 in male Swiss Wistar mice. Turmerin forms 0.1% of the dry weight of turmeric and is obtained in a crystalline form. It is a heat stable, noncyclic peptide containing 40 amino acid residues, with a blocked N-terminal and leucine at the C-terminal. It is insensitive to trypsin and pepsin, heat, and UV radiation (Srinivas, L. *et al.*, 1992). Turmerin contains 3 residues of methionine which are partly responsible for its antioxidant activity. At 183 nM, it offers 80% protection to membranes and DNA against oxidative injury. ROS-induced arachidonate release and the mutagenic activity of t-butyl hydroperoxide are substantially inhibited by Turmerin. it is non-cytotoxic at up to milligram concentrations, as tested by Ames assay and in human lymphocytes (Srinivas, L. *et al.*, 1992).

6. BGS-HARIDRIN

BGS-Haridrin is a 28kDa glycoprotein isolated, purified and characterized from boiling water extract of Turmeric (*Curcuma longa* L.) and named as BGS-Haridrin (2011). Ramadas and Srinivas (2011) reported that, BGS-Haridrin scavenges hydroxyl, Diphenyl-picrylhydrazyl (DPPH) radicals, superoxide radicals and inhibited lipid peroxidation 78% at a maximum dosage of 0.9 nM concentration when compared to Butylated hydroxyanisole (BHA), Curcumin (400 μM) and α-tocopherol (400μM). It has also effectively protected H_2O_2 induced cell death in human peripheral lymphocytes and prevented H_2O_2 caused calf thymus DNA damage as evidenced by agarose gel electrophoresis. In view of this, BGS-Haridrin exhibits different radical scavenging activities and thus can be used as an effective antioxidant agent to cellular components.

7. SNAKIN-Z

Snakin-Z is a new peptide isolated, purified and characterized by Zare-Zardini *et al.*,., (2013) from *Ziziphus jujuba* fruit and was investigated for its inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. *Zizyphus jujuba* is a small tree with eatable fruit (Yoon, J. I. *et al.*, 2010). Its dried fruits are used as sedatives, anticancer, antipyretic, analgesic, appetizer, anti-haemorrhage and as a tonic agent (Memarpoor-Yazdi, M. *et al.*, 2013).

Primary structural characterization of this peptide revealed that it was a member of defensin-like Snakin-2. Thus, based on a recent nomenclature recommendation by Conlon (Conlon, J.M. 2008), the peptide was named as Snakin-Z.

Zare-Zardini *et al.*,., (2013) postulated that, the inhibition of cholinesterase enzyme is through the formation of a complex between the snaking-Z and toxic cholinesterase.

8. MP-4

MP-4 is the dominant protein isolated, purified and characterized by Kumar *et al.*,., (2016) from the seed proteome of *Mucuna pruriens* and named MP-4 with crystal structure and molecular weight of 20.9kDa. The full-length sequence of this protein was determined

using N-terminal Edman degradation method and bioinformatic analysis of the derived sequence suggested that this protein may belong to the Kunitz-type protease inhibitor (KTPI) family. This observation raised the possibility that MP-4 may neutralize snake venom through direct inhibition of the proteases present in snake venom. However, *in vivo* and biochemical assays showed that the protein does not directly neutralize the toxic effects of snake venom. The structure of this protein showed that a residue critical for protease inhibition is missing in the reactive site loop. In line with the structural observation, the protein does not inhibit the proteolytic activity of trypsin and chymotrypsin. However, it was observed that immunization of mice with this protein provided significant protection against the toxic effects of venom from *Echis carinatus*. Overall, Kumar *et al.*,., (2016) studies showed that the MP-4 protein contributes substantially to the protection against snake venom through an antibody-mediated mechanism and not through direct inhibition of venom proteases. It was shown that antibodies rose in mice against *Mucuna pruriens* seed protein (MP-4) also react with venom components. It was also revealed that MP-4 is monomeric in nature, based on the comparison of reducing, non-reducing SDS-PAGE and native PAGE. It was therefore unlikely that MP-4 belongs to water-soluble chlorophyll binding protein (WSCP) class as these proteins are normally tetrameric (Horigome, D. *et al.*, 2007).

9. PHYTO-PEPTIDE STRUCTURE AND ANTIOXIDANT ACTIVITY

Studies indicated that the antioxidant effect of plant peptides is closely related to some structural characteristic of the peptides, such as their molecular mass, amino acid compositions, sequences, and hydrophobicity (Ren, Y. *et al.*, 2014).

9.1 Molecular Weight

The high activity against lipid peroxidation in a linoleic acid model system of BNH-P7 was due to the small size of peptides (Cai, L. *et al.*, 2015). High peptide sequences leads to a dilution effect of the peptide, thereby exhibiting hydroxyl radical scavenging activity. Therefore, appropriately low molecular weight can exert a significant effect on the antioxidant activities of peptides.

9.2 Amino Acid Composition

High proportion of hydrophobic amino acids (Leu, Val and Phe) has been reported in peptides with high antioxidant activity compared to other hydrophilic amino acids (His, Pro and Lys) (Saidi, S. *et al.*, 2014). This is considered as a key factor in peptide ability to scavenge radicals. For instance, the nano filtration fraction (1–4 kDa) from tuna dark muscle by-product showed the highest superoxide radical and reducing power activities, which contained antioxidant amino acids such as Tyr, Phe, Pro, Ala, His, and Leu, which accounted for 30.3% of the total amino acids (Saidi, S. *et al.*, 2014).

9.3 Amino Acids Sequence

The interaction between amino acids may be an important factor for the antioxidant activity. Besides the hydrophobicity of peptides with high antioxidant activity, the amphiphilic nature of peptides also seems to enhance the radical-scavenging activities by increasing peptide solubility while facilitating interaction and proton exchanges with radical species. In amino acid sequence of a peptide, the presence of hydrophobic amino acids (Leu, Val and Phe), hydrophilic and basic amino acids (His, Pro and Lys), and aromatic amino acids (Phe and Tyr) is believed to contribute to the overall high antioxidant activity of the peptide (Najafian, L., & Babji, A. S. 2015).

9.4 Secondary Structure

Taking the secondary structure into consideration, Agrawal, P. *et al.*,. (2018) found through bioinformatics that 58 out of 77 bioactive peptides contain at least one β-turn, followed by helices (60%), and β-strands were present in just 13% of the total peptides studied (Kaur, H. *et al.*, 2007). α-Helices and β-sheet secondary structures exist in the structure of antioxidant peptides.

10. CONCLUSION

This review highlighted some of the anti-snake venom plant peptides isolated, purified and characterized from different parts of some medicinal plants with known anti-snake venom properties. As reported, these peptides have shown different effects against snake venoms through different mechanisms. Some of them prevent oxidative organ damage against *Naja naja* venom phospholipase A_2, while some serve as inhibitors of other enzymes and toxins of the snake venom. MP-4 has been found to neutralize the snake venom through antibody mediated mechanism. This might serve as a lead in establishing effective, affordable, readily available and storable antidotes against snakebite envenomations.

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