CHAPTER I

INTRODUCTION

1.1 Snake envenomation: A neglected tropical disease

1.1.1 Global burden of snake envenomation

Snake envenomation is a neglected public health concern in tropical and subtropical countries, often leading to life-threatening conditions. Every year, about 5.4 million snake bites are reported, which results in 1.8-2.7 million cases of envenoming [1]. Globally, South and Southeast Asia, sub-Saharan Africa, and Central and South America carry the significant burden of snakebite [2]. According to the estimations by Kasturiratne and co-workers, the highest number of envenomings was recorded in South Asia (121,000), followed by Southeast Asia (111,000) and East Sub-Saharan Africa (43,000). In contrast, Central Europe and Central Asia recorded the lowest numbers of snakebite cases. Further, the highest number of deaths recorded were in South Asia (14,000), followed by West sub-Saharan Africa (1,500), East sub-Saharan Africa (1,400), and Southeast Asia (790) [2] (Fig. 1.1). The incidence of snakebite envenomation is rare in Europe, the Middle East, Canada, and North America [3].

However, it is a rather cumbersome and challenging exercise to estimate the global incidence of snakebite accurately, and its associated mortality since the majority of the incidences occur in rural areas of developing countries with poor health and transportation facilities and these bites are not recorded [2,4]. This is further influenced by the dependence of many people on traditional medicinal systems to treat snakebite. In general, treatment-seeking behaviour from conventional healers is often guided by local culture, ignorance of modern medical treatments against snakebites, and inaccessibility of healthcare facilities to treat snakebites [5]. Moreover, resource-poor rural areas usually lack a well-documented central registry system of record keeping, which further adds to the under-documentation of snake envenomation in developing nations [6,7]. The highest cases of snake envenomation are recorded in India, which is approximately 1.11 to 1.77 million per year, followed by Sri Lanka (33,000), Vietnam (30,000), Brazil (30,000), Mexico (28,000), and Nepal (20,000) [2,8].

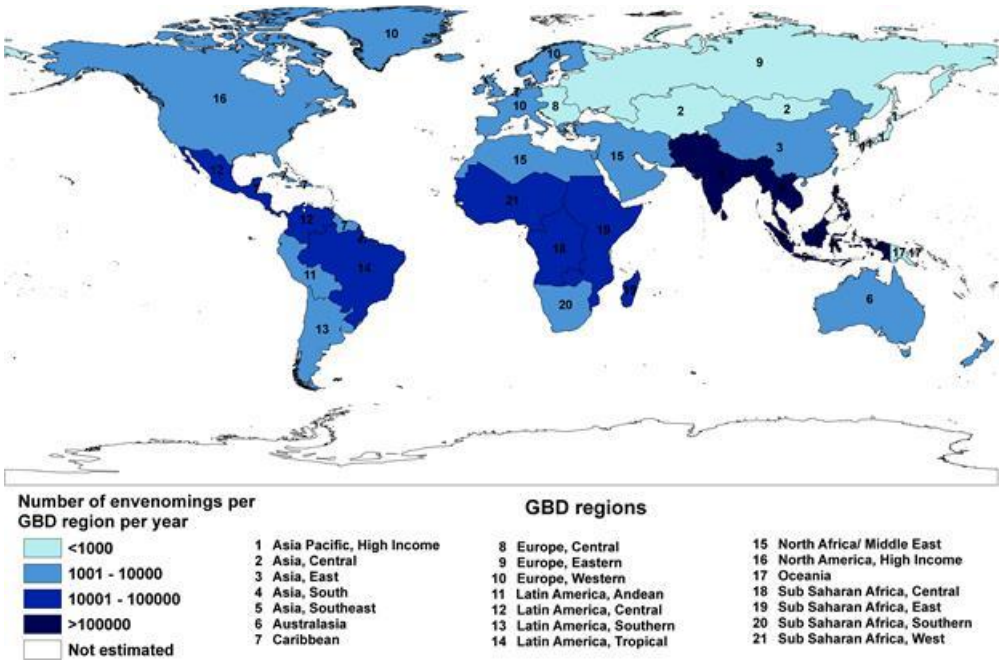

Fig. 1.1 Regional estimates of envenomings due to snakebite (low estimate). (Figure adapted from Kasturiratne et al., [2]).

Out of the around 3596 snake species worldwide, about 768 species are venomous; however, bite by every snake species is not fatal, and every venomous snake is not found in each locality. A region's medically essential snake species is categorised into Category 1 and 2. The most common highly venomous snake species causing numerous snakebites and resulting in high morbidity, disability, or mortality are included in Category 1. In contrast, Category 2 comprises of highly venomous snakes capable of causing morbidity, disability, or death, yet they lack exact epidemiological or clinical data and/or get implicated in lesser frequency [1]. Depending on the number of envenoming and fatalities, the most dangerous species of medically essential snakes inhabiting the four different geographical regions of the globe have been identified and listed in Table 1.1.

Table 1.1. List of medically important venomous snakes distributed in different parts of the world.

Region	**Snake species**	**Family**	**Venom type**	**Ref.**
Southern Asian countries	*Naja naja,* and *Naja kaouthia*	Elapidae	Neurotoxic	[7,9-13]
	Daboia russelii russelii,	Viperidae	Hemotoxic	
	Bungarus caeruleus, Bungarus walli, and *Bungarus sindanus,*	Elapidae	Neurotoxic	
	Ophiophagus hannah	Elapidae	Neurotoxic	
	Echis carinatus	Viperidae	Hemotoxic	
	Hypnale hypnale	Viperidae	Hemotoxic	
	Protobothrops spp.	Viperidae	Hemotoxic	
African and the middle-eastern region	*Naja oxiana, Naja nigricollis,* and *Naja mossambica*	Elapidae	Neurotoxic	[3,14]
	Dendroaspis polylepis, and *Dendroaspis mauritanica*	Elapidae	Neurotoxic	
	Echis borkini	Viperidae	Hemotoxic	
	Bitis arietans	Viperidae	Hemotoxic	
Asian (other than Southern Asian countries) and Australian regions	*Naja atra, Naja kaouthia,* and *Naja naja*	Elapidae	Neurotoxic	[14]
	Bungarus multicinctus	Elapidae	Neurotoxic	
	Oxyuranus scutellatus	Elapidae	Neurotoxic	
	Pseudechis australis	Elapidae	Neurotoxic	
	Pseudonaja textilis	Elapidae	Neurotoxic	
	Daboia siamensis	Viperidae	Hemotoxic	
	Hypnale hypnale	Viperidae	Hemotoxic	
	Calloselasma rhodostoma	Viperidae	Hemotoxic	

Europe	*Vipera ammodytes, Vipera berus* and *Vipera aspis*	Viperidae	Hemotoxic	[14,15]
America	*Agkistrodon bilineatus*	Viperidae	Hemotoxic	[14,16]
	Bothrops asper, Bothrops jararaca, and *Bothrops atrox*	Viperidae	Hemotoxic	
	Crotalus scutulatus, and *Crotalus durissus*	Viperidae	Hemotoxic	

1.1.2 Indian scenario of snake envenomation

The highest cases of snake envenomation have been recorded in India at approximately 1.11-1.77 million cases annually [8]. The Registrar General of India-Million Death Study (RGI-MDS) has estimated that there are around 46,900 deaths per year due to venomous snakebites in India [17]. The magnitude of the snake envenomation issue is far greater than documented in the published material.

The Indian subcontinent is inhabited by more than 52 species of venomous snakes. Among them, the Indian cobra (*Naja naja*), Indian common krait (*Bungarus caeruleus*), Indian Russell's viper (*Daboia russelii russelii*), and Indian Saw-scaled viper (*Echis carinatus*), commonly known as "Big Four" venomous snakes of India, account for the majority of snakebite deaths and morbidity in these regions; therefore, they are considered as category I medically significant snakes the bite by which deserves immediate medical attention [3,11,14,18-21]. Other than these deadly species, Indian monocled cobra (*Naja kaouthia*), Wall's krait (*Bungarus walli*), Sind krait (*Bungarus sindanus*), King cobra (*Ophiophagus hannah*), and several species of Pit vipers (*Hypnale hypnale*, *Protobothrops* spp.) are also found in different parts of India and cause fatalities [11,12,21].

The majority of the snakebite deaths reported from the rural areas (97%) during the monsoon months of June to September were more frequent in males (59%) than females (41%), and highest in the age group of 15–29 years (25%) [4]. In the period between 2001-2014, a few states of India (Bihar, Jharkhand, Madhya Pradesh, Odisha, Uttar

Pradesh, Andhra Pradesh, Telangana, Rajasthan and Gujarat) were identified as high-burden states, and the age-standardised death rate was recorded as approximately six per 100,000. On the other hand, all the country's different states were identified as low-burden states that recorded age-standardised death rates of roughly 3.7 per 100,000 at the start of the study, which fell over time [8]. The distribution of the absolute risk of dying from snakebite is shown in Fig. 1.2. The figure has been reported using data from 7400 small areas (the small sampling units used in the RGI's Sample Registration System for the MDS) from 2004 to 2013 [8].

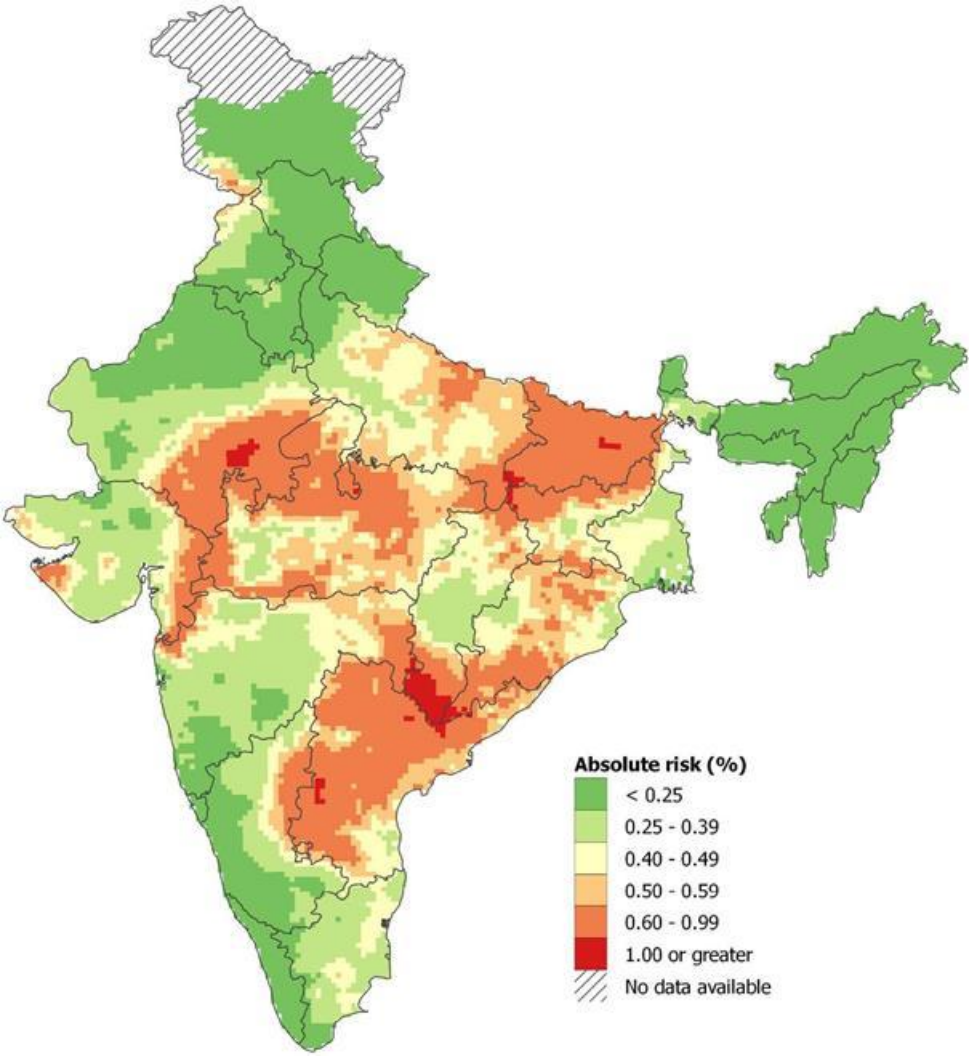

Fig. 1.2 Spatial distribution of snakebite mortality risk in India for 2004-13. (Figure adapted from Suraweera et al., [8]).

1.2 Scorpion envenomation: a neglected tropical disease

1.2.1 Global burden of scorpion envenomation

Across the globe, approximately 1.23 million scorpion stings (average rate of envenomation is 20 per 100,000 inhabitants) worldwide result in about 3250 deaths yearly; therefore, scorpion sting envenomation is considered a neglected public health disease in tropical and sub-tropical countries [22-24]. The regions where individuals often come into contact with scorpions are North-Saharan Africa, South Africa, South-India, Near and Middle-East, Sahelian Africa, East of the Andes, South Latin America, and Mexico; and therefore designated high-risk areas for scorpion stings [24,25]. Due to inadequate reporting systems and limited accessibility to health institutions in the endemic regions, it is challenging to pinpoint the exact incidence of envenomation cases [24].

The scorpion species *Androctonus australis*, *Leiurus quinquestriatus*, *Androctonus mauretanicus*, *Androctonus aeneas*, *Buthus occitanus* and *Hottentota franzwerneri*, in Northern Africa are considered deadly or potentially deadly [26]. Morocco annually records an incidence of approximately 50 scorpion stings per 100,000 inhabitants, with a mortality rate of 0.27 per 100,000 inhabitants [27]. The scorpion sting incidences are highest in the central part of the country, whereas the northern part has low cases of envenomation [28]. Algeria has about 170 scorpion stings per 100,000 inhabitants annually, and the annual mortality is 0.38 per 100,000 inhabitants [29]. Tunisia, on the other hand, has a yearly incidence of 420 scorpion stings per 100,000 inhabitants, which leads to about 40,000 stings and 50 deaths a year [30,31]. With the predominant scorpion species of *Parabuthus transvalues*, Zimbabwe records an estimated 195 scorpion stings per 100,000 inhabitants per year, and the annual mortality is 2.8 per 100,000 inhabitants [32].

In Turkey, the most dangerous scorpions are *Androctonus crassicauda* and *Mesobuthus eupeus*. The annual incidence is about 36 scorpion stings per 100,000 inhabitants (mortality is 0.01 per 100,000) [33]. Israel has approximately 200 cases of scorpion envenomation per year [34]. Saudi Arabia is home to the dangerous scorpion species *Leiurus quinquestriatus*, *Androctonus crassicauda* and *Parabuthus liosoma,* and annual incidence and mortality were found to be 90 and 0.001, respectively, per 100,000 inhabitants [23].

The predominantly deadly scorpions found in South America belong to the genus *Tityus*. In the year 2017, Brazil, a South American country, has recorded the number of confirmed deaths from scorpion stings as 90 in 2017, with 124,982 cases of scorpion stings [35]. The fatality rate for victims under 10 and over 75 was 0.32% and 0.13%, respectively [35]. Mexico sees more activity from *Centruroides* spp. scorpions during the warmer months [36]. Some states in the central and west of Mexico record an annual incidence of 1350 scorpion stings per 100,000 inhabitants [23]. This situation was similar to Argentina, where scorpion sting incidence increased between October and April due to *Tityus trivittatus* scorpion [37]. A correlation has been observed between the decline in scorpion stings and the arrival of the rainy season [38,39].

Scorpion stings were observed all over Iran, and among the 51 species of scorpions found in different parts of the country, the Buthidae family comprises the majority of the scorpion fauna in Iran, accounting for 88.5% of all species and 82% of all genera [40]. A study in Iran between the period of 2002 to 2011 reported 54.8 to 66 scorpion stings per 100,000 people [41]. According to Chippaux and Goyffon (2008), the global annual mean rate of sting occurrences is approximately 17.14 per 100,000 people [28]; thus, Iran was documented to have higher scorpion stings than the worldwide average. There have been two recent studies on scorpion stings in Pakistan, one in the Sargodha district of Punjab [42] and the other in the Lasbella district of Balochistan [43]. The distribution and incidence of significant scorpion species worldwide are depicted in Fig. 1.3.

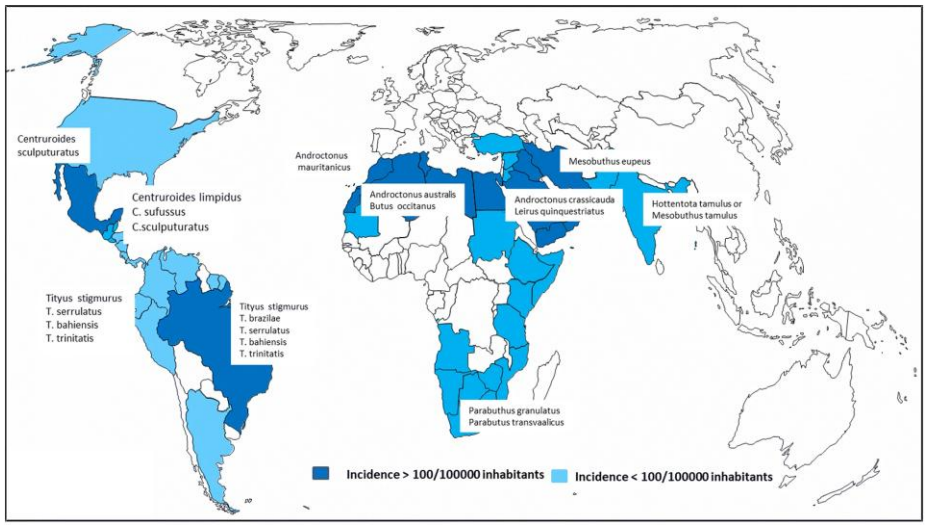

Fig 1.3 Distribution and incidence of Old World and New World scorpion species. (Figure adapted from Abroug et al., [24]).

1.2.2 Indian scenario of scorpion envenomation

Among the 86 scorpion species found throughout India, only the Indian red scorpion (*Mesobuthus tamulus*) belonging to the family Buthidae, and the Indian black scorpion (*Heterometrus swammerdami,* formerly *Palamneus gravimanus*) of the Scorpionidae family pose a significant threat to humans; however, limited clinical reports suggest that *Mesobuthus tamulus* venom is more toxic compared to any venomous scorpion in this subcontinent [22,44-49].

In India, morbidity and mortality due to scorpion stings frequently occur in western Maharashtra, Saurashtra, Kerala, Tamil Nadu, Andhra Pradesh and Karnataka. The most adverse effects of scorpion envenomation were observed in children at 1-3 and 7-12 years of age [50].

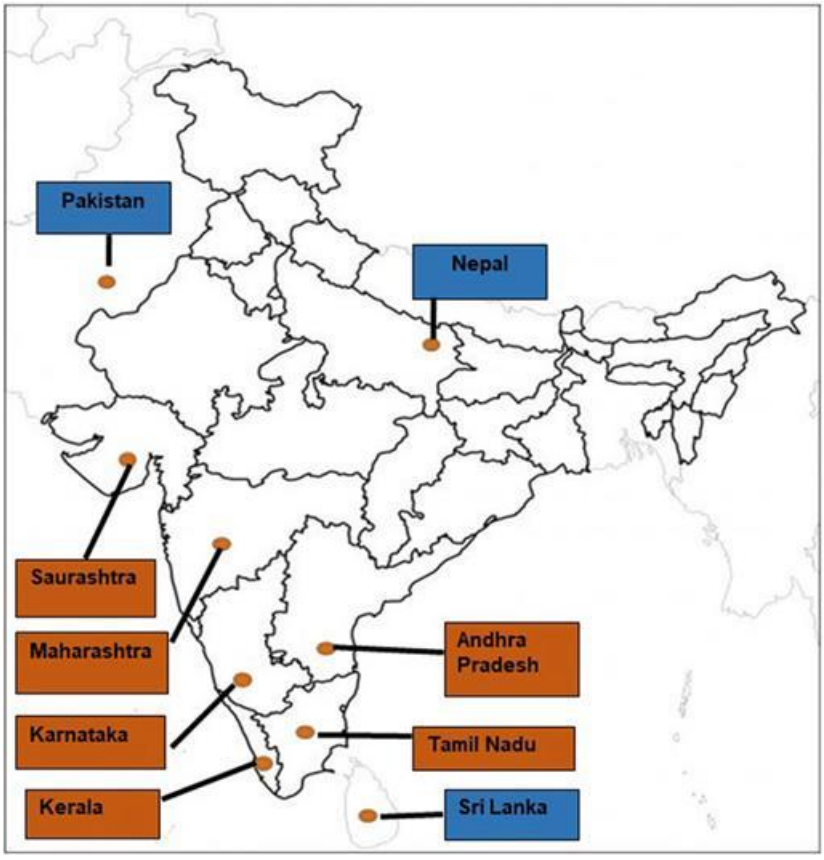

Fig. 1.4 The geographical distribution of the Indian red scorpion throughout the Indian sub-continent [Brown fill: Indian states; Blue fill: neighbouring countries of India]. (Figure adapted from Das et al., [45]).

1.3 Classical or contemporary methods for detection

1.3.1 Snake envenomation

Snake envenomation has been a significant health concern for the rural population of most of the tropical and sub-tropical countries [51]. Management of snake envenomation is essential to curb the dangerous effects of snake venom on snakebite victim. Generally, the bitten snake species are identified through the description or photograph of the snake provided by the patient and/or their family or friends or witnesses, an examination of the bite site, local symptoms of envenomation, biochemical analysis of urine, and 20-min whole blood clotting test (20WBCT) [52-55]. The patient or their witnesses often do the initial identification of the culprit snake. In numerous cases, the witnesses kill the snake, causing envenomation and the dead snake, creating additional danger to the people involved and a risk of affecting the snake population ecology. There are also cases where no good evidence exists to identify the culprit snake. If a patient shows signs and symptoms of a venomous snakebite, these cases are considered "unknown venomous" [56]. However, healthcare providers are not snake experts; therefore, they often struggle to identify snakes, leading to cases of misidentification [55].

The 20WBCT is a famous test used to indicate the extent of snake envenoming by determining the clinically significant coagulopathy [57-59]. It is a simple, effective, and affordable bedside examination for the initial assessment. In 20WBCT, a few millilitres of venous blood are placed in a clean, dry glass test tube and left undisturbed for 20 minutes. The test tube is tipped to discover if the blood clots. The test is negative or positive depending on the formation or non-formation of the clot [54,59]. Because bites by the Viperidae family of snakes are responsible for showing coagulopathy, this test applies only against Viperidae bites [59,60]. Further, it has been reported that due to the poor detectability of 20WBCT, the result of this test alone should not influence the decision on antivenom administration.

1.3.2 Scorpion envenomation

There are no specific diagnostic tests or methods for scorpion envenomation [61-63]. Clinical practitioners and physicians examine the possible ramifications associated with scorpion envenomation. They rely on evaluating the potential for renal failure by

measuring creatinine levels and assess pancreatic enzyme levels in cases of scorpion sting-induced pancreatitis [62]. In cases of severe systemic envenomation, an electrocardiogram is ordered to detect frequent occurrence of electrocardiographic abnormalities [62].

The most common manifestation of scorpion stings is localised pain, with only a low percentage of stings resulting in severe systemic envenomation. The sting site may exhibit oedema, paresthesias, erythema, muscle fasciculations, and numbness [62,64,65]. Systemic envenomation by *Centruroides* and *Parabuthus* scorpion species is primarily associated with neuromuscular toxicity; however, severe envenomation from *Androctonus*, *Buthus*, and *Mesobuthus* scorpions is related to cardiovascular toxicity [45,62,66-69].

1.3.3 Key issues about clinical diagnosis of snake and scorpion envenomation

Notably, most snakebite and scorpion sting cases occur in the rural areas of underdeveloped and developing countries [4,62]. Thus, while developing snake or scorpion venom detection kits, it has to be kept in mind that the maximum intended use of these kinds of kits would be in the rural health centres of such countries. Consequently, the following key issues must be addressed for the practical effectiveness of such kits in improving the hospital management of snakebite and scorpion sting patients.

(i) The snake venom detection test must be very rapid, perhaps within 30 min, because clinicians generally consider the first 2 h post-bite as the "Golden Period" for successful antivenom treatment. Administration of within this period significantly increases the probability of saving the life of the bite victim [21]. After time passes, bite-induced symptoms may vary depending on the quantity of venom injected, bite site, age and weight (body mass ratio) and response/immunity of the bitten patient [7].

In cases of scorpion envenomation, the detection should also be rapid as the symptoms of the affected patients change gradually [70].

(ii) In the case of a snakebite, the venom is delivered very quickly, either subcutaneously or occasionally intramuscularly. Then, the toxins get absorbed into systemic circulation until they reach their target to exert toxicity. The blood vascular endothelium absorbs the low-molecular-weight toxins of mostly elapid venoms, whereas the lymphatic system absorbs the high-molecular-weight toxins of viperid venoms [71]. Since most patients

arrive late at the health centres after a long, exhaustive journey, by that time, several components of venom, most notably the non-enzymatic toxins of venom, bind very tightly to their target tissues and/or circulatory proteins and due to this reappropriation, a small quantity of venom may be available in circulation and/or body fluids to be detected [72].

The stung patients from rural areas often arrive at health centres 1-2 h after the incident due to their initial preference for traditional medicine or inaccessible health centres. The pharmacokinetics studies of a few scorpion species venoms have demonstrated that the peak concentration of venom reaches in blood between 30 and 60 min after venom injection, and after that, the venom is distributed in the organs and eliminated rapidly from the blood [73,74].

Therefore, the detection tests for both snake and scorpion venoms should allow the determination of a trace quantity of venom in the body fluids/swab from the patient. This test will also assist the clinicians in determining the success of treatment (quantity of remaining venom in the blood and body fluids) at different time intervals post-antivenom administration.

(iii) Several closely related or unrelated venomous snakes may inhabit a particular region. Extensive cross-reactivity among the components of venoms from different groups of snakes can lead to confusion in selecting the species-specific monovalent antivenom, which may further lead to fatal outcomes [21,75,76]. Therefore, the diagnosis of snake-bitten species should be accurate in administering species-specific monovalent or bivalent antivenom [21,52].

(iv) Venomous snakebites typically result in varying degrees of toxicity, from mild local symptoms to systemic toxicity like severe coagulopathy, neuroparalysis, multiple organ failure, shock and finally death. But, sometimes, during an envenomation by a venomous snake, the victim does not show local or systemic symptoms, and these kinds of snakebites are termed dry bites. Patients diagnosed with a 'dry bite' should be hospitalised and observed closely for any indications of envenomation for at least 12-24 h to avoid inappropriate antivenom use [77]. Therefore, a diagnostic device that can differentiate between a life-threatening bite and a dry bite would be much more helpful in providing confidence for clinicians, including the less experienced trainee doctors in rural health centres [52].

(v) The detection kits should occupy minimal storage space, be affordable for developing countries, be capable of storage at room temperature, and not require maintaining the cold chain during transportation. The detection test should be simple to perform [78]. Thus, snake and scorpion venom detection methods that do not involve costly instruments, reagents, and skilled or highly qualified technicians to perform the diagnostic test and data analysis would be a highly welcome approach in the rural health centres of the countries.

(vi) The test kit should be stable and has long shelf life.

1.4 Proteome composition

1.4.1 Indian snake venoms

Snake venom contains a cocktail of enzymatic and non-enzymatic toxins in various proportions. However, the venom of a particular snake species may not contain all the toxins. In addition, depending on the age, sex, zoogeographic and species and/or genus of the snakes, the relative proportion of different toxins may also vary [79-83]. The proteomic analyses of snake venoms have demonstrated that the most common enzymes of venom are phospholipase A_2s (PLA_2s), phospholipase B (PLB), snake venom serine proteases (SVSP), aspartic protease (ASPro), snake venom metalloproteases (SVMP), acetylcholinesterases (AChEs), cholinesterases (ChEs), L-amino acid oxidases (LAAO), nucleotidases (5'-nucleotidases, ATPases, phosphodiesterases, PDE; and DNases), glutaminyl cyclase (GC) and hyaluronidases (Hya), snake venom thrombin-like enzyme (SVTLE) whereas non-enzymatic venom components such as three-finger toxins (3FTx), cysteine-rich secretory proteins (CRISPs), kunitz-type proteinase inhibitors (KSPI), snake C-type lectin-like proteins (snaclecs), snake C-type lectins (CTL), cobra venom factor (CVF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), Ohanin-like protein (OLP), Vespryn (Vesp), natriuretic peptides (NP) and disintegrins are present in several venoms (Table 1.2).

However, proteomic analysis has shown that geographical and species-specific variation in snake venom composition is a common phenomenon, resulting in differences in toxicity and clinical manifestations following a bite by a venomous snake [84-87].

Table 1.2. A summary of the common enzymes found in venomous species of Indian snakes from different geographical locations of India where maximum snakebite takes place. The proteins (toxins) were identified by proteomic analysis of venoms.

Venoms (common name)	**Geographic origin**	**Protein classes identified**	**Ref.**
Naja naja (Indian spectacled cobra)	Northern India (Rajasthan and Gujarat)	PLA_2, SVMP, 3FTx, KSPI, NGF, CRISP	[85]
	Southern India	PLA_2, SVMP, LAAO, PDE, NT, SVSP, APase, 3FTx, CRISP, CVF, KSPI, NGF, OLP, Vespryn	[88]
	Southern India	PLA_2, SVMP, LAAO, NT, SVSP, AChE, APase, 3FTx, CRISP, CVF, KSPI, NGF, OLP, Cys	[89]
	Eastern India (Nadia, West Bengal)	PLA_2, SVMP, LAAO, NT, SVSP, AChE, ChE, PDE, 3FTx, CRISP, NP, OLP, CVF, NGF, KSPI,	[90]
	Eastern India (Burdwan, West Bengal)	PLA_2, SVMP, LAAO, NT, PDE, ChE, PLB, AChE, 3FTx, NGF, CRISP, CVF, OLP, Cys	[91]
	Western India	PLA_2, SVMP, NT, PDE, LAAO, SVSP, AChE, APase, PLB, 3FTx, OLP, KSPI, CRISP, NGF, Cys, CVF	[92]
	Western India (Maharastra)	CVF, SVMP, NT, LAAO, SVSP, CRISP, PLA_2, NP, Vesp, 3FTx, KSPI	[93]
Naja kaouthia (Monocled cobra)	Eastern India (Burdwan, West Bengal)	PLA_2, SVMP, LAAO, PDE, NT, AChE, Hya, PLB, 3FTx, CRISP, CVF, OLP, NGF	[91]
	Eastern India (West Bengal)	CVF, SVMP, NT, PLB, LAAO, CRISP, SVSP, CTL, NP, PLA_2, NGF, Vesp, 3FTx, KSPI	[93]
	North-Eastern India (Assam)	PLA_2, SVMP, LAAO, NGF, Vesp, 3FTx, KSPI, Vesp, Waprin	[94]
	North-Eastern India (Arunachal Pradesh)	CVF, PDE, SVMP, AChE, NT, LAAO, CRISP, PLA_2, NGF, Vesp, 3FTx	[93]
Bungarus caeruleus (Common krait)	Southern India	PLA_2, LAAO, AChE, Hya, SVMP, NT, 3FTx, CRISP	[88]
	Southern India	SVMP, PLA_2, SVSP, PLB, AChE, LAAO, 3FTx, CRISP, NGF, CVF, KSPI, Vespryn, β-Bungarotoxin	[95]

	Northern India (Punjab)	SVMP. AChE, NT, LAAO, Hyla., NGF, CRISP, Vesp., PLA_2, 3FTx, KSPI	[93]
Bungarus sindanus (Sind krait)	North-western India (Rajasthan)	SVMP. AChE, NT, PLB, LAAO, NGF, CRISP, Vesp., CTL, PLA_2, 3FTx, KSPI	[93]
Bungarus faciatus (Banded krait)	Eastern India (West Bengal)	SVMP. AChE, NT, PLB, LAAO, NGF, SVSP, CTL, PLA_2, 3FTx, KSPI	[93]
Daboia russelii (Russell's viper)	Southern India	SVSP, SVMP, PLA_2, NT, PDE, LAAO, KSPI, VEGF, snaclec, NGF, Dis, CRISP	[96]
	Southern India	SVSP, SVMP, PLA_2, NT, PDE, LAAO, PLB, GC, KSPI, VEGF, snaclec, NGF, CRISP	[86]
	Southern India	SVSP, SVMP, PLA_2, NT, PDE, LAAO, PLB, GC, KSPI, VEGF, snaclec, NGF, CRISP	[95]
	Eastern India (Burdwan)	SVSP, SVMP, PLA_2, NT, PDE, LAAO, APase, GC, Hya, KSPI, VEGF, snaclec, NGF, Dis, CRISP	[97]
	Eastern India (Nadia)	SVSP, SVMP, PLA_2, NT, PDE, LAAO, PLB, GC, Hya, KSPI, VEGF, snaclec, NGF, Dis, CRISP	[97]
	Western India	SVSP, SVMP, PLA_2, NT, PDE, LAAO, PLB, KSPI, VEGF, snaclec, NGF, Dis, CRISP	[98]
Echis carinatus (Saw scaled-viper)	Southern India	SVMP, PLA_2, SVSP, LAAO, NT, APase, PLB, GC, ASPro, PDE, snaclec, Dis, KSPI, VEGF, CRISP, NGF	[99]
	Western India (Maharashtra)	Hya, SVMP, LAAO, SVSP, snaclec, PLA_2, Dis.	[93]
Echis carinatus sochureki (Sochurek's saw-scaled viper)	North-western India (Rajasthan)	Hya, SVMP, PLA_2, SVSP, LAAO snaclec, Dis, CTL, CRISP	[93]
Hypnale hypnale (Hump-nosed viper)	Southern India (Kerala)	SVSP, SVMP, PLA_2, snaclec, NGF, Dis, CRISP	[100]
Trimeresurus malabaricus (Malabar pit viper)	Western Ghats, India	LAAO, SVMP, PLA_2, SVSP, PLB, APase, SVTLE, PDE, NT, GC, Dis, CRISP, NGF, snaclec	[101]

1.4.2 Indian red scorpion venom

Scorpion venom is a cocktail of enzymatic and non-enzymatic proteins. The proteins can be further classified into two categories based on the number of amino acids in their sequences: i) short toxins which are comprised of 30-40 amino acids, and ii) long toxins with 60-70 amino acids [102]. The non-enzymatic proteins are divided into four groups based on their biological functions and pharmacological activity, namely Na^+, K^+, Cl^-, and Ca^{2+} channel toxins [103-105].

Proteomic analysis of the Indian red scorpion (*Mesobuthus tamulus*) venom revealed the predominance of low molecular mass K^+ and Na^+ channel toxins, accounting for 38.2% and 38.4% of the proteome, respectively (Fig. 1.5) [106]. The Na^+ channel toxins are mainly responsible for the neurotoxic effects of scorpion stings. The K^+ channel toxins, short peptides comprised of 30-40 amino acids, show a high affinity toward one or more subtypes of potassium channels [106].

The Na^+ channel toxins based on their sensitivity to voltage changes post-target binding, the Na+ channel toxins are classified as α-type and β-type toxins, and the proteomic analysis of *Mesobuthus tamulus* venom identified both toxins [106]. Based on sequence homologies, 3D folding pattern, and activity, the K^+ channel toxins are classified into six families [102]. Proteomic analysis showed that *Mesobuthus tamulus* venom predominately comprises α-K^+ channel toxins containing about 200 peptides [102,106]. Additionally, β-K+ venom toxins and γ-K^+ channel toxins have also been identified in *Mesobuthus tamulus* venom proteome [106].

Proteomic analysis identified several other minor toxin classes in less abundance in the *Mesobuthus tamulus* venom proteome, such as serine protease-like protein (SPLP), serine protease inhibitor (SPI), antimicrobial peptide, makatoxin, hyaluronidase, lipolysis potentiating peptides (LPP), parabutoporin, Cl^--channel toxin, Ca^{2+}-channel toxin, bradykinin potentiating peptide (BPP), HMG CoA reductase inhibitor, and some other toxins with unknown targets or pharmacological activity [106].

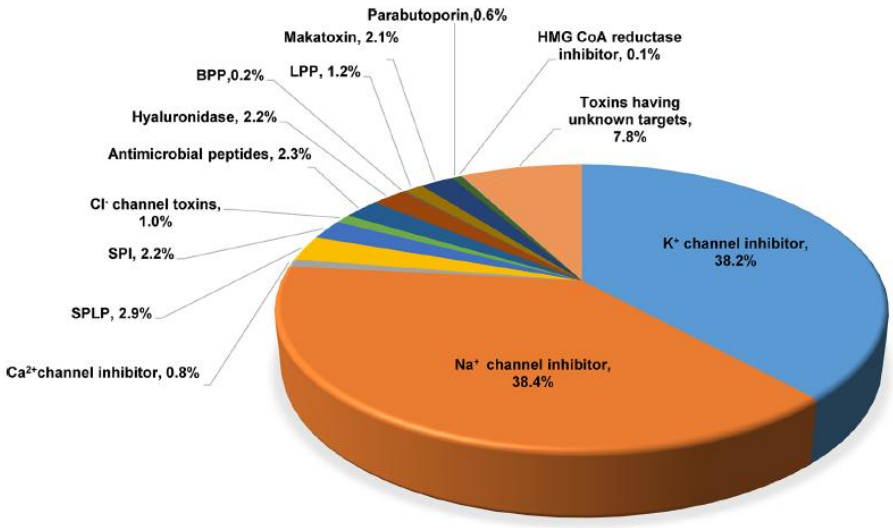

Fig. 1.5 Protein family composition of *Mesobuthus tamulus* venom. The relative abundance of different venom protein families is expressed as an average of relative abundances calculated using MSI (summed peptide-spectrum Match Precursor Intensity) based on label-free quantitation techniques. (Figure adapted from Das et al., [106]).

1.5 Peptide antigens and antibodies in detection

Recent decades have seen a rise in the extensive use of synthetic peptide antigens to generate specific antibodies for detection. Synthetic peptides are generated by mimicking selected regions of the protein of interest, and they are preferred for their high purity and ease of handling [107]. After that, antibodies may be produced using the epitopes on these synthetic peptide antigens.

1.5.1 Antigenic peptide design and synthesis

Designing a synthetic peptide to raise antipeptide antibodies must start with studying the native protein sequence and structure using protein structure databases like Protein Data Bank (PDB) [www. rcsb.org/pdb]. The protein sequences should also be studied to determine and avoid any potential cross-reactivity with other closely related proteins using protein homology tools like those available at NCBI (www.ncbi. nlm.nih.gov), UniProtKB (www.uniprot.org), PIR (pir.georgetown.edu), ExPASy (us.expasy.org/tools), or HomoloGene (www. ncbi.nlm.nih.gov/homologene).

Further, if 3D structure of a protein is not available at PDB,

I-Tasser (zhanglab.ccmb.med.umich.edu/I-TASSER) may help predict the 3D structure of a particular protein [108,109].

Peptide immunogens for antibody production are selected based on several factors, sequential epitopes, physicochemical characteristics like accessibility, hydrophilicity, flexibility, and structural features [110-112]. Several B-cell epitope databases/prediction servers can help predict peptide sequences that elicit a robust immune response [108]. Synthetic peptide sequences selected should be potentially exposed, as well as immunogenic internal sequences of the native protein, as antibodies generally bind to epitopes exposed on protein surfaces and those flexible enough to move to accessible positions since accessibility is crucial for antipeptide antibodies to recognise native proteins, exposed regions such as the N- or C-termini, protruding loops or connecting regions are preferred as these regions are enriched with charged and polar amino acids [111-113]. Thus, with the help of algorithms, protein characteristics such as hydrophilicity/hydrophobicity and secondary structural regions can be predicted, aiding in selecting and designing antigenic peptide sequences [108,112].

Peptide sequences are generally designed using 10-20 amino acids long as sequences at this length have minimal issues during synthesis and may show reasonable solubility in aqueous solutions. Notably, peptide sequences with smaller than eight amino acids may not be feasible for immunisation as they may elicit antibodies with insufficient affinity towards the native proteins. In contrast, peptides with longer than 20 amino acids may sometimes lose specificity and adopt conformations that do not resemble those of native protein [108,112,114].

Antigenic peptides with less than 50 amino acid residues can be synthesised by the widely used method of 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis (SPPS), in which the N^{α}-amino group gets protected by base-labile Fmoc group and side-chains get blocked by acid-labile groups [115]. After the synthesis process, the synthetic peptides obtained are analysed purity by liquid chromatography-mass spectrometry (LC-MS) or analytical reverse-phase high-performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) [112,116].

1.5.2 Antigenic peptide antibody

One of the main applications of synthetic peptides in immunology arises from their capability to elicit antipeptide antibodies that cross-react with the native protein [117]. Immunising animals with a particular synthetic peptide will produce polyclonal antiserum with a heterologous mixture of immunoglobulin Gs (IgGs) against the concerned protein [118]. Immunisation procedures for producing polyclonal antipeptide antibodies use peptide conjugates for successful animal immunisation. The animals are injected with peptide-carrier conjugates mixed with adjuvant, which enhance the immune response [112,119,120].

Animals such as mice, guinea pigs, hamsters, rats, rabbits, chickens, goats, pigs, sheep, donkeys, cows, and horses may be used as host animals for producing peptide antibodies. The most suitable animal for the project is generally selected based on the presence of homologous proteins in the immunised species, the amount of peptide available for immunisation, the amount of antibody required, and the time needed for an antibody response. Assessing all the factors, the most common host for immunisation projects is the New Zealand White rabbit because rabbits can respond to a broader class of antigens and require a shorter period to produce good yields [112,121].

The antiserum obtained undergoes purification to prevent non-specific interactions with the other proteins. Purification to obtain the antipeptide antibodies may be performed by performing primary precipitation followed by chromatography or an antibody affinity purification using protein A or G [122,123].

1.6 Gold nanoparticles as colorimetric sensor

Any particle of matter with a diameter from 1 to 100 nm that behaves as a single unit regarding its transport and other properties is defined as a nanoparticle. Owing to their unique optical, photothermal and electrical properties, one of the most widely studied nanoparticles is gold nanoparticles (AuNPs) [124,125]. Colourimetric sensing by AuNPs depends on their interparticle distance-dependent localised surface plasmon resonance (LSPR) properties [125,126]. LSPR is explained by the absorption band from the radiation absorbed when the incident photon frequency resonates with the collective oscillation of conduction band electrons [125].

Synthesizing AuNPs is simple, and their versatile surface chemistry offers linkage capability to probes for chemical and biological target detection [127,128]. The main criteria for naked-eye colourimetric sensing are that AuNPs can confine their electrons, producing quantum effects that lead to visible surface charge modifications as colour changes from red/pink to blue. Thus, colourimetric sensing by AuNPs exploits the colour changes associated with their aggregation [124,125,129-131] (Fig. 1.6). External stimuli can initiate aggregation or dispersion of AuNPs, which is accompanied by a shift in the absorption spectrum, leading to colour changes of the colloidal solution. Thus, there has been a wide range of aggregation-based colourimetric assays for sensing proteins, small molecules, enzyme activities, inorganic ions, and oligonucleotides [132-137].

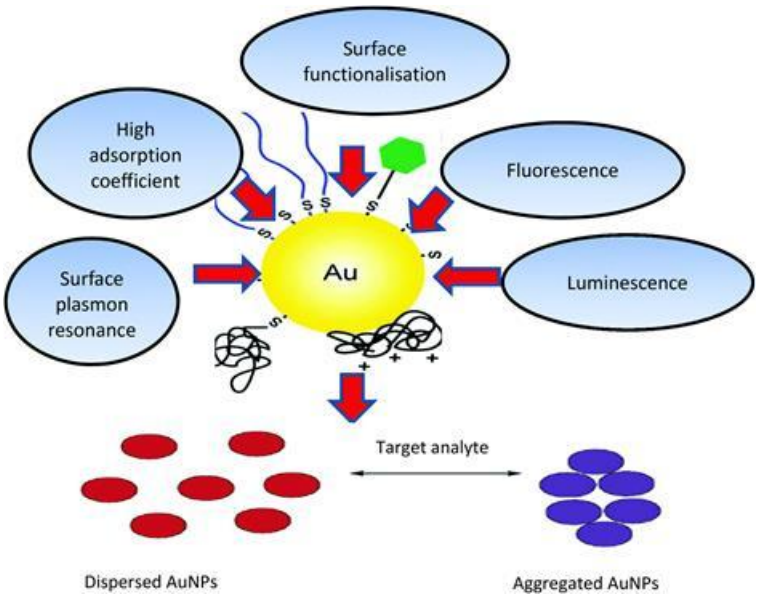

Fig. 1.6 Physical and chemical properties of AuNPs and schematic illustration of AuNPs (aggregation/dispersion) colourimetric-based detection systems. (Figure adapted from Aldewachi et. al., [125]).

1.7 Colorimetric assays

As the study of chemistry evolved, the scientific community discerned that specific chemical reactions involve solutions changing colours, and the colour intensity recorded may be used to determine the concentration of the analytes in the reaction. Further, colour vision theory led to colourimetric reactions becoming used for qualitative analysis [138-140].

Colourimetric assays play a significant role in producing sensors or sensing technologies that are simple (without any hefty instrumentations), miniature, rapid and cost-effective by relying on instantaneous visual detection of the analyte. The optical property, SPR, of nanoparticles is central to visual detection. There are numerous studies where colour change detection by colourimetric assays has been used [129,131,132,141].

Shifts in plasmon bands due to aggregation and dispersion of AuNPs result in colour change from red to blue and vice versa. Thus, the colourimetric assays are based on the controlled decrease of particle distance due to the target analyte [142-144]. The extent of aggregation of AuNPs is proportional to the absorption peak shift, which can be measured using a UV-Vis spectrophotometer, and the importance of signal change offers a quantitative measure of the aggregation inducer or the analyte. For example, AuNPs may have a plasmonic peak at 520 nm. Upon progressively increasing particle aggregation, the peak at 520 nm may gradually decrease, and a new peak appears at ~600-700 nm when colour of the solution changes from red to purple/blue. The extent of aggregation is quantified using the aggregation parameter, i.e., the ratio of maximum absorbance at the more extended and original wavelength (A_{600}/A_{520}) [125,145,146].

Another method for based colourimetric assays involves digital images captured by digital cameras, webcams, scanners, or smartphones, offering portable and in situ analysis [147-149]. Smartphone cameras initially divide the images captured into two-dimensional grids called pixels. The pixel values represented by colour spaces are processed using Android or iOS operating systems, resulting in the images displayed on smartphone screens [150]. For AuNP-based colourimetric assays where there is colour change due to analyte-reagent reaction, smartphone filters assign colours to each pixel responsible for recording the sample signal. The most frequently used filters are CMYK (cyan, magenta, yellow and black) or RGB (red, green, and blue). The RGB colour system, which mimics human vision, uses 3 matrices, R, G, and B, to store the colour intensity values of these components. These intensity values may be used to elucidate colour components and analyte concentration relationships [148,151].

1.8 Gap in the study

Snake envenomation constitutes an occupational health risk in underdeveloped nations, such as India, leading to both death and morbidity. The injection of antivenom is the sole treatment for snake envenomation, yet it is not without adverse effects. Despite the hazards linked to managing snakebites, there exists a deficiency of snake venom detection kits suitable for the clinical diagnosis of snake envenomation in rural regions with inadequate healthcare resources, which could assist patients in circumventing complications arising from unwarranted antivenom treatment.

Scorpion envenomation, a significant public health concern, is often identified by clinical symptoms, with the conventional treatment including intravenous scorpion antivenom and supportive symptomatic care. The Indian red scorpion, *Mesobuthus tamulus*, causes significant fatalities in India and Sri Lanka. Nonetheless, there is no definitive diagnostic technique to identify this scorpion's venom in envenomed plasma or bodily fluids, nor to quantify the venom administered to patients to establish an effective therapy regimen.

1.9 Aim of the present study

I. Characterisation of the specificity of antibodies produced against unique major toxin(s) and/or their peptide immunogens from specific Indian ‘Big Four’ snake venom, *Naja kaouthia* venom and Indian red scorpion venom for unambiguous detection of that particular venom.

II. Conjugation of the toxin-specific antibodies with nanoparticles and assessment of the increase in antibody-nanoparticle conjugate efficiency to detect specific unique toxin(s) in venom.

III. Development of analytical techniques for detecting species-specific venom toxin(s) in biological fluids for identification and/or clinical diagnosis of Indian ‘Big Four’ snake venom, *Naja kaouthia* venom and Indian red scorpion venom.

Bibliography

[1] World Health Organization, W. WHO Expert Committee on Biological Standardization: Sixty-Ninth Report. Technical Report, Geneva, 2019.

[2] Kasturiratne, A., Wickremasinghe, A. R., de Silva, N., Gunawardena, N. K., Pathmeswaran, A., Premaratna, R., Savioli, L., Lalloo, D. G., and De Silva, H. J. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Med*, 5(11): e218, 2008.

[3] Chippaux, J. P. Snake-bites: appraisal of the global situation. *Bull World Health Organ*, 76(5): 515, 1998.

[4] Mohapatra, B., Warrell, D. A., Suraweera, W., Bhatia, P., Dhingra, N., Jotkar, R. M., Rodriguez, P. S., Mishra, K., Whitaker, R., and Jha, P. Snakebite mortality in India: a nationally representative mortality survey. *PLoS Negl Trop Dis*, 5(4): e1018, 2011.

[5] Mukherjee, A. K. and Mackessy, S. P. Prevention and improvement of clinical management of snakebite in Southern Asian countries: a proposed road map. *Toxicon*, 200: 140-152, 2021.

[6] Bhargava, S., Kumari, K., Sarin, R., and Singh, R. Call for accurate statistical data to solve snakebite conundrum in India. *Asian Pac J Trop Med* 13(4): 141-142, 2020.

[7] Chanda, A. and Mukherjee, A. K. Mass spectrometric analysis to unravel the venom proteome composition of Indian snakes: Opening new avenues in clinical research. *Exp Rev Proteomics* 17(5): 411-423, 2020.

[8] Suraweera, W., Warrell, D., Whitaker, R., Menon, G., Rodrigues, R., Fu, S. H., Begum, R., Sati, P., Piyasena, K., and Bhatia, M. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. *Elife*, 9: e54076, 2020.

[9] Kalita, B. and Mukherjee, A. K. Recent advances in snake venom proteomics research in India: a new horizon to decipher the geographical variation in venom proteome composition and exploration of candidate drug prototypes. *J Proteins Proteomics*, 10: 149-164, 2019.

[10] Mukherjee, A. and Maity, C. Biochemical composition, lethality and pathophysiology of venom from two cobras—*Naja naja and N. kaouthia*. *Comp Biochem Physiol, Part B: Biochem Mol Biol*, 131(2): 125-132, 2002.

[11] Whitaker, R. *Common Indian snakes: a field guide*. Macmillan, 2006.

[12] Warrell, D. A., Gutiérrez, J. M., Calvete, J. J., and Williams, D. New approaches & technologies of venomics to meet the challenge of human envenoming by snakebites in India. *Indian J Med Res*, 138(1): 38-59, 2013.

[13] Ariaratnam, C., Thuraisingam, V., Kularatne, S., Sheriff, M., Theakston, R. D. G., De Silva, A., and Warrell, D. Frequent and potentially fatal envenoming by hump-nosed pit vipers (*Hypnale hypnale* and *H. nepa*) in Sri Lanka: lack of effective antivenom. *Trans R Soc Trop Med Hyg*, 102(11): 1120-1126, 2008.

[14] World Health Organization WHO, E. C. o. B. S. WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. Technical Report, WHO, Geneva, Switzerland, 2010.

[15] Chippaux, J.-P. Epidemiology of snakebites in Europe: a systematic review of the literature. *Toxicon*, 59(1): 86-99, 2012.

[16] Russell, F., Walter, F., Bey, T., and Fernandez, M. Snakes and snakebite in Central America. *Toxicon*, 35(10): 1469-1522, 1997.

[17] Menon, J. C., Bharti, O. K., Dhaliwal, R. S., John, D., Menon, G. R., Grover, A., and Chakma, J. K. ICMR task force project-survey of the incidence, mortality, morbidity and socio-economic burden of snakebite in India: A study protocol. *PLoS one*, 17(8): e0270735, 2022.

[18] Warrell, D. A. Guidelines for the Clinical Management of Snake Bites in the South-East Asia Region. Technical Report, South East Asia Regional Office, New Delhi, 2005.

[19] Warrell, D. A. Guidelines for the Management of Snake-Bites, World Health Organization. Technical Report, Geneva, Switzerland, 2010.

[20] Brunda, G., Rao, B. S., and Sarin, R. K. Quantitation of Indian krait (*Bungarus caeruleus*) venom in human specimens of forensic origin by indirect competitive inhibition enzyme-linked immunosorbent assay. *J AOAC Int*, 89(5): 1360-1366, 2006.

[21] Mukherjee, A. K., Kalita, B., Dutta, S., Patra, A., Maity, C. R., and Punde, D. Snake envenomation: therapy and challenges in India In S.P. Mackessy, *Handbook of Venoms and Toxins of Reptiles*, of, pages. CRC Press, USA, 2020.

[22] Bawaskar, H. Scorpion sting. *Trans R Soc Trop Med Hyg*, 78: 414-415, 1984.

[23] Chippaux, J.-P. and Goyffon, M. Epidemiology of scorpionism: a global appraisal. *Acta Trop*, 107(2): 71-79, 2008.

[24] Abroug, F., Ouanes-Besbes, L., Tilouche, N., and Elatrous, S. Scorpion envenomation: state of the art. *Int Care Med*

46(3): 401-410, 2020.

[25] Shah, P. T., Ali, F., Qayyum, S., Ahmed, S., Haleem, K. S., Tauseef, I., Hayat, A. H., Malik, A. A., Ramzan, R., and Khan, I. Scorpion venom: a poison or a medicine-mini review. *Indian J Mar Sci*, 47(04): 773-778, 2018.

[26] Goyffon, M. and Guette, C. Scorpions dangereux du Niger. *Bull. Soc. Pathol. Exot*, 98(4): 293-295, 2005.

[27] Bencheikh, R. S., Faraj, Z., Semlali, I., Ouammi, L., and Badri, M. Stratégie nationale de lutte contre les piqûres et. *Bull Soc Pathol Exot*, 96(4): 317-319, 2003.

[28] Chippaux, J. P. and Goyffon, M. Epidemiology of scorpionism: a global appraisal. *Acta tropica*, 107(2): 71-79, 2008.

[29] Benguedda, A., Laraba-Djébari, F., Ouahdi, M., Hellal, H., Griene, L., Guerenik, M., and Laid, Y. Expérience de quinze années de lutte contre l'envenimation scorpionique en Algérie. *Bull Soc Pathol Exot*, 95(3): 205-208, 2002.

[30] Mansour, N. Délai et caractéristiques de la prise en charge des piqués par scorpion dans la région de Sidi-Bouzid. *Arch Inst Pasteur de Tunis*, 78(1-4): 25-31, 2001.

[31] Njah, M., Abdelaziz, A. B., Abdouli, M., Zaher, M., and Garaoui, A. Health program and use of community health workers: the example of scorpion envenomation in Tunisia. *Cahiers Santé*, 11(1): 57-62, 2001.

[32] Bergman, N. J. Scorpion sting in Zimbabwe. *S Afr Med J*, 87(2): 163-167, 1997.

[33] Ozkan, O., Uzun, R., Adiguzel, S., Cesaretli, Y., and Ertek, M. Evaluation of scorpion sting incidence in Turkey. *J Venom Anim Toxins Incl Trop Dis*, 14: 128-140, 2008.

[34] Bentur, Y., Taitelman, U., and Aloufy, A. Evaluation of scorpion stings: the poison center perspective. *Vet Hum Toxicol*, 45(2): 108-111, 2003.

[35] Saúde, M. d. *Sistema de Informação de Agravos de Notificação (SINAN).* Brasília, 2018.

[36] Dehesa-Dávila, M. and Possani, L. D. Scorpionism and serotherapy in Mexico. *Toxicon*, 32(9): 1015-1018, 1994.

[37] de Roodt, A. R., García, S. I., Salomón, O. D., Segre, L., Dolab, J. A., Funes, R. F., and de Titto, E. H. Epidemiological and clinical aspects of scorpionism by *Tityus trivittatus* in Argentina. *Toxicon*, 41(8): 971-977, 2003.

[38] Dehesa-Dávila, M. Epidemiological characteristics of scorpion sting in Leon, Guanajuato, Mexico. *Toxicon*, 27(3): 281-286, 1989.

[39] Chowell, G., Díaz-Dueñas, P., Bustos-Saldaña, R., Mireles, A. A., and Fet, V. Epidemiological and clinical characteristics of scorpionism in Colima, Mexico (2000–2001). *Toxicon*, 47(7): 753-758, 2006.

[40] Dehghani, R. and Fathi, B. Scorpion sting in Iran: a review. *Toxicon*, 60(5): 919-933, 2012.

[41] Dehghani, R., Rafinejad, J., Fathi, B., Shahi, M. P., Jazayeri, M., and Hashemi, A. A retrospective study on Scropionism in Iran (2002–2011). *J Arthropod Borne Dis* 11(2): 194, 2017.

[42] Ahsan, M. M., Tahir, H. M., and Naqi, J. A. First report of scorpion envenomization in District Sargodha, Punjab, Pakistan. *Biologia*, 61(2): 279-285, 2015.

[43] Khan, M. F. and Ullah, H. Multi-organ dysfunction secondary to Yellow Scorpion Sting. *Journal of Ayub Medical College Abbottabad*, 29(2): 347-349, 2017.

[44] Badhe, R., Thomas, A., Deshpande, A., Salvi, N., and Waghmare, A. The action of red scorpion (*Mesobuthus tamulus coconsis,* Pocock) venom and its isolated protein fractions on blood sodium levels. *J Venom Anim Toxins Incl Trop Dis*, 13: 82-93, 2007.

[45] Das, B., Saviola, A. J., and Mukherjee, A. K. Biochemical and proteomic characterization, and pharmacological insights of indian red scorpion venom toxins. *Front. Pharmacol.*, 12: 710680, 2021.

[46] Bhadani, U., Tripathi, M., Sharma, S., and Pandey, R. Scorpion sting envenomation presenting with pulmonary edema in adults: a report of seven cases from Nepal. *Ind J Med Sci*, 60(1): 19-23, 2006.

[47] Kularatne, S. A., Dinamithra, N. P., Sivansuthan, S., Weerakoon, K. G., Thillaimpalam, B., Kalyanasundram, V., and Ranawana, K. B. Clinico-epidemiology of stings and envenoming of *Hottentotta tamulus* (Scorpiones: Buthidae), the Indian red scorpion from Jaffna Peninsula in northern Sri Lanka. *Toxicon*, 93: 85-89, 2015.

[48] Senthilvelan, M., Reddy, P. P. K., Resveen, H., Chandramohan, M., Santhosh, S., Abraham, D. J., Suganya, P., Senthilkumar, S., Kanagaraj, D., and Jayaram, K. A Case Report of Severe Acute Myocarditis and Pulmonary Odema Following Scorpion Sting. *Int J Modn Res Revs*, 3(10): 815-817, 2015.

[49] Bawaskar, H. and Bawaskar, P. Indian red scorpion envenoming. *Indian J Pediatr*, 65: 383-391, 1998.

[50] Yuvaraja, K., Chidambaram, N., Umarani, R., Bhargav, K. M., Kumar, S. P., Prabhu, T., and Balasubramaian, E. A study on clinical features, complications and

management of scorpion sting envenomation at a tertiary care hospital, in rural South India. *J Clin Sci Res*, 8(3): 140-144, 2019.

[51] Organization, W. H. *Rabies and envenomings: a neglected public health issue: report of a consultative meeting*. World Health Organization, Geneva, 2007.

[52] Williams, H. F., Layfield, H. J., Vallance, T., Patel, K., Bicknell, A. B., Trim, S. A., and Vaiyapuri, S. The urgent need to develop novel strategies for the diagnosis and treatment of snakebites. *Toxins*, 11(6): 363, 2019.

[53] Warrell, D. A. Snake bite. *The lancet*, 375(9708): 77-88, 2010.

[54] Warrell, D. A. Venomous bites, stings, and poisoning. *Infect Dis Clin North Am*, 26(2): 207-223, 2012.

[55] Alirol, E., Sharma, S. K., Bawaskar, H. S., Kuch, U., and Chappuis, F. Snake bite in South Asia: a review. *PLoS Negl Trop Dis*, 4(1): e603, 2010.

[56] Spiller, H. A. and Bosse, G. M. Prospective study of morbidity associated with snakebite envenomation. *J Toxicol Clin Toxicol*, 41(2): 125-130, 2003.

[57] Warrell, D., Davidson, N. M., Greenwood, B., Ormerod, L., Pope, H. M., Watkins, B. J., and Prentice, C. Poisoning by bites of the saw-scaled or carpet viper (*Echis carinatus*) in Nigeria. *QJM*, 46(1): 33-62, 1977.

[58] Sano-Martins, I., Fan, H. W., Castro, S., Tomy, S., França, F., Jorge, M., Kamiguti, A., Warrell, D., and Theakston, R. Reliability of the simple 20 minute whole blood clotting test (WBCT20) as an indicator of low plasma fibrinogen concentration in patients envenomed by *Bothrops* snakes. *Toxicon*, 32(9): 1045-1050, 1994.

[59] Isbister, G., Maduwage, K., Shahmy, S., Mohamed, F., Abeysinghe, C., Karunathilake, H., Ariaratnam, C., and Buckley, N. Diagnostic 20-min whole blood clotting test in Russell's viper envenoming delays antivenom administration. *QJM*, 106(10): 925-932, 2013.

[60] Ratnayake, I., Shihana, F., Dissanayake, D. M., Buckley, N. A., Maduwage, K., and Isbister, G. K. Performance of the 20-minute whole blood clotting test in detecting venom induced consumption coagulopathy from Russell's viper (*Daboia russelii*) bites. *Thromb Haemostasis*, 117(03): 500-507, 2017.

[61] Abroug, F., Ouanes-Besbes, L., Tilouche, N., and Elatrous, S. Scorpion envenomation: state of the art. *Intensive Care Med*, 46(3): 401-410, 2020.

[62] Isbister, G. K. and Bawaskar, H. S. Scorpion envenomation. *New England J Med*, 371(5): 457-463, 2014.

[63] Godoy, D. A., Badenes, R., Seifi, S., Salehi, S., and Seifi, A. Neurological and systemic manifestations of severe scorpion envenomation. *Cureus*, 13(4), 2021.

[64] Freire-Maia, L., Campos, J., and Amaral, C. Approaches to the treatment of scorpion envenoming. *Toxicon*, 32(9): 1009-1014, 1994.

[65] Bahloul, M., Chaari, A., Dammak, H., Samet, M., Chtara, K., Chelly, H., Hamida, C. B., Kallel, H., and Bouaziz, M. Pulmonary edema following scorpion envenomation: mechanisms, clinical manifestations, diagnosis and treatment. *Int J Cardiol*, 162(2): 86-91, 2013.

[66] Boyer, L. V., Theodorou, A. A., Berg, R. A., Mallie, J., Chávez-Méndez, A., García-Ubbelohde, W., Hardiman, S., and Alagón, A. Antivenom for critically ill children with neurotoxicity from scorpion stings. *N Engl J Med*, 360(20): 2090-2098, 2009.

[67] Bouaziz, M., Bahloul, M., Kallel, H., Samet, M., Ksibi, H., Dammak, H., Ahmed, M. N. B., Chtara, K., Chelly, H., and Hamida, C. B. Epidemiological, clinical characteristics and outcome of severe scorpion envenomation in South Tunisia: multivariate analysis of 951 cases. *Toxicon*, 52(8): 918-926, 2008.

[68] Bawaskar, H. and Bawaskar, P. Vasodilators: scorpion envenoming and the heart (an Indian experience). *Toxicon*, 32(9): 1031-1040, 1994.

[69] Pandi, K., Krishnamurthy, S., Srinivasaraghavan, R., and Mahadevan, S. Efficacy of scorpion antivenom plus prazosin versus prazosin alone for *Mesobuthus tamulus* scorpion sting envenomation in children: a randomised controlled trial. *Arch Dis Child*, 99(6): 575-580, 2014.

[70] Krifi, M. N., Kharrat, H., Zghal, K., Abdouli, M., Abroug, F., Bouchoucha, S., Dellagi, K., and El Ayeb, M. Development of an ELISA for the detection of scorpion venoms in sera of humans envenomed by *Androctonus australis garzonii* (Aag) and *Buthus occitanus tunetanus* (Bot): correlation with clinical severity of envenoming in Tunisia. *Toxicon*, 36(6): 887-900, 1998.

[71] D. Paniagua, I. Vergara, L. Boyer, and Alagón, A. Role of the lymphatic system on

snake venom absorption: review of mechanism and clinical implications. In P. Gopalakrishnakone, H. Inagaki, A.K. Mukherjee, T.R. Rahmy, & C.-W. Vogel, *Snake Venoms*, of, pages 454-469 Springer, Netherlands, 2014.

[72] Girish, K. and Kemparaju, K. Overlooked issues of snakebite management: time for strategic approach. *Curr Top Med Chem*, 11(20): 2494-2508, 2011.

[73] Revelo, M. P., Bambirra, E. A., Ferreira, A. P., Diniz, C. R., and Chávez-Olórtegui, C. Body distribution of *Tityus serrulatus* scorpion venom in mice and effects of scorpion antivenom. *Toxicon*, 34(10): 1119-1125, 1996.

[74] Krifi, M. N., Miled, K., Abderrazek, M., and El Ayeb, M. Effects of antivenom on *Buthus occitanus tunetanus* (Bot) scorpion venom pharmacokinetics: towards an optimization of antivenom immunotherapy in a rabbit model. *Toxicon*, 39(9): 1317-1326, 2001.

[75] Sutherland, S. K. and Leonard, R. L. Snakebite deaths in Australia 1992-1994 and a management update. *Med J Australia*, 163(11-12): 616-618, 1995.

[76] Steuten, J., Winkel, K., Carroll, T., Williamson, N. A., Ignjatovic, V., Fung, K., Purcell, A. W., and Fry, B. G. The molecular basis of cross-reactivity in the Australian Snake Venom Detection Kit (SVDK). *Toxicon*, 50(8): 1041-1052, 2007.

[77] Naik, B. S. "Dry bite" in venomous snakes: A review. *Toxicon*, 133: 63-67, 2017.

[78] Cox, J. C., Moisidis, A. V., Shepherd, J. M., Drane, D. P., and Jones, S. L. A novel format for a rapid sandwich EIA and its application to the identification of snake venoms. *J Immunol Methods*, 146(2): 213-218, 1992.

[79] Menezes, M. C., Furtado, M. F., Travaglia-Cardoso, S. R., Camargo, A. C., and Serrano, S. M. Sex-based individual variation of snake venom proteome among eighteen *Bothrops jararaca* siblings. *Toxicon*, 47(3): 304-312, 2006.

[80] Casewell, N. R., Wagstaff, S. C., Harrison, R. A., Renjifo, C., and Wüster, W. Domain loss facilitates accelerated evolution and neofunctionalization of duplicate snake venom metalloproteinase toxin genes. *Mol. Biol. Evol.*, 28(9): 2637-2649, 2011.

[81] Modahl, C. M., Mukherjee, A. K., and Mackessy, S. P. An analysis of venom ontogeny and prey-specific toxicity in the Monocled Cobra (*Naja kaouthia*). *Toxicon*, 119: 8-20, 2016.

[82] Kalita, B., Mackessy, S. P., and Mukherjee, A. K. Proteomic analysis reveals geographic variation in venom composition of Russell's Viper in the Indian subcontinent: implications for clinical manifestations post-envenomation and antivenom treatment. *Expert Rev Proteomics*, 15(10): 837-849, 2018.

[83] Chanda, A. and Mukherjee, A. K. Mass spectrometric analysis to unravel the venom proteome composition of Indian snakes: opening new avenues in clinical research. *Expert Review of Proteomics*, 17(5): 411-423, 2020.

[84] Tan, K. Y., Tan, C. H., Fung, S. Y., and Tan, N. H. Venomics, lethality and neutralization of Naja kaouthia (monocled cobra) venoms from three different geographical regions of Southeast Asia. *J Proteomics*, 120: 105-125, 2015.

[85] Sintiprungrat, K., Watcharatanyatip, K., Senevirathne, W., Chaisuriya, P., Chokchaichamnankit, D., Srisomsap, C., and Ratanabanangkoon, K. A comparative study of venomics of *Naja naja* from India and Sri Lanka, clinical manifestations and antivenomics of an Indian polyspecific antivenom. *J Proteomics*, 132: 131-143, 2016.

[86] Kalita, B., Singh, S., Patra, A., and Mukherjee, A. K. Quantitative proteomic analysis and antivenom study revealing that neurotoxic phospholipase A2 enzymes, the major toxin class of Russell's viper venom from southern India, shows the least immuno-recognition and neutralization by commercial polyvalent antivenom. *Int. J. Biol. Macromol*, 118: 375-385, 2018.

[87] Senji Laxme, R., Attarde, S., Khochare, S., Suranse, V., Martin, G., Casewell, N. R., Whitaker, R., and Sunagar, K. Biogeographical venom variation in the Indian spectacled cobra (*Naja naja*) underscores the pressing need for pan-India efficacious snakebite therapy. *PLoS Negl Trop Dis*, 15(2): e0009150, 2021.

[88] Choudhury, M., McCleary, R. J., Kesherwani, M., Kini, R. M., and Velmurugan, D. Comparison of proteomic profiles of the venoms of two of the 'Big Four'snakes of India, the Indian cobra (*Naja naja*) and the common krait (*Bungarus caeruleus*), and analyses of their toxins. *Toxicon*, 135: 33-42, 2017.

[89] Chanda, A. and Mukherjee, A. K. Quantitative proteomics to reveal the composition of Southern India spectacled cobra (*Naja naja*) venom and its immunological cross-reactivity towards commercial antivenom. *Int J Biol Macromol*, 160: 224-232, 2020.

[90] Dutta, S., Chanda, A., Kalita, B., Islam, T., Patra, A., and Mukherjee, A. K. Proteomic analysis to unravel the complex venom proteome of eastern India *Naja naja*: Correlation of venom composition with its biochemical and pharmacological properties. *J Proteomics*, 156: 29-39, 2017.

[91] Chanda, A., Patra, A., Kalita, B., and Mukherjee, A. K. Proteomics analysis to compare the venom composition between *Naja naja* and *Naja kaouthia* from the same geographical location of eastern India: Correlation with pathophysiology of

envenomation and immunological cross-reactivity towards commercial polyantivenom. *Expert review of proteomics*, 15(11): 949-961, 2018.

[92] Chanda, A., Kalita, B., Patra, A., Senevirathne, W. D. S. T., and Mukherjee, A. K. Proteomic analysis and antivenomics study of Western India *Naja naja* venom: correlation between venom composition and clinical manifestations of cobra bite in this region. *Expert review of proteomics*, 16(2): 171-184, 2019.

[93] Laxme, R. S., Khochare, S., de Souza, H. F., Ahuja, B., Suranse, V., Martin, G., Whitaker, R., and Sunagar, K. Beyond the 'big four': Venom profiling of the medically important yet neglected Indian snakes reveals disturbing antivenom deficiencies. *PLOS Negl. Trop. Dis.* , 13(12): e0007899, 2019.

[94] Deka, A., Gogoi, A., Das, D., Purkayastha, J., and Doley, R. Proteomics of *Naja kaouthia* venom from North East India and assessment of Indian polyvalent antivenom by third generation antivenomics. *J Proteomics*, 207: 103463, 2019.

[95] Pla, D., Sanz, L., Quesada-Bernat, S., Villalta, M., Baal, J., Chowdhury, M. A. W., León, G., Gutiérrez, J. M., Kuch, U., and Calvete, J. J. Phylovenomics of *Daboia russelii* across the Indian subcontinent. Bioactivities and comparative *in vivo* neutralization and *in vitro* third-generation antivenomics of antivenoms against venoms from India, Bangladesh and Sri Lanka. *J Proteomics*, 207: 103443, 2019.

[96] Sharma, M., Das, D., Iyer, J. K., Kini, R. M., and Doley, R. Unveiling the complexities of *Daboia russelii* venom, a medically important snake of India, by tandem mass spectrometry. *Toxicon*, 107: 266-281, 2015.

[97] Kalita, B., Patra, A., Das, A., and Mukherjee, A. K. Proteomic analysis and immuno-profiling of eastern India Russell's Viper (*Daboia russelii*) venom: correlation between RVV composition and clinical manifestations post RV bite. *J Proteome Res*, 17(8): 2819-2833, 2018.

[98] Kalita, B., Patra, A., and Mukherjee, A. K. Unraveling the proteome composition and immuno-profiling of western India Russell's viper venom for in-depth understanding of its pharmacological properties, clinical manifestations, and effective antivenom treatment. *J Proteome Res*, 16(2): 583-598, 2017.

[99] Patra, A., Kalita, B., Chanda, A., and Mukherjee, A. K. Proteomics and antivenomics of *Echis carinatus carinatus* venom: Correlation with pharmacological properties and pathophysiology of envenomation. *Sci Rep*, 7(1): 1-17, 2017.

[100] Vanuopadath, M., Sajeev, N., Murali, A. R., Sudish, N., Kangosseri, N., Sebastian, I. R., Jain, N. D., Pal, A., Raveendran, D., and Nair, B. G. Mass spectrometry-assisted

venom profiling of *Hypnale hypnale* found in the Western Ghats of India incorporating de novo sequencing approaches. *Int J Biol Macromol*, 118: 1736-1746, 2018.

[101] Vanuopadath, M., Shaji, S. K., Raveendran, D., Nair, B. G., and Nair, S. S. Delineating the venom toxin arsenal of Malabar pit viper (*Trimeresurus malabaricus*) from the Western Ghats of India and evaluating its immunological cross-reactivity and in vitro cytotoxicity. *Int J Biol Macromol*, 148: 1029-1045, 2020.

[102] Srairi-Abid, N., Othman, H., Aissaoui, D., and BenAissa, R. Anti-tumoral effect of scorpion peptides: Emerging new cellular targets and signaling pathways. *Cell calcium*, 80: 160-174, 2019.

[103] Possani, L. D., Becerril, B., Delepierre, M., and Tytgat, J. Scorpion toxins specific for Na+-channels. *Eur J Biochem*, 264(2): 287-300, 1999.

[104] Tytgat, J., Chandy, K. G., Garcia, M. L., Gutman, G. A., Martin-Eauclaire, M.-F., Van Der Walt, J. J., and Possani, L. D. A unified nomenclature for short-chain peptides isolated from scorpion venoms: α-KTx molecular subfamilies. *Trends Pharmacol Sci*, 20(11): 444-447, 1999.

[105] de la Vega, R. C. R. g. and Possani, L. D. Current views on scorpion toxins specific for K+-channels. *Toxicon*, 43(8): 865-875, 2004.

[106] Das, B., Patra, A., and Mukherjee, A. K. Correlation of venom toxinome composition of Indian red scorpion (*Mesobuthus tamulus*) with clinical manifestations of scorpion stings: failure of commercial antivenom to immune-recognize the abundance of low molecular mass toxins of this venom. *J. Proteome Res.*, 19(4): 1847-1856, 2020.

[107] Saravanan, P., Kataria, J., and Rasool, T. Detection of Infectious bursal disease virus by ELISA using an antipeptide antibody raised against VP3 region. *Acta Virol*, 48(1): 39-45, 2004.

[108] Lee, B.-S., Huang, J.-S., Jayathilaka, L. P., Lee, J., and Gupta, S. Antibody production with synthetic peptides. In Steven D. Schwartzbach, Omar Skalli, & Thomas Schikorski, *High-Resolution Imaging of Cellular Proteins: Methods Protocols*, 1474 of, pages 25-47, 978-1-4939-6352-2. Humana New York, NY, New York, 2016.

[109] Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., and Zhang, Y. The I-TASSER Suite: protein structure and function prediction. *Nat Methods*12(1): 7-8, 2015.

[110] Thornton, J., Edwards, M., Taylor, W., and Barlow, D. Location of 'continuous' antigenic determinants in the protruding regions of proteins. *EMBO*, 5(2): 409-413, 1986.

[111] Shinnick, T. M., Sutcliffe, J. G., Green, N., and Lerner, R. A. Synthetic peptide immunogens as vaccines. *Annu Rev Microbiol*, 37: 425-446, 1983.

[112] Trier, N. H., Hansen, P., and Houen, G. Production and characterization of peptide antibodies. *Methods*, 56(2): 136-144, 2012.

[113] Rubinstein, N. D., Mayrose, I., Halperin, D., Yekutieli, D., Gershoni, J. M., and Pupko, T. Computational characterization of B-cell epitopes. *Mol Immunol*, 45(12): 3477-3489, 2008.

[114] Hancock, D. C. and OReilly, N. J. Synthetic peptides as antigens for antibody production. In R Burns, *Immunochemical Protocols. Methods In Molecular Biology™*, 295 of, pages 13-25, 1588292746. Humana Press, 2005.

[115] Atherton, E., Fox, H., Harkiss, D., Logan, C., Sheppard, R., and Williams, B. A mild procedure for solid phase peptide synthesis: use of fluorenylmethoxycarbonylamino-acids. *J Chem Soc, Chem Commun*, (13): 537-539, 1978.

[116] Hansen, P. R. and Oddo, A. Fmoc solid-phase peptide synthesis. In G. Houen, *Peptide Antibodies. Methods in Molecular Biology*, 1348 of, pages 33-50, 1493929984. Humana Press, New York, NY, 2015.

[117] Van Regenmortel, M. and Muller, S. Synthetic Peptides as Antigens. In, *Laboratory techniques in biochemistry and molecular biology*, of, pages 1-381. Amsterdam : Elsevier, 1999.

[118] Denery-Papini, S., Popineau, Y., Quillien, L., and Van Regenmortel, M. Specificity of antisera raised against synthetic peptide fragments of highMrglutenin subunits. *J Cereal Sci* 23(2): 133-144, 1996.

[119] Van Regenmortel, M. H. Antigenicity and immunogenicity of synthetic peptides. *Biologicals*, 29(3-4): 209-213, 2001.

[120] Helling, F., Zhang, S., Shang, A., Adluri, S., Calves, M., Koganty, R., Longenecker, B. M., Yao, T.-J., Oettgen, H. F., and Livingston, P. O. GM2-KLH conjugate vaccine: increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21. *Cancer Res*, 55(13): 2783-2788, 1995.

[121] Lee, B.-S., Huang, J.-S., Jayathilaka, G. L. P., Lateef, S. S., and Gupta, S. Production of antipeptide antibodies. In S Schwartzbach & T Osafune, *Immunoelectron Microscopy. Methods in Molecular Biology*, 657 of, pages 93-108, 160761782X. Humana Press, Totowa, NJ, 2010.

[122] Steward, M. and Petty, R. The use of ammonium sulphate globulin precipitation for determination of affinity of anti-protein antibodies in mouse serum. *Immunology*, 22(5): 747, 1972.

[123] Eliasson, M., Andersson, R., Olsson, A., Wigzell, H., and Uhlén, M. Differential IgG-binding characteristics of staphylococcal protein A, streptococcal protein G, and a chimeric protein AG. *J Immunol* 142(2): 575-581, 1989.

[124] Sabela, M., Balme, S., Bechelany, M., Janot, J. M., and Bisetty, K. A review of gold and silver nanoparticle-based colorimetric sensing assays. *Adv Eng Mater*, 19(12): 1700270, 2017.

[125] Aldewachi, H., Chalati, T., Woodroofe, M., Bricklebank, N., Sharrack, B., and Gardiner, P. Gold nanoparticle-based colorimetric biosensors. *Nanoscale*, 10(1): 18-33, 2018.

[126] Baptista, P., Pereira, E., Eaton, P., Doria, G., Miranda, A., Gomes, I., Quaresma, P., and Franco, R. Gold nanoparticles for the development of clinical diagnosis methods. *Anal Bioanal Chem*, 391: 943-950, 2008.

[127] Chen, Y., Xianyu, Y., and Jiang, X. Surface modification of gold nanoparticles with small molecules for biochemical analysis. *Acc Chem Res*, 50(2): 310-319, 2017.

[128] Cao-Milán, R. and Liz-Marzán, L. M. Gold nanoparticle conjugates: recent advances toward clinical applications. *Expert Opin Drug Deliv*, 11(5): 741-752, 2014.

[129] Schofield, C. L., Haines, A. H., Field, R. A., and Russell, D. A. Silver and gold glyconanoparticles for colorimetric bioassays. *Langmuir*, 22(15): 6707-6711, 2006.

[130] Chang, C.-C., Chen, C.-P., Wu, T.-H., Yang, C.-H., Lin, C.-W., and Chen, C.-Y. Gold nanoparticle-based colorimetric strategies for chemical and biological sensing applications. *Nanomaterials*, 9(6): 861, 2019.

[131] Jazayeri, M. H., Aghaie, T., Avan, A., Vatankhah, A., and Ghaffari, M. R. S. Colorimetric detection based on gold nano particles (GNPs): An easy, fast, inexpensive, low-cost and short time method in detection of analytes (protein, DNA, and ion). *Sens. Bio-sens. Res.*, 20: 1-8, 2018.

[132] Yue, G., Su, S., Li, N., Shuai, M., Lai, X., Astruc, D., and Zhao, P. Gold nanoparticles as sensors in the colorimetric and fluorescence detection of chemical warfare agents. *Coord Chem Rev*, 311: 75-84, 2016.

[133] Wang, F., Lu, Y., Yang, J., Chen, Y., Jing, W., He, L., and Liu, Y. A smartphone readable colorimetric sensing platform for rapid multiple protein detection. *Analyst*, 142(17): 3177-3182, 2017.

[134] Liu, G., Zhang, R., Li, L., Huang, X., Li, T., Lu, M., Xu, D., and Wang, J. Anti-agglomeration behavior and sensing assay of chlorsulfuron based on acetamiprid-gold nanoparticles. *Nanomaterials*, 8(7): 499, 2018.

[135] Chang, C.-C., Lee, C.-H., Wu, T.-H., Chen, C.-P., Chen, C.-Y., and Lin, C.-W. Reversion of gold nanoparticle aggregates for the detection of Cu 2+ and its application in immunoassays. *Analyst*, 142(24): 4684-4690, 2017.

[136] Wu, B., Zou, F., Wang, X., Koh, K., Wang, K., and Chen, H. The colorimetric assay of diamine oxidase activity with high sensitivity based on calixarene derivative-capped gold nanoparticles. *Anal Methods*, 9(14): 2153-2158, 2017.

[137] Zou, L., Shen, R., Ling, L., and Li, G. Sensitive DNA detection by polymerase chain reaction with gold nanoparticles. *Anal Chim Acta*, 1038: 105-111, 2018.

[138] Beer. Bestimmung der Absorption des rothen Lichts in farbigen Flüssigkeiten. *Ann Phys*, 162(5): 78-88, 1852.

[139] Müller, A. Neues colorimeter. *J Für Prakt Chem*, 60(1): 474-476, 1853.

[140] Ohta, N. and Robertson, A. *Colorimetry: fundamentals and applications*. John Wiley & Sons, 1-329, 2006.

[141] Oliveira, J. P., Prado, A. R., Keijok, W. J., Antunes, P. W. P., Yapuchura, E. R., and Guimarães, M. C. C. Impact of conjugation strategies for targeting of antibodies in gold nanoparticles for ultrasensitive detection of 17β-estradiol. *Sci. Rep.*, 9(1): 13859, 2019.

[142] Tang, L. and Li, J. Plasmon-based colorimetric nanosensors for ultrasensitive molecular diagnostics. *ACS sensors*, 2(7): 857-875, 2017.

[143] Peng, C., Duan, X., Khamba, G. W., and Xie, Z. Highly sensitive "signal on" plasmonic ELISA for small molecules by the naked eye. *Anal Methods*, 6(24): 9616-9621, 2014.

[144] Mauriz, E. Clinical applications of visual plasmonic colorimetric sensing. *Sensors*, 20(21): 6214, 2020.

[145] Baptista, P. V., Koziol-Montewka, M., Paluch-Oles, J., Doria, G. a., and Franco, R. Gold-nanoparticle-probe–based assay for rapid and direct detection of *Mycobacterium tuberculosis* DNA in clinical samples. *Clin Chem*, 52(7): 1433-1434, 2006.

[146] VS, A. P., Joseph, P., SCG, K. D., Lakshmanan, S., Kinoshita, T., and Muthusamy, S. Colorimetric sensors for rapid detection of various analytes. *Mater Sci Eng C*, 78: 1231-1245, 2017.

[147] Fernandes, G. M., Silva, W. R., Barreto, D. N., Lamarca, R. S., Gomes, P. C. F. L., da S Petruci, J. F., and Batista, A. D. Novel approaches for colorimetric measurements in analytical chemistry–a review. *Anal Chim Acta*, 1135: 187-203, 2020.

[148] Aqillah, F., Permana, M. D., Eddy, D. R., Firdaus, M. L., Takei, T., and Rahayu, I. Detection and quantification of Cu2+ ion using gold nanoparticles via Smartphone-based digital imaging colorimetry technique. *Results Chem*, 7: 101418, 2024.

[149] Solra, M., Das, S., and Rana, S. Point-of-care detection of hydroxyurea drug in serum using a supramolecular enzyme mimetic. *Sens Actuators B: Chem*, 406: 135424, 2024.

[150] Albizu, G., Bordagaray, A., Davila, S., Garcia-Arrona, R., Ostra, M., and Vidal, M. Analytical control of nickel coating baths by digital image analysis. *Microchem J*, 154: 104600, 2020.

[151] dos Santos Benedetti, L. P., dos Santos, V. B., Silva, T. A., Benedetti Filho, E., Martins, V. L., and Fatibello-Filho, O. A digital image-based method employing a spot-test for quantification of ethanol in drinks. *Anal Methods*, 7(10): 4138-4144, 2015.