PUBLICATIONS

A. Publications in peer-reviewed international journals from thesis work

1. **Puzari, U.,** & Mukherjee, A. K. (2020). Recent developments in diagnostic tools and bioanalytical methods for analysis of snake venom: A critical review. ***Analytica Chimica Acta***, 1137, 208-224. ***IF - 5.7.***
2. **Puzari, U.**, Khan, M. R., & Mukherjee, A. K. (2024). Development of a gold nanoparticle-based novel diagnostic prototype for in vivo detection of Indian red scorpion (*Mesobuthus tamulus*) venom. ***Toxicon: X***, 23, 100203. ***IF- 3.6.***
3. **Puzari, U.,** Das, B., & Mukherjee, A. K. (2024). Advancements in Diagnostic Techniques for Scorpion Venom Identification: A Comprehensive Review. ***Toxicon****,* 253, 108191. ***IF - 2.6.***
4. **Puzari, U.,** Khan, M. R., & Mukherjee, A. K. (2025). Diagnosis of Indian Big Four and monocled Cobra snakebites in envenomed plasma using smartphone-based digital imaging colorimetry method. ***PLOS Neglected Tropical Diseases,*** 19(3), e0012913. ***IF - 3.4.***

B. Indian Patents Filed

1. Mukherjee, A. K., **Puzari, U.**, and Khan, M. R. Indian Patent on "**Antibodies for the Detection of Indian Red Scorpion Venom and Composition Thereof**" Patent application no. 202331015487 filed on 08.03.2023 (Granted; Patent Number 563930).
2. Mukherjee, A. K., **Puzari, U.**, and Khan, M. R. Indian Patent on "**Antibodies for the Detection of Indian Snake Venoms and Composition Thereof**" Patent application no. 202431065376 filed on 29.08.2024 (Application not yet published).

C. Publications in National and International conferences

1. **Puzari, U.,** and Mukherjee, A. K. (2022) "Snakebite Diagnosis: an Age Old Dilemma and an Insight on the Current Aspects in Research and Development of Tools and Bio-analytical Methods for Snake Venom Detection" at the **14th Annual Meeting of the Proteomics Society, India and International**

Conference on Proteins & Proteomics (PSI-ICPP 2022), CSIR-Indian Institute of Chemical Biology, Kolkata, 03-05, November, 2022.

2. **Puzari, U.,** and Mukherjee, A. K. (2024) "Snake Venom Diagnosis: Insight on the Current Scenario**"** at the **2-Day Symposium on From Venom Pharmacology to Drug Discovery: National and International perspective (SnakeSymp-2024**), Institute of Advanced Study in Science and Technology, Guwahati, Assam, 09-10, February, 2024.
3. **Puzari, U.,** Khan, M. R., and Mukherjee, A. K. (2024) "Gold nanoparticle-based novel diagnostic prototype for in vivo detection of Indian red scorpion (*Mesobuthus tamulus*) venom" at the **National Conference on Polymers and Advanced Functional Materials (NCPAFM 2024)**, Institute of Advanced Study in Science and Technology, Guwahati, Assam, 13-14 December, 2024.

D. Other publications in peer-reviewed international journals

1. **Puzari, U.**, Fernandes, P. A., & Mukherjee, A. K. (2021). Advances in the therapeutic application of small-molecule inhibitors and repurposed drugs against snakebite: Miniperspective. ***Journal of Medicinal Chemistry***, 64(19), 13938-13979. ***IF – 6.9.***

2. **Puzari, U.**, Fernandes, P. A., & Mukherjee, A. K. (2022). Pharmacological re-assessment of traditional medicinal plants-derived inhibitors as antidotes against snakebite envenoming: A critical review. ***Journal of Ethnopharmacology***, 292, 115208. ***IF – 4.8.***

3. **Puzari, U.,** Goswami, M., Rani, K., Patra, A., & Mukherjee, A. K. (2023). Computational and *in vitro* analyses to identify the anticoagulant regions of Echicetin, a snake venom anticoagulant C-type lectin (snaclec): possibility to develop anticoagulant peptide therapeutics? ***Journal of Biomolecular Structure and Dynamics***, 41(24), 15569-15583**.** ***IF-2.7.***

4. Das, B., Patra, A., **Puzari, U.,** Deb, P., & Mukherjee, A. K. (2022). *In vitro* laboratory analyses of commercial anti-scorpion (*Mesobuthus tamulus*)

antivenoms reveal their quality and safety but the prevalence of a low proportion of venom-specific antibodies. ***Toxicon***, 215, 37-48. ***IF- 2.6.***

5. Das, B., Madhubala, D., Mahanta, S., Patra, A., **Puzari, U.**, Khan, M. R., & Mukherjee, A. K. (2023). A Novel Therapeutic Formulation for the Improved Treatment of Indian Red Scorpion (*Mesobuthus tamulus*) Venom-Induced Toxicity-Tested in *Caenorhabditis elegans* and Rodent Models. ***Toxins***, 15(8), 504. ***IF – 4.2.***

6. Chanda, A., Salvi, N. C., Shelke, P. V., Kalita, B., Patra, A., **Puzari, U**., Khadilkar, M.V. & Mukherjee, A. K. (2024). Supplementation of polyclonal antibodies, developed against epitope-string toxin-specific peptide immunogens, to commercial polyvalent antivenom, shows improved neutralization of Indian Big Four and *Naja kaouthia* snake venoms. ***Toxicon: X***, 24, 100210. ***IF- 3.6.***

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Review

Recent developments in diagnostic tools and bioanalytical methods for analysis of snake venom: A critical review

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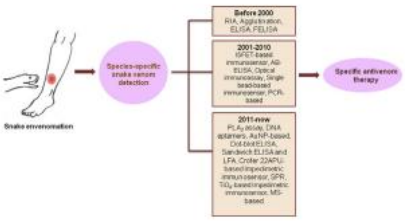
HIGHLIGHTS

- Concerns involved in developing effective snake venom diagnostic kit underlined.
- Developments in snake venom detection assays till most recent are summarized.
- The pros and cons of the lone diagnostic kit available are discussed.
- Practical hurdles in commercialization of diagnostic kits in rural areas are summarized.

GRAPHICAL ABSTRACT



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ABSTRACT

Snakebite is a neglected medical emergency causing fatalities and long-term disabilities throughout the world, especially in tropical countries. The effectiveness of therapy against snakebite is reliant on the unambiguous identification of bitten species of snake followed by immediate administration of venom-specific monovalent antivenom. However, this is a challenging task and therefore, over the several years scientists are constantly trying to address this issue by developing species-specific snake venom diagnostic kits as an alternative to classical methods of snake identification in clinics. Recently quite a few modern tools and techniques have been deployed for the development of simple, inexpensive, rapid, specific, and sensitive snake venom detection kits. However, despite these efforts a lone snakebite diagnostic kit is available until now which is a severe concern for efficacious snakebite therapy. In this article, we have reviewed the key issues pertaining to the rapid diagnosis of snake envenomation, tools and techniques developed and/or invented particularly over the past 40 years for the detection of snakebite as well as quantity of venom in the body fluids and/or tissues of victims. To overcome the practical constraints against the successful commercialization of these diagnostic kits, much more intensive studies for their improvement in terms of efficacy, affordability, storage stability, and usability, in addition to standardization of techniques for use in clinics are required to fulfil the objectives of the user-friendliness and commercial viability of snakebite diagnostic kits particularly in the rural and underdeveloped areas of tropical countries showing the maximum incidence of snakebite.

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Development of a gold nanoparticle-based novel diagnostic prototype for *in vivo* detection of Indian red scorpion (*Mesobuthus tamulus*) venom

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ABSTRACT

Indian red scorpion *Mesobuthus tamulus* is responsible for substantial mortality in India and Sri Lanka; however, no specific diagnostic method is available to detect the venom of this scorpion in envenomed plasma or body fluid. Therefore, we have proposed a novel, simple, and rapid method for detecting *M. tamulus* venom (MTV) in the plasma of envenomed animals using polyclonal antibodies (PAb) raised against three modified custom peptides representing the antigenic epitopes of K^+ (Tamapin) and Na^+ (α-neurotoxin) channel toxins, the two major MTV toxins identified by proteomic analysis. The optimum PAb formulation containing PAb 1, 2, and 3 in proportion (1:1:1, w/w/w) acted synergistically, demonstrating significantly higher immunological recognition of MTV than anti-scorpion antivenom (developed against native toxins) and individual antibodies against peptide immunogens. The PAb formulation could detect MTV optimally in envenomed rat plasma (intravenous and subcutaneous routes) at 30–60 min post-injection. The acetonitrile precipitation method developed in this study to augment the MTV detection sensitivity enriched the low molecular mass peptide toxins in envenomed rat plasma, which was ascertained by mass spectrometry analysis. The gold nanoparticles conjugated PAb formulation, characterised by biophysical techniques such as Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM), demonstrated their interaction with low molecular mass MTV peptide toxins in envenomed rat plasma. This interaction results in the accumulation of the gold nanoparticles, thus leading to signal change in absorbance spectra that can be discerned within 10 min. From a standard curve of MTV spiked plasma, the quantity of MTV in envenomed rat plasma could be determined by gold nanoparticle-PAb formulation conjugate.

1. Introduction

Across the globe, approximately 1.23 million scorpion stings worldwide result in about 3250 deaths yearly; therefore, scorpion sting envenomation is considered a neglected public health disease in tropical and sub-tropical countries (Bawaskar, 1984; Chippaux and Goyffon, 2008). Among the scorpion species found in India, only the Indian red scorpion (*Mesobuthus tamulus*) and Indian black scorpion (*Heterometrus bengalensis*) pose a significant threat to humans; however, clinical reports show that the *M. tamulus* venom (MTV) is more toxic compared to any venomous scorpion in this subcontinent (Badhe et al., 2007; Bawaskar, 1984; Bhadani et al., 2006; Das et al., 2021; Kularatne et al., 2015; Senthilvelan et al., 2015).

Most of those affected by scorpion envenomation are elderly adults, immuno-compromised individuals, and small children whose immune systems are still maturing (Badhe et al., 2007; Das et al., 2020; Laustsen et al., 2016; Ortiz et al., 2015; Santos et al., 2016; Tiwari and Deshpande, 1993). 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. Unfortunately, there is no commercial diagnostic tool or technique for identifying scorpion venom in human bodily fluids like blood. As a result, doctors mostly follow the traditional diagnosis approach, which concentrates on the clinical signs of scorpion stings (Ailani et al., 1999; Sivak et al., 1983; Sofer and Gueron, 1988). However, because the symptoms of the affected patients change gradually, these diagnosis methods may result in an inadequate treatment plan (Krifi et al., 1998).

Administration of anti-scorpion antivenom (ASA) and alpha-adrenergic receptor inhibitors such as prazosin have been used to treat

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Advancements in diagnostic techniques for scorpion venom identification: A comprehensive review

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ABSTRACT

Scorpion envenomation's ignored public health problem in tropical and subtropical countries is alarming. Particularly dangerous for small children and the elderly, it can cause severe problems and even death. Recent studies have proposed the creation of rapid, easy, species-specific, and sensitive detection kits as an alternative to the methods currently used to identify scorpions. Unfortunately, there is currently no commercially available technology for detecting scorpion envenomation in clinical settings, especially in remote tropical health centres. This study delineates the most dangerous scorpion species globally and the advancements in identifying their stings in vitro or in envenomed plasma. Furthermore, we have highlighted the practical challenges associated with scorpion venom detection and the necessity for innovative, expedited, and more accessible detection kits in countries where scorpion envenomation poses a significant issue.

1. Introduction

Scorpion envenomation, a medical emergency posing severe threats to life, is a neglected public health concern in Latin America, North Africa, the Middle East, and India (Bawaskar and Bawaskar, 2011; Reddy, 2013). Globally, annual reports of scorpion stings are estimated at 1.2 million, leading to about 3250 fatalities (Abroug et al., 2020; Chippaux and Goyffon, 2008). Among the 86 scorpion species found throughout India, only the Indian red scorpion (*Mesobuthus tamulus*) and the Indian black scorpion (*Heterometrus swammerdami*) belonging to the family Buthidae and Scorpionidae family, respectively pose a significant threat to humans (Badhe et al., 2007; Das et al., 2021; Reddy, 2013; Tiwari and Deshpande, 1993). The distribution of medically important scorpion species worldwide is depicted in Fig. 1.

The known examples of scorpion stings indicate that scorpions belonging to the Buthidae, Hemiscorpiidae, and Scorpionidae families are medically significant or may cause harm to humans (Hauke and Herzig, 2017; Lourenço, 2018; Ward et al., 2018; White, 2016). Nevertheless, the precise documentation of worldwide occurrences of scorpion stings is unclear because of inadequate infrastructure, limited availability of healthcare services, and the absence of a well-structured central registration record. They are implementing a resource-constrained rural system (Abroug et al., 2020).

Scorpion envenomation management involves antivenom therapy and supportive treatment against hypertension, pulmonary oedema, and cardiogenic shock (Bawaskar and Bawaskar, 1994; Chippaux and Goyffon, 2008; Deshpande et al., 2008). However, considering the complications associated with these treatments, a diagnosis method to detect and assess the toxins in the patient's body fluids is essential for efficient therapy against scorpion stings (Mars et al., 2018; Puzari et al., 2024). In this study, we have reviewed the most dangerous scorpion species found around the world that cause the most harm to humans and have provided an analysis of the research conducted on developing techniques or kits for detecting the venom of these scorpion species. It focuses on the identified scorpion species, the limit of detection, and the time required for detection. Our analysis emphasizes the practical constraints of accurately detecting scorpion venom in the bloodstream.

2. Methods

Through a comprehensive search of public databases such as PubMed, Google Scholar, and Web of Science, we identified published papers until September 30, 2024. The identified search terms were

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PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Diagnosis of Indian Big Four and monocled Cobra snakebites in envenomed plasma using smartphone-based digital imaging colourimetry method

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Abstract

Background

Venomous or dry bites can result from snake envenomation. Therefore, developing a detection test for venomous snakebites in envenomed patients can prevent from unnecessary antivenom therapy for dry bites, thereby, saving them from adverse effects and cost of antivenom therapy.

Methodology

This study demonstrates a method for the diagnosis of medically significant 'Big Four' Indian snake venoms (*Naja naja, Bungarus caeruleus, Daboia russelii, Echis carinatus*) in the plasma of experimentally envenomed animals (envenomed under laboratory conditions). Rabbit polyclonal antibodies (PAbs) were produced by generating modified bespoke peptides identified by computational analysis from the antigenic sites of the main toxins found in the proteome of India's 'Big Four' venomous snakes. The polyclonal antibody formulation (FPAb) prepared by mixing the five representative PAbs in the ratio of 1:1:1:1:1 demonstrated synergistic immune recognition of the 'Big Four' snakes and *Naja kaouthia* venoms. The recognition for these venoms under *in vitro* and *in vivo* conditions by FPAb was significantly higher ($p<0.05$) than commercial polyvalent antivenom produced against native venom toxins. The FPAb was tested to detect the venoms in subcutaneously envenomed rat plasmas until 240 minutes post-injection. Fourier-transform infrared spectroscopy, zeta potential, transmission electron microscopy, and atomic force microscopy characterised gold nanoparticles (AuNP) conjugated with FPAb. The FPAb-conjugated AuNP demonstrated aggregation upon interaction with venom toxins, changing the colour from red through burgundy to blue, monitored using a smartphone. From the digital image colourimetry analysis of the images, calibration curves for venoms were obtained, and each venom in the envenomed plasma at different time intervals was quantified using these curves.

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पेटेंट प्रमाण पत्र | Patent Certificate

(पेटेंट नियमावली का नियम 74) | (Rule 74 of The Patents Rules)

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आविष्कारकों का नाम /Name of Inventor(s)	:	1.MUKHERJEE, ASHIS KUMAR 2.PUZARI, UPASANA 3.KHAN, MOJIBUR R.

प्रमाणित किया जाता है कि पेटेंटी को, उपरोक्त आवेदन में यथाप्रकटित *ANTIBODIES FOR THE DETECTION OF INDIAN RED SCORPIONVENOM AND COMPOSITION THEREOF* नामक आविष्कार के लिए, पेटेंट अधिनियम, 1970 के उपबंधों के अनुसार आज तारीख मार्च 2023 के आठवें दिन से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled *ANTIBODIES FOR THE DETECTION OF INDIAN RED SCORPIONVENOM AND COMPOSITION THEREOF* as disclosed in the above mentioned application for the term of 20 years from the 8th day of March 2023 in accordance with the provisions of the Patents Act,1970.

अनुदान की तारीख : 27/03/2025
Date of Grant :

पेटेंट नियंत्रक
Controller of Patents

टिप्पणी - इस पेटेंट के नवीकरण के लिए शीर्ष, यदि देय करार रखा जाना है, वर्ष 2025 के आठवें दिन को और उसके पश्चात प्रत्येक वर्ष में उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained, will fall / has fallen due on 8th day of March 2025 and on the same day in every year thereafter.

APPENDIX

Appendix table A1. List of proteins identified in LMMPT-enriched *M. tamulus* venom treated plasma by LC-MS/MS analysis followed by database search against Buthidae family (taxid: 6855) protein entries of the non-redundant NCBI databases.

Accession No.	Protein Description
P0C175	Potassium channel toxin epsilon-KTx 1.2 OS=Tityus serrulatus OX=6887 PE=1 SV=1
A0A2I9LNI8	Actin OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A510ACK2	Dscam11 OS=Mesobuthus martensii OX=34649 PE=2 SV=1
A0A2I9LNY0	Elongation factor 1-alpha OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A2I9LPV8	Tubulin alpha chain OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A2I9LP59	Malate dehydrogenase OS=Centruroides hentzi OX=88313 PE=3 SV=1

Appendix table A2. List of proteins identified in *M. tamulus* venom treated plasma by LC-MS/MS analysis followed by database search against *M. tamulus* (taxid: 34647) protein entries of the non-redundant NCBI databases.

Accession No.	Protein Description
P82811	Insect toxin BsIT1 (sodium channel inhibitor)

Appendix table A3. List of proteins identified in Control plasma (untreated with MTV) by LC-MS/MS analysis followed by database search against Buthidae family (taxid: 6855) protein entries of the non-redundant NCBI databases.

Accession No.	Protein Description
A0A2I9LNY0	Elongation factor 1-alpha OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A2I9LNI8	Actin OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A8K1NFR4	Triosephosphate isomerase OS=Androctonus crassicauda OX=122909 PE=2 SV=1
A0A2I9LPV8	Tubulin alpha chain OS=Centruroides hentzi OX=88313 PE=3 SV=1
A0A0U1SA87	Histone H4 (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1
A0A2I9LPE3	Paramyosin OS=Centruroides hentzi OX=88313 PE=4 SV=1
W0I6I5	Cytochrome c oxidase subunit 1 (Fragment) OS=Buthus sp. D DP-2013 OX=1441938 GN=CO1 PE=3 SV=1
A0A0U1SST2	Uncharacterized protein (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1