# ABSTRACT

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The scorpion is one of the earliest animals to acclimate to the terrestrial environment fully. Due to the scarcity of their early fossils, it has been challenging to provide definitive answers to critical concerns, such as when and how they evolved to live on land. Scorpions first appeared as aquatic organisms during the Silurian (approximately 450 million years ago) and have experienced few morphological changes since then. The evolutionary history of the scorpion has been further revealed by peptides discovered in its venom.

Although scorpions are present on all continents except Antarctica, the severity and incidence of envenoming are higher in northern Saharan Africa, African Sahel, South Africa, the Middle East, Southern India, Mexico, Brazil, and the Amazon basin area. However, there is a lack of reports of symptoms induced by the venom of most of these scorpion species. Scorpionism is a severe public health issue in many parts of the world, as scorpion stings are often severe and lethal to humans and cause severe health complications. The incidence and severity of scorpionism vary significantly by geographical location. Thus, identifying the local population's specific concerns and the significant risk factors is essential. Around 95% of the scorpion stings are caused by species of the family Buthidae C. L. Koch (Buthidae family was established by Carl Ludwig Koch in 1837), which includes the genera Tityus, Centruroides, Mesobuthus, Parabuthus, Leiurus, Buthus, Hottentota, and Androctonus.

The Indian red scorpion (*Mesobuthus tamulus*) is one of the world’s deadliest scorpions, with stings representing a life-threatening medical emergency. This species is distributed throughout the Indian subcontinent, including eastern Pakistan, eastern Nepal, and Sri Lanka. In India, Indian red scorpions are broadly distributed in western Maharashtra, Saurashtra, Kerala, Andhra Pradesh, Tamil Nadu, and Karnataka; however, fatal stings have been recorded primarily in the Konkan region of Maharashtra. *M. tamulus* (MT) stings induce the release of catecholamine, which leads to pathophysiological abnormalities in the victim. A strong correlation has been observed between venom proteome composition and local (swelling, redness, heat, and regional lymph node involvement) and systemic (tachycardia, mydriasis, hyperglycemia, hypertension, toxic myocarditis, cardiac failure, and pulmonary edema) manifestations.

Immediate administration of antivenom is the preferred treatment for MT stings. Nevertheless, the accomplishment of in-patient scorpion sting management is highly dependent on the safety, efficacy, and homogeneity of scorpion antivenom preparation. Production of antivenom is a multistep protocol, and each step is crucial for guaranteeing its quality. Therefore, any deviation from the standard manufacturing process or a failure to comply with Good Manufacturing practices (GMPs) may result in inferior products with adverse effects on patients. The assessment of *in vitro* neutralization of lethality and toxic activities of venom is a benchmark for assessing antivenom's efficacy.

Nevertheless, laboratory assessment of the quality of commercial antivenom can significantly improve the quality and safety that leads to improvement of scorpion sting treatment. Along with the antivenom's efficacy (venom neutralisation potency) against the venoms for which it is intended to be used, these products' quality depends on the homogeneity of preparation (active substances), fulfilment of required physicochemical standards, and parameters that ensure a good safety profile (for example, stability, sterility, and lack of endotoxin contamination). In addition to manufacturing laboratories, the national regulatory agencies must also confirm that these standards are accomplished.

Stimulation of the α1-adrenergic receptor by *M. tamulus* venom (MTV) plays a significant role in its pharmacology, resulting in clinical symptoms such as hypertension, tachycardia, myocardial dysfunction, pulmonary oedema, and cool extremities in patients. Therefore, α1-adrenoreceptor agonists (AAAs), such as Prazosin, are also used alone or in combination with commercial ASA for treating scorpion stings. The effect of scorpion venom to induce tissue damage to organs (heart, liver, kidney, lung, etc.) owing to the generation of free radicals has been reported, and antioxidants are found to protect them from damage partially. Furthermore, the failure of commercial ASA to immunorecognized the most abundant low molecular toxins of MTV due to the presence of a low proportion of venom-specific antibodies in commercial ASAs is another hurdle for efficient hospital management of scorpion sting victims. Therefore, a higher volume of ASA must be administered to scorpion sting patients, which can cause adverse serum reactions in treated patients. Thus there is an immediate need to discover a potent drug formulation to treat scorpion stings better.

For easy understanding, this thesis is structured into following seven chapters-

**Chapter I:** This chapter introduces the scorpion species' origin and evolution and the scorpion stings' global burden. This chapter also briefly introduces the Indian red scorpion (*Mesobuthus tamulus*), its geographical distribution, epidemiology, and clinical manifestations upon stings by this scorpion species. Further, this chapter discusses the fundamental aspects of antivenom production and adverse reactions associated with antivenom therapy. Besides, this chapter also illustrates the discovery of a novel formulation drug as an alternative approach for better treatment of scorpion stings clinical manifestations. The aim and objectives of the present study are also described in this chapter.

**Chapter II:** This chapter reviews the published literature on scorpion venom proteomics around the globe and also introduces the clinical study of scorpion stings. This chapter also reviews the usefulness of different analytical techniques used for the quality assessment of commercial antivenoms. This chapter also reviews the limitation of antivenom therapy and its improvement protocol.

**Chapter III:** This chapter enlists the chemical and consumables used in the study and the methods and protocols employed for performing various experiments.

**Chapter IV, V, and VI:** These chapters include results and discussions, and the content of each chapter is briefly discussed below:

**Chapter IV:** This chapter depicts the proteomic characterization of MTV. MTV proteome composition also correlates with the pathophysiology and clinical manifestations of MT stings in India. In addition, this chapter also emphasizes the immunological profiling of MTV against commercial ASAs by ELISA and immunoblot to study its efficacy against MTV.

**Chapter V:** This chapter focuses on assessing the quality and safety of commercial ASAs produced in India by physiochemical characterization, determination of homogeneity, purity of active substance, endotoxin contamination, preservative load, complement activation properties, and proportion of venom-specific antibodies.

**Chapter VI:** Illustrates the discovery of a potent drug formulation as an alternative approach for efficiently treating scorpion stings clinical manifestations. Different pharmacological effects induced by MTV were studied using *Caenorhabditis elegans* and Wistar strain albino rats as model organisms and their neutralization by the formulated drug.

**Chapter VII:** This chapter presents the conclusion of this study and visualizes the prospects of the study's findings.

In this study, the proteome composition of MTV was studied for the first time by tandem mass spectrometry analysis. A total of 110 venom toxins were identified from searching the MS data against the Buthidae family (taxid: 6855) of toxin entries in non-redundant protein databases. The $Na^+$ and $K^+$, ion channel toxins, taken together, are the most abundant toxins (76.7%), giving rise to the neurotoxic nature of this venom. The other minor toxin classes in the *M. tamulus* venom proteome are serine protease-like protein (2.9%), serine protease inhibitor (2.2%), antimicrobial peptide (2.3%), hyaluronidase (2.2%), makatoxin (2.1%), lipolysis potentiating peptides (1.2%), neurotoxin affecting $Cl^-$ channel (1%), parabutoporin (0.6%), $Ca^{2+}$ channel toxins (0.8%), bradykinin potentiating peptides (0.2%), HMG CoA reductase inhibitor (0.1%), and other toxins with unknown pharmacological activity (7.7%). Several of these toxins are promising drug candidates.

MTV does not show enzymatic activity (phospholipase $A_2$, L-amino acid oxidase, adenosine tri-, di-, and monophosphatase, hyaluronidase, metalloproteinase, and fibrinogenolytic), *in vitro* hemolytic activity, interference with blood coagulation, or platelet modulation properties. The clinical manifestations post MT stings have been described in the literature and correlate well with its venom proteome composition. An abundance of low molecular mass toxins (3−15 kDa) is responsible for exerting the significant pharmacological effects of MTV. However, they are poorly immune-recognized by commercial anti-scorpion-antivenoms (ASAs). This result is a significant concern for developing effective antivenom therapy against scorpion stings.

The WHO has recommended evaluating the quality and safety of commercial antivenom by *in vitro* laboratory tests before their pre-clinical evaluation in animal models and therapeutic use. Therefore, in this study, the qualities of commercial ASAs manufactured in India were assessed by *in vitro* laboratory analyses. Biophysical characterization of MTV by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion chromatography, and proteomics analysis demonstrated that ASAs mostly contain $F(ab')_2$ molecules with a trace amount of undigested immunoglobulin (Ig) G. The physicochemical characterization, electron microscopy, and dynamic light scattering studies revealed that ASAs were prepared according to the guidelines of WHO, and were devoid of aggregate content and virus particles. ASAs did not show IgE contamination

and bacterial endotoxin but demonstrated moderate complement activation properties, which may have adverse effects in treated patients. Spectrofluorometric and atomic force microscopy analyses showed poor venom binding with commercial ASAs. The percent of antibodies raised against the venom toxins in commercial ASAs was determined at the range of 5.3–6.3%, which is a reason for their poor efficacy. This study advocates the importance of *in vitro* laboratory analyses for assessing commercial antivenom quality and safety parameters before pre-clinical research and clinical use to treat Indian red scorpion sting.

However, the drawbacks of conventional therapies using commercial ASAs and α1-adrenoreceptor antagonists (AAA) have prompted us to search for an adequate formulation to improve treatment against *M. tamulus* sting. A therapeutic drug formulation (TDF) of low doses of commercial ASA, AAA, and ascorbic acid has remarkably improved in neutralising the *in vivo* toxic effects MTV tested in *C. elegans* and Wistar strain albino rats *in vivo* models. The neutralisation of MTV-induced production of free radicals, alteration of the mitochondrial transmembrane potential, and upregulated expression of genes involved in apoptosis, detoxification, and stress response in *C. elegans* by TDF surpassed the same effect shown by individual components of the TDF. Further, TDF efficiently neutralized the MTV-induced increase in blood glucose level within 30 to 60 min post-treatment, organ tissue damage, and necrosis, pulmonary edema in Wistar rats, indicating its clinical application for affecting treating MTV stings. This study demonstrates for the first time that *C. elegans* can be a model organism for screening the neutralization potency of the drug molecules against a neurotoxic scorpion venom.