Dedicated to my beloved parents, Mr. Gobinda Das (Deta) and Mrs. Rebati Das (Maa)

for their blessings, endless support and encouragement

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis "Characterization of Mesobuthus tamulus Venom (MTV), commercial anti-scorpion-antivenom, and assessment of MTV neutralization potency of a formulated drug" being submitted to Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam in partial fulfillment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology, has previously not formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title or recognition. Due to unavailability of proper facilities in Tezpur University, the following experiments/sample analyses were carried out at other institutes:

- LC-MS/MS analysis of protein samples were performed at Centre for Cellular and Molecular Platforms (C-CAMP), NCBS-TIFR, Bangalore, India, and Kalinga Institute of Industrial Technology-Technology Business Incubator (KIIT-TBI), Bhubaneswar, India.
- 2. RT-PCR and *in vivo* experiments are performed at animal experiment facility, IASST, Guwahati 781035, India

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TEZPUR UNIVERSITY

CERTIFICATE OF SUPERVISOR

This is to certify that the thesis entitled "Characterization of *Mesobuthus tamulus* venom (MTV), commercial anti-scorpion-antivenom, and assessment of MTV neutralization potency of a formulated drug" submitted to the School of Sciences, Tezpur University in requirement of partial fulfilment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of research work carried out by Ms. Bhabana Das under my supervision and guidance. All help received by her from various sources have been duly acknowledged. No part of this thesis has been submitted elsewhere for award of any other degree.

Ammyco

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	Significance of difference between control and MTV,	
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	(Abbreviations: LC: lethal concentration; ASAs: anti-	
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MTV-induced ROS generation in C. elegans after 6 h of MTV (LC₅₀) treatment and its neutralisation by (a) Optimum dose of individual components of the formulation, their combinations and different concentrations of formulations, (b) Individual components of the formulation and their combinations compared with formulation 2. ROS level in the positive control (CCCP1) C. elegans was considered baseline (100%), and other values were compared. Fluorescence intensities were determined by ImageJ software. Data represent \pm SD of three Determination. In fig. 9a shows the significance of the difference compared to formulation 2, ${}^{\#}p \leq 0.05$. In fig. 9b shows the significance of the difference compared to formulation 2, ${}^{4}p \leq 0.05$. There was no significant difference (p>0.05) between formulations 2 and 3. (Abbreviations: LC: lethal concentration; ROS: reactive oxygen species; MTV: M. tamulus venom; CCCP1: Carbonyl cyanide 3-chlorophenylhydrazone 1)

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ABBREVIATIONS

Abbreviation	Full form
1D SDS-PAGE	One dimensional sodium dodecyl sulfate-
	polyacrylamide gel electrophoresis
2D SDS-PAGE	Two dimensional sodium dodecyl sulfate-
	polyacrylamide gel electrophoresis
AAA	α1-adrenoreceptor agonists
ACM	Anococcygeus muscle
ADP	Adenosine diphosphate
AF	Alpha Fold
AFM	Atomic force microscopic
AI	Artificial intelligence
ALKP	Alkaline phosphatise
AMP	Adenosine monophosphate
ASA	Anti-scorpion antivenom
ATP	Adenosine triphosphate
BA	Binding affinity
BCIP/NBT	5-bromo-4-chloro-3-indolyl-phosphate/ nitro blue tetrazolium
BPP	Bradykinin potentiating peptide
BSA	Bovine serum albumin
Csαβ	Cysteine-stabilized α/β motif
CC	Corpus cavernosum
CCCP	Carbonyl cyanide m-chlorophenylhydrazone
CGC	Charge coupled device
CCD	Caenorhabditis Genetics Center
CTAB	Cetyltrimethylammonium bromide
DBP	disulfide bridge peptide
DCF	2',7'-dichlorodihydrofluorescein
DLS	Dynamic light scattering
DT	Diffusion coefficient
DTT	dithiothreitol
DPPH	2,2-diphenyl-1-picrylhydrazyl

Abbreviation	Full form
DR	Docking rank
ED	Erectile dysfunction
ELISA	Enzyme-linked immunosorbent assay
FESEM	Field emission scanning electron microscopy
FPLC	Fast Protein Liquid Chromatography
HBC	Haffkine Biopharmaceutical Corp. Ltd., Mumbai, India
HCL	Hydrochloride
H ₂ DCFDA	2',7'-dichlorofluorescein-diacetate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horse radish peroxidise
IAA	Iodoacetamide
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
Kv	Potassium ion channel
LAL	Limulus amebocyte lysate
LAAO	L-amino acid oxidase
LC ₅₀	Median lethal concentration
LC/ES-MS	Liquid chromatography-electronspray tandem mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LD ₅₀	Median lethal dose
LPP	Lipolysis potentiating peptides
MALDI-TOF-	Matrix-assisted laser desorption/ionization -Time of flight - mass
	spectrometry
MTV	Mesobuthus tamulus venom
MYA	Million years ago
NaV	Sodium ion channel
NCBI	National Center for Biotechnology Information
NDBP	Non-sulfide bridge peptide
NGM	Nematode growth media
NTU	Nephelometric turbidity unit
NO	Nitric oxide

Abbreviation	Full form
PBS	Phosphate buffered saline
PD	Pore-forming domain
PLA_2	Phospholipase A ₂
PPP	Platelet poor plasma
PRP	Platelet rich plasma
PSVPL	Premium Serum and Vaccine Pvt. Ltd.
PVDF	Polyvinylidne fluoride
Q-RT PCR	Quantitative reverse transcription polymerase chain reaction
Q-TOF	Quadrapole time of flight
RCSB	Research collaborator for structural bioinformatics
RH	Hydrodynamic radius
RNA	Ribonuclic acid
ROS	Reactive oxygen species
RP-HPLC	Reversed-phase high-performance liquid chromatography
RP-UHPLC	Reversed-phase ultra high-performance liquid chromatography
SEC	Size-exclusion chromatography
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SPI	Serine protease inhibitor
SPLP	Serine protease-like protein
STX	Saxitoxin
TBS	Tris buffered saline
TCA	Trichloroacetic acid
TDF	Therapeutic drug formuat
TEMED	Tetramethylethylenediamine
TFA	Trifluoroacetic acid
TMB/H_2O_2	3,3,5,5'-tetramethylbenzidine/hydrogen peroxide
ТМ	Transmembrane
TTX	Tetrodotoxin
VSD	Voltage-sensing domain
WHO	World health organization