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# Botulinum Toxin Type B: Microbiological Foundations And Translational Prospects

Aditi Kulshreshtha[1,] *, Sataditya Jana[1,] *, Ritika Rana[2], Azhar Khan[2,] ✉

[1] School of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Kasuali Hills, Solan, HP, India 173229

[2] Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Kasuali Hills, Solan, HP, India 173229

*These authors have contributed equally and both will be awarded with first authorship for the manuscript

**Abstract**

*Clostridium botulinum* serotype B produces botulinum neurotoxin type B (BTX-B), a zinc-dependent endopeptidase with unique structural and receptor-binding characteristics. Although BTX-B is FDA-approved for select clinical indications, its microbial diversity, gene organization, and structural adaptations remain comparatively underexplored. This mini-review consolidates current knowledge on the taxonomy, strain variation, and genetic architecture of BTX-B–producing *C. botulinum*, including the role of toxin gene clusters and accessory proteins in modulating yield, stability, and host specificity. Structural analysis is linked to the dual receptor-binding mechanism of the toxin and its enzymatic cleavage of vesicle-associated membrane protein (VAMP), which collectively underpin its biological activity. By integrating these microbiological insights with an overview of current and emerging applications, this review highlights BTX-B as both a model bacterial protein toxin and a promising candidate for biotechnological innovation. Understanding its microbial basis offers new opportunities for safe production, targeted delivery, and the development of next-generation neurotoxin-based tools.

**Keywords:** Clostridium botulinum; botulinum neurotoxin type B; microbial genetics; toxin gene clusters; neurotoxin structure; biotechnology.

## I. Introduction

Botulinum Toxin B (BTX-B), a neurotoxin derived from the food-borne pathogenic strain *Clostridium botulinum*, has been explored in the past by a few clinical studies for its unique analgesic effects as a neuro-transmission-inhibitor(1). While few of the studies highlighted its high potential as an analgesic in the medical industry for first-aid and surgical usage(1), some of them have also conveyed the possibility of its utility as a local anesthetic as well(2). Unfortunately, to our knowledge, not much research has been done on this drug mostly because of its local-paralysis-inducing side effects, thus limiting its usage and potential in the modern medical industry(3). Though cosmetic studies have adopted this drug for various works(4), the therapeutics have only explored its potential in the fields of muscle relaxation and headache pain reduction(5), prompting a significant gap between the prediction of its theoretical usage and practical implementation. Thereby, this study aims evaluate its potential and convey its future usage through a thorough review of the past studies and linking them with analysis of the mechanism-of-action of BTX-B in different scenarios.

## II. Origins

The anerobic bacterial strain, *Clostridium botulinum*, was first introduced as a food-borne pathogen in late 1800s and early 1900s in Germany through several local outbreaks, presumably originating from sausage poisoning. During the frequent outbreaks, Belgian microbiologist Emile van Ermengem discovered the strain *Bacillus botulinus* in 1895, later named *Clostridium botulinum*, by isolating the bacterial strain from contaminated ham sample. His findings from the bacterial isolation conveyed the association of a neurotoxin with the bacterial food-poisoning, thus introducing the concept of BOTOX in microbiology. Later in 1919, the BOTOX was segregated into two distinct types, namely Type A and Type B, based on their toxin-antitoxin reactions as per the experiments conducted by Georgina Burke. Though medical studies till early 20$^{th}$ century only conveyed about its pathogenic effect in food poisoning, Justinus Kerner was the one to hypothesize about its unique therapeutic potential in early 1800s, following which Dr. Alan Scott started its therapeutic usage in 1960-70 to treat strabismus. Though Botulinum Toxin Type A was first to receive approval by FDA, BTX-B also gained FDA-approval by December 2000 for its therapeutic utilities. Since then, it is being continuously used in the cosmetic industry for hyperhidrosis and facial wrinkle reduction, while its off-label therapeutic usages include migraine pain relief and muscle relaxation(6,7).

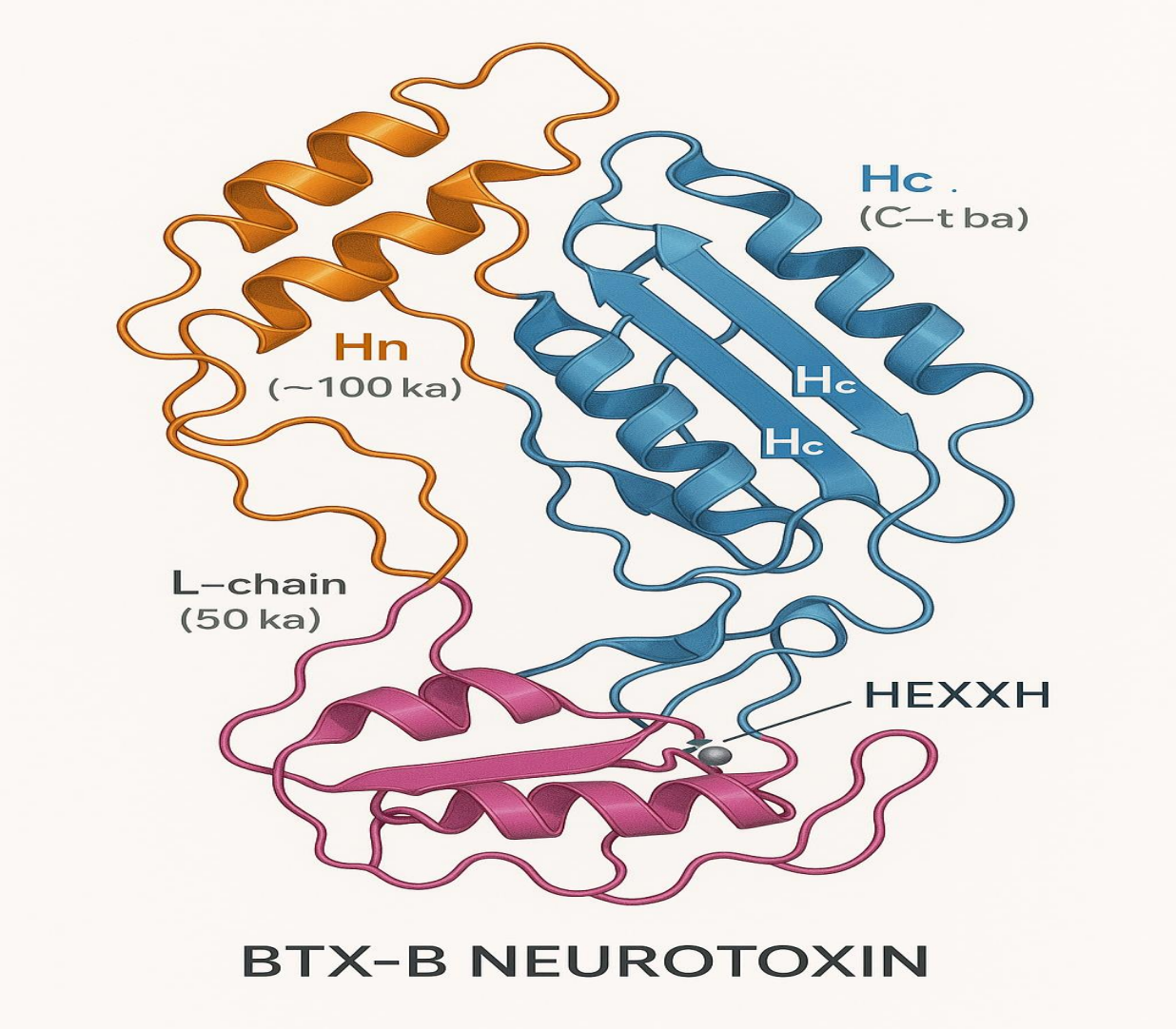
## III. Structure

Before diving deep into its mechanism, a brief introduction to its structure may help understanding BTX-B mechanism. BTX-B neurotoxin is biochemically categorized as a protein or specifically a single polypeptide chain of ~150 kDa, consisting of a heavy chain (H-chain) of ~100kDa and a light chain (L-chain) of ~50 kDa. As structure basis, the heavy chain is constructed of two domains, namely the N-terminal (Hn) domain for the translocation of the light chain into the neuronal cytosol and the C-terminal (Hc) domain for binding of specific receptors to the presynaptic nerve terminal. Here, Hc domain encompasses of two sub-domains, namely HcC (C-terminal subdomain) for binding to gangliosides and HcN (N-terminal subdomain) for binding to protein receptors. The light chain of BTX-B neurotoxin is a zinc-dependent endopeptidase, responsible for enzymatic activity of the toxin. Maintain a similar structure to other metalloproteases, this light chain includes a characteristic HEXXH motif in its active site, which

coordinates an essential zinc ion for its proteolytic activity(8). Figure 1 visualizes the 3D morphological structure of BTX-B.

**Figure 1.** 3D morphological structure of BTX-B with labelling of chains, domains, and sub-domains (8).

## IV. Mechanism




Before discussing about the current and usage of BTX-B, a brief explanation of its mechanism can deliver clarity about the particular fields it can be used in. Figure 2 illustrates the mechanism of action of BTX-B neurotoxin in inhibiting the neurotransmitter release.

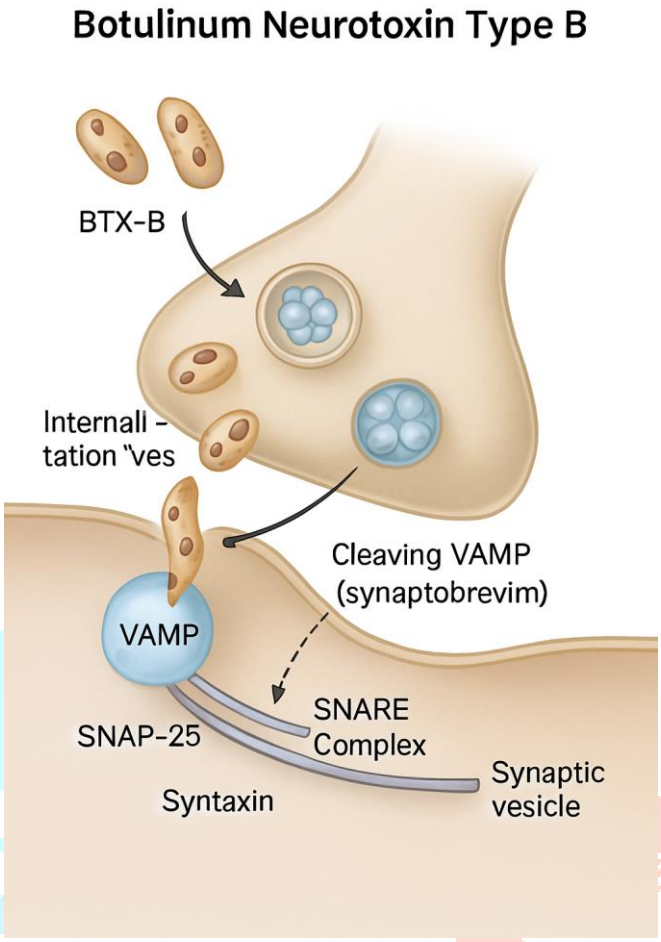



**Figure 2.** Mechanism of action of BTX-B neurotoxin in inhibiting neurotransmitter release (9,10).

As shown in Figure 2, the neurotoxic mechanism of action of BTX-B starts with the Hc domain of BTX-B, with a high affinity, specifically binding to the outer surface of the presynaptic membrane of cholinergic neurons, where this binding follows a two-step process. Here, the HcC domain firstly binds to polysialic acid gangliosides embedded in the neuronal membrane. Thereafter, the HcN domain binds to a specific protein receptor, mainly Synaptotagmin I/II, on the neuronal surface, where the dual receptor binding ensures the correct neuron type target for the toxin. After binding, a receptor-mediated endocytosis process executes the internalization of the toxin-receptor complex into the nerve terminal, where an endosome, containing the toxin, is formed. This endosome then acidifies due to the action of V-ATPase proton pump, where the acidic environment induces a conformational change in the heavy chain, especially in the Hn domain. Thereafter, the Hn domain forms a protein-conducting channel or pore through inserting itself into the endosomal membrane. This formed channel helps in the translocation of the light chain (L-chain) from the endosomal lumen into the neuronal cytosol. Following this process, when the disulfide linkage between the heavy chain and the light chain is reduced in the cytosol by cellular reductase, the light chain gets free, which allows its full activation. This light chain of BTX-B, as a highly specific zinc-dependent endopeptidase, targets vesicle-associated membrane protein (VAMP) or in simple terms syaptobrevin. This process triggers the cleavage of VAMP at a specified single peptide bond, typically in between Gln76 and Phe77. As VAMP being one of the three essential SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor) proteins for synaptic vesicle fusion and neurotransmitter release, alongside SNAP-25 and Syntaxin, its cleavage hinders the formation of SNARE complex, which is referred as a stable four-helix bundle complex that acts as a molecular machinery for pulling synaptic vesicles close to the presynaptic membrane and facilitating their fusion for neurotransmitter release into the synaptic cleft.

This event renders the functionality of VAMP in SNARE complex assembling or function, thus precluding the exocytosis of neurotransmitter, acetylcholine, from the presynaptic nerve terminal. With no acetylcholine release, this scenario inhibits the signal-transmission of muscle contraction across neurotransmitter, thus facilitating flaccid paralysis(9,10).

## V. Literature Review on Existing Uses

BTX-B as a comparatively new drug introduced in the early 2000s(7) has limited therapeutic usage in the current medical industry(3,10), where there are some off-label utilities(10) of this drug in recent clinical era. BTX-B has a prominent use in reducing the severity of abnormal head and neck pain associated with cervical dystonia(4), especially where the patient is either resistant to or have a suboptimal response to BTX-A. Another of its significant use remains in treating sialorrhea(11), where it targets the salivary glands to reduce saliva production. Though the process remains under research, BTX-B, similar to BTX-A, has also showed its potential to treat various forms of spasticity ad focal dystonia(12) rather than cervical dystonia. Emerging research on BTX-B has also conveyed its capability in improving symptoms of Raynaud's phenomenon(13) and healing digital ulcers, mostly because of its ability to relax overactive sympathetic nerve fibers constructing the blood vessels. Several studies have also suggested the alternative of BTX-A as BTX-B in hyperhidrosis(14) through clinical experimentations, specially where the patient is resistant to BTX-A. Some research studies have also efficiently explored its ability to treat urinary incontinence(15) in BTX-A-resistant patients. Recent research has also indicated the unique analgesic potential of BTX-B, where it has been readily tested successful in curing certain types of neuropathic pain and chronic myofascial pain(5,10), though this field remains yet to be explored properly. Though, some hypothetical studies suggest its further usages in clinical therapeutics, its immunogenicity and some side-effects remain as the main hurdles from its proper implementation(3,10), where future research advances may find effective ways to tackle this problem.

## VI. Outlook on Off-Label BTX-B Usage and Future Directions

Though the recent usage of BTX-B, especially the off-label uses(10), promises significant advancements of BTX-B in the modern therapeutic industry, a lot of its potential are yet to be explored as suggested by the past studies. Thereby, this study, here, provides a brief outline on which particular fields BTX-B might be used as an alternative or main therapeutic.

### 6.1. Future Analgesic Usage

BTX-B, as a unique neurotoxic neuro-transmitter-inhibitor, has already portrayed a high potential among all the analgesic therapeutic drugs, though a lot about it remains unexplored. Its renowned acetylcholine blocking mechanism have the potential to not only help breaking spasm-pain cycle, like cervical dystonia or myofascial pain, but also to initiate neuro-transmitter-blockade to reduce mechanical stress in the tissues, joints, and nerves, which can be a potential game-changer for treating neuronal diseases, muscle dystrophies, and chronic joint pains, like arthritis. Another of its utilities lies in its nociceptive-neurotransmitter-release-inhibition process, where, similar to acetylcholine-blockade, this mechanism allows BTX-B to block several pain-sensation-transmitter neuropeptides, including Substance P (SP), Calcitonin Gene-Related Peptide (CGRP), and Glutamate, thus resulting in lesser delivery of pronociceptive substances at the peripheral nerve endings. Thereby, BTX-B acts a peripheral-sensitization-decreasing substance, thus prompting sensory nerves to become less hypersensitive to painful stimuli. A few studies have also suggested about the anti-inflammatory characteristics of BTX-B alongside its ability to affect the expression or function of certain pain-sensing-receptors on the cell surface, including TRPV1,

which can further enhance its analgesic capabilities. BTX-B can also modulate sympathetic overflow which could potentially contribute to pain-relief by improving blood-flow and reducing sympathetically mediated pain, thus highlighting another of its potential contribution chances in analgesic therapeutics(9,10,16).

### 6.2. Current Limitations and Overcoming Possibilities

While BTX-B has demonstrated significant potential for analgesic therapeutics, as a rapid-action-painkiller, BTX-B is still limited due to its delayed effects on nervous system while some patients may require immediate pain-relief. The introduction of a super-binding-substrate may change this perspective in BTX-B analgesic therapy, while allowing the possibility of further and novel future research opportunities of BTX-B. Thereby, this scenario can prompt the novel utilities of BTX-B as a painkiller in treating acute exacerbations of chronic pain, acute dystonic crises, acute flare-ups of neuropathic pain, and phantom limb pain, while it may also act as an emergency muscle relaxations for diagnostics or procedures. This scenario also opens a chance for BTX-B to be used as a local anesthetic for various causes, which includes its involvement in post-procedural pain relief, symptom control in chronic pain management, easier pain relief during the facilitation of physical rehibition therapies, and targeted nerve block for specific conditions. While significantly promising, during these experimentations, the effect of BTX-B needs to be closely monitored to identify any potential side effects that can harm patients. Though requires thorough clinical trials for its future implementation in these novel fields, BTX-B may promise several advancements than existing opioids and painkiller in nerve and pain-sensation related cases(7,10,17,18).

## VII. Conclusion

Despite decades of clinical use, Botulinum Toxin Type B remains underappreciated in therapeutics with unused potential far beyond its current indications. This review not only reaffirms its validated roles in neuromuscular and autonomic disorders but also delivers a compelling future for BTX-B as a next-generation analgesic and localized anesthetic alternative. Its unique mechanism, targeted neurotransmission blockade, offers a paradigm shift in managing chronic and neuropathic pain without the systemic risks associated with opioids. By identifying mechanistic pathways, clinical gaps, and translational opportunities, an urgent call is forwarded for engaging researchers in the broader applications of BTX-B. Harnessing its full therapeutic potential could redefine pain medicine and usher in a safer, more precise era of neuropharmacology.

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**Author Contributions**

***Aditi Kulshreshtha*:*** Writing, Conceptualization, Reviewing and Editing; ***Sataditya Jana*:*** Writing, Conceptualization, Reviewing and Editing; ***Ritika Rana:*** Review and Editing ***Azhar Khan ✉:*** Conceptualization, Critical Review of Manuscript, Editing, Finalizing the Draft.

**These authors have contributed equally and will be awarded as the first author of the manuscript.*

*✉ The correspondence will be addressed to Dr. Azhar Khan (mk.azhar1@gmail.com)*

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Correspondence and requests for materials should be addressed to Dr. Azhar Khan (mk.azhar1@gmail.com)

**Aditi Kulshreshtha:** B.Tech. Biotechnology Research, 4th Year, School of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Kasuali Hills, Solan, HP, India 173229; Email: aditikul2202@gmail.com; ORCID: 0009-0008-5386-6592

**Sataditya Jana:** B.Tech. Biotechnology SRP, 4th Year, School of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Kasuali Hills, Solan, HP, India 173229; Email: satadityajana13@gmail.com; ORCID: 0009-0009-8517-8177

**Ritika Rana:** Research scholar, faculty of applied sciences and biotechnology and management sciences, Kasuali Hills, Solan, HP, india; Email: ritika1995rana@gmail.com: ORCID: 0000-0003-0058-4037

**Azhar Khan:** Professor, Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Kasuali Hills, Solan, HP, India 173229; Email: mk.azhar1@gmail.com; ORCID: 0000-0003-2570-8219