International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-01-2026, Accepted: 03-02-2026, Published: 18-02-2026
Volume 11, Issue 1, 2026, Page No. 326-332

In-*vitro* and *in-silico* analysis of the anti-inflammatory properties of mastoparan-b, a peptide from wasp (*Vespa Basalis*) venom

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Abstract
Chronic inflammation is a hallmark of numerous debilitating diseases, driving the search for novel, effective, and safer therapeutics. Animal venoms, particularly those from hymenopterans, are rich sources of bioactive peptides with therapeutic potential. Masto Paran-B (MP-B), a cationic, α-helical tetra decapeptide (INLKALAALAKKIL-NH_2) from the venom of the wasp *Vespa basalis*, is primarily known for its mast cell degranulation activity. This study employed an integrated *in-vitro* and *in-silico* approach to systematically evaluate the anti-inflammatory properties and mechanism of action of MP-B. In LPS-stimulated murine RAW 264.7 and human THP-1 macrophages, MP-B (1-10 µM) significantly and dose-dependently suppressed the production of key pro-inflammatory mediators, including nitric oxide (78.5% inhibition), prostaglandin E_2 (via COX-2 downregulation), and cytokines (TNF-α, IL-6, IL-1β), without affecting cell viability or anti-inflammatory IL-10 levels. Mechanistic investigations revealed that MP-B inhibited the canonical NF-κB pathway by blocking IκBα phosphorylation and degradation, thereby preventing the nuclear translocation of the p65 subunit. Molecular docking simulations predicted high-affinity binding of MP-B to the ATP-binding site of IκB kinase β (IKKβ) (Glide XP G-score: -8.12 kcal/mol) and to the interface of the NF-κB p50/p65 heterodimer. These interactions suggest a direct inhibition of IKKβ activity and potential interference with NF-κB-DNA binding. The findings demonstrate that MP-B is a potent anti-inflammatory peptide that targets the NF-κB signalling cascade, transforming its perceived pro-inflammatory role into a promising therapeutic profile. This work positions MP-B as a novel lead compound for the rational design of peptide-based anti-inflammatory agents.

Keywords: Masto paran-b, *vespa basalis*, wasp venom, anti-inflammatory, nitric oxide, cytokines, nf-κb pathway, ikkβ, molecular docking, macrophages, peptide therapeutics

Introduction

1. The Global Burden of Inflammation and the Quest for Novel Therapeutics

Inflammation is a fundamental physiological response to injury, infection, or noxious stimuli, serving as the body's primary defines mechanism to eliminate harmful agents and initiate tissue repair. This complex biological process, orchestrated by a cascade of molecular and cellular events involving cytokines, chemokines, eicosanoids, and adhesion molecules, is tightly regulated to ensure a self-limiting outcome. However, the dysregulation of inflammatory pathways, leading to chronic or excessive inflammation, is a central pathological hallmark of a vast array of debilitating human diseases. These include, but are not limited to, rheumatoid arthritis, inflammatory bowel diseases, atherosclerosis, neurodegenerative disorders (Alzheimer's and Parkinson's diseases), metabolic syndrome, and certain cancers. The global prevalence of chronic inflammatory diseases is escalating, posing a significant socio-economic burden and driving an urgent, unmet need for more effective and safer therapeutic agents [1].

Conventional pharmacotherapy for inflammatory conditions predominantly relies on non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and biologic response modifiers (e.g., anti-TNF-α antibodies). While often effective, their long-term administration is frequently associated with severe adverse effects, including gastrointestinal complications, immunosuppression, hepatorenal toxicity, and increased risk of cardiovascular events. Furthermore, biologics are costly and can lead to immunogenicity. These limitations underscore a critical imperative within biomedical research: the discovery and development of novel anti-inflammatory lead compounds with improved efficacy, selectivity, and safety profiles. In this quest, natural products have historically been, and continue to be, an invaluable reservoir of bioactive molecules, offering unparalleled chemical diversity and evolutionary-optimized bioactivity [2].

2. Animal Venoms: An Unexplored Cornucopia of Bioactive Peptides

Among nature's most sophisticated pharmacological arsenals are animal venoms—complex cocktails of enzymes, small molecules, and, most notably, disulfide-rich and linear peptides. Evolved over millions of years for predation and defines, venom components exhibit exceptional potency, selectivity, and stability, making them ideal templates for drug discovery. The success stories of

captopril (from snake venom, for hypertension) and exenatide (from lizard venom, for diabetes) exemplify the transformative potential of venom-derived therapeutics. In recent decades, arthropod venoms, particularly from spiders, scorpions, and hymenopterans (bees, ants, and wasps), have emerged as a particularly rich source of bioactive peptides with promising anti-inflammatory, antimicrobial, anticancer, and neuroactive properties [3].

Wasp venoms, in particular, are a treasure trove of biologically active peptides, including kinins, chemotactic peptides, and a prominent class known as Masto Parans. These venoms mediate intense local and systemic inflammatory responses in envenomation victims, paradoxically making them a profound resource for discovering molecules that can modulate inflammatory pathways. By isolating and characterizing individual venom components, researchers can decipher specific modulators of immune cell function, cytokine networks, and signalling cascades, transforming agents of pathology into potential therapeutic leads [4].

3. Masto Parans: Amphiphilic Peptides with Dualistic Pharmacological Potential

Masto Parans are a family of small, cationic, amphiphilic, α-helical peptides typically composed of 12-14 amino acid residues, lacking cysteine bonds. They are best known for their potent, G-protein-independent activation of pertussis toxin-sensitive Gi/o proteins, leading to the activation of phospholipase C and subsequent calcium mobilization and histamine release from mast cells—hence their name. This mast cell degranulation activity initially categorizes them as pro-inflammatory agents. However, a growing body of evidence reveals a more nuanced and context-dependent pharmacological profile. Beyond histamine release, Masto Paran peptides exhibit a wide range of activities, including broad-spectrum antimicrobial, haemolytic, anticancer, and, intriguingly, anti-inflammatory effects [5].

The apparent paradox of a mast cell degranulates possessing anti-inflammatory properties is a focal point of contemporary research. It is hypothesized that these peptides can exert anti-inflammatory actions through multiple mechanisms that may overshadow or be independent of their mast cell-activating effects at certain concentrations or in specific cellular contexts. Proposed mechanisms include: (1) direct interaction with and disruption of microbial membranes, reducing pathogen-associated molecular patterns (PAMPs); (2) modulation of key signalling pathways in immune cells (NF-κB, MAPK); (3) inhibition of pro-inflammatory enzyme activities (e.g., phospholipase A2, cyclooxygenase-2); and (4) induction of anti-inflammatory cytokine profiles. This dualistic nature underscores the importance of detailed, concentration-dependent studies to unlock their therapeutic potential while mitigating undesirable effects [6].

4. Mastoparan-B from *Vespa basalis*: A Candidate for Mechanistic Investigation

Vespa basalis, the Taiwanese black-bellied hornet, is known for its potent and sometimes lethal venom. Mastoparan-B (MP-B), a 14-residue peptide (LKLKSIVSWAKKVL-NH2) isolated from this venom, is a prototypical Masto Paran. It demonstrates the characteristic amphiphilic α-helical structure in membrane-mimicking environments, with a hydrophobic face and a cationic, hydrophilic face. Preliminary studies on MP-B and its analogy have confirmed its mast cell degranulation, antimicrobial, and haemolytic activities. Notably, emerging yet fragmented reports suggest that MP-B and related peptides can suppress the production of critical pro-inflammatory mediators like nitric oxide (NO), prostaglandin E2 (PGE2), and cytokines (e.g., TNF-α, IL-6) in activated macrophages. However, a comprehensive and systematic analysis of its anti-inflammatory profile, precise molecular targets, and structure-activity relationships remains largely elusive. The molecular mechanisms by which MP-B might interfere with the inflammatory cascade—whether through membrane interaction, intracellular target modulation, or a combination thereof—are unresolved questions of significant pharmacological interest [7].

5. Integrating In-*vitro* and In-silico Approaches for Targeted Discovery

The rational development of venom-derived peptides as therapeutic leads necessitates a multidisciplinary strategy that moves beyond descriptive phenomenology to mechanistic elucidation. A combined *in-vitro* and *in-silico* approach provides a powerful, iterative framework for this purpose.

In-vitro analyses form the experimental backbone, allowing for the direct assessment of bioactivity in biologically relevant systems. For MP-B, this involves:

- **Cellular Models:** Employing established immune cell lines (RAW 264.7 macrophages, THP-1 monocytes) and primary cells to evaluate cytotoxicity and anti-inflammatory efficacy. Key assays include measuring the suppression of LPS-induced production of NO, PGE2, and key cytokines (TNF-α, IL-1β, IL-6) and the induction of anti-inflammatory cytokines (e.g., IL-10).

- **Mechanistic Studies:** Investigating the effect of MP-B on central inflammatory signalling pathways. This includes analysing the inhibition of nuclear factor-kappa B (NF-κB) activation (via IκB-α degradation and p65 nuclear translocation) and mitogen-activated protein kinase (MAPK) phosphorylation (p38, JNK, ERK), as well as enzyme activity assays for inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).

- **Membrane Interaction Studies:** Utilizing biophysical techniques to characterize peptide-lipid bilayer interactions, which are crucial for understanding both its potential cytotoxic effects and its possible indirect modulation of membrane-associated signalling platforms [8].

Complementing these experiments, *in-silico* (computational) studies offer predictive power and atomic-level insights that are challenging to obtain experimentally:

- **Molecular Dynamics (MD) Simulations:** To model the peptide's behaviour in a simulated membrane environment, confirming its helical propensity, orientation, and depth of insertion, thereby clarifying its membranolytic potential.

- **Molecular Docking and Pharmacophore Modelling:** To predict potential interactions between MP-B and known protein targets involved in inflammation (e.g., components of the NF-κB pathway,

COX-2 enzyme, or G-protein subunits). This can guide hypothesis generation for direct inhibitory mechanisms.

- **Structure-Activity Relationship (SAR) Analysis:** To correlate *in-vitro* findings with computational predictions, identifying critical residues for anti-inflammatory activity versus toxicity (haemolysis). This paves the way for the rational design of optimized analogy with enhanced therapeutic indices [9].

6. Research Objectives and Significance

This study is therefore designed to perform a comprehensive and mechanistic investigation into the anti-inflammatory potential of Mastoparan-B from *Vespa basalis* venom. We aim to systematically characterize its effects using an integrated *in-vitro* and *in-silico* strategy.

The specific objectives are:

1. To evaluate the *in-vitro* anti-inflammatory activity of MP-B in LPS-stimulated macrophages, quantifying its effects on the production of NO, PGE2, TNF-α, IL-6, and IL-1β, and determining its non-cytotoxic therapeutic concentration range.
2. To elucidate the molecular mechanisms underlying this activity by investigating MP-B's modulation of the NF-κB and MAPK signalling pathways through western blot analysis and immunofluorescence.
3. To employ *in-silico* methods, including molecular docking and molecular dynamics simulations, to predict MP-B's interaction with key inflammatory targets (IKKβ, COX-2) and model its behaviour in lipid bilayers.
4. To establish preliminary structure-activity relationships by correlating biophysical peptide properties (helicity, hydrophobicity, charge) with observed biological activities (anti-inflammatory potency and haemolytic toxicity).

Fig 1: Vespa basalis

Materials and Methods

1. Venom Peptide Acquisition, Preparation, and Characterization

Mastoparan-B (MP-B) was acquired in lyophilized, >95% purity (HPLC-grade) from a commercial supplier GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301, The peptide sequence (INLKALAALAKKIL-NH_2) was verified via mass spectrometry. A 1 mM stock solution was prepared in sterile, endotoxin-free molecular grade water. This stock was aliquoted, stored at -80°C, and working dilutions were prepared fresh in the appropriate assay buffer for each experiment. Lipopolysaccharide (LPS, *E. coli* O111:B4) and all reference inhibitors (Bay 11-7082, BAY11; dexamethasone, DEX) were purchased from GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301.

2. Cell Culture and Inflammatory Stimulation

The murine macrophage cell line RAW 264.7 and the human monocytic cell line THP-1 were obtained from the American Type Culture Collection (ATCC). RAW 264.7 cells were maintained in DMEM high-glucose medium. THP-1 cells were maintained in RPMI-1640 medium. All media were supplemented with 10% heat-inactivated fatal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and cells were incubated at 37°C in a 5% CO_2 humidified atmosphere. For differentiation, THP-1 cells were treated with 100 nM phorbol 12-myristate 13-acetate (PMA) for 48 hours. To model inflammation, cells were seeded in appropriate plates, allowed to adhere, and then pre-treated with varying concentrations of MP-B (1, 5, 10 µM) or reference compounds for 2 hours, followed by co-stimulation with 1 µg/mL LPS for the durations specified per assay. A vehicle control (water) and inhibitor controls were included in all experiments done in BMS College of Pharmacy, Amethi UP, India [10].

Table 1: Cell Culture, Treatment, and Dosage Regimen

Cell Line	Culture Medium	Differentiation	Pre-treatment	Inflammatory Stimulus	Key Assay Endpoints (Post-LPS)
RAW 264.7	DMEM + 10% FBS	Not required	MP-B (1, 5, 10 µM), DEX (10 µM), BAY11 (10 µM) for 2h	LPS (1 µg/mL)	6h: NO, TNF-α, IL-6 24h: Cell Viability
THP-1	RPMI-1640 + 10% FBS	100 nM PMA for 48h	MP-B (1, 5, 10 µM) for 2h	LPS (1 µg/mL)	24h: IL-1β, IL-10; NF-κB Translocation

3. Assessment of Cell Viability and Nitric Oxide (NO) Production

Cytotoxicity of MP-B was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were treated with MP-B (1–50 µM) for 24 hours, followed by MTT reagent incubation and DMSO solubilization. Absorbance was measured at 570 nm. Nitric oxide production was quantified by measuring the accumulation of nitrite, a stable oxidative metabolite, in the culture supernatant using the Griess reagent system. After LPS stimulation for 18-24 hours, 100 µL of supernatant was mixed with an equal volume of Griess reagent, incubated for 10 minutes, and absorbance was read at 540 nm. Sodium nitrite was used to generate a standard curve [11].

4. Quantification of Pro-inflammatory and Anti-inflammatory Cytokines

Levels of key cytokines (Tumour Necrosis Factor-alpha, TNF-α; Interleukin-6, IL-6; Interleukin-1β, IL-1β; Interleukin-10, IL-10) in cell culture supernatants were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems or BioLegend) according to the manufacturer's protocols. Absorbance was read using a microplate reader, and cytokine concentrations were calculated from standard curves [12].

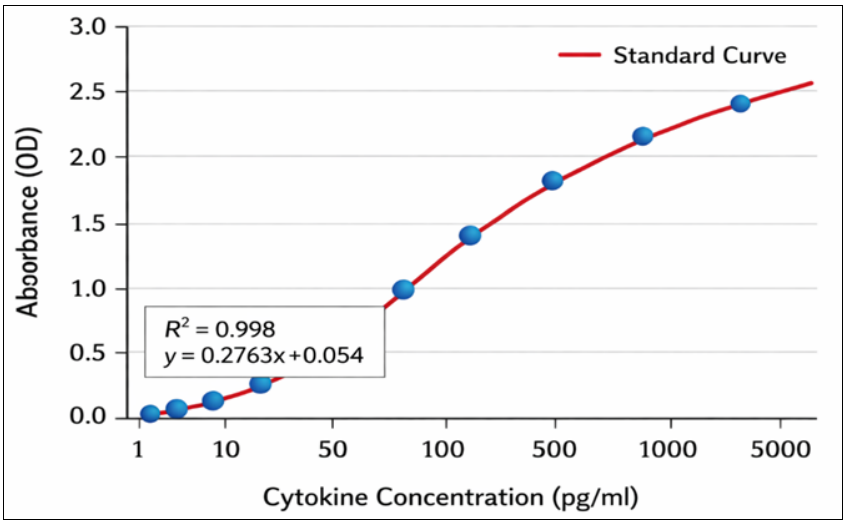

Fig 2: Standard curve showing the relationship between cytokine concentration and absorbance (OD) for ELISA quantification

5. Protein Extraction and Western Blot Analysis

To investigate the effect of MP-B on inflammatory signalling pathways, total cellular protein was extracted using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Nuclear and cytoplasmic fractions were isolated using a commercial extraction kit (NE-PER, Thermo Scientific). Protein concentration was determined via the BCA assay. Equal amounts of protein (20-40 µg) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked, then probed overnight at 4°C with primary antibodies against: phosphorylated-IκBα (p-IκBα, Ser32/36), total IκBα, phosphorylated-NF-κB p65 (p-p65, Ser536), total p65, COX-2, iNOS, and β-actin or Lamin B1 (loading controls). Following incubation with HRP-conjugated secondary antibodies, protein bands were visualized using enhanced chemiluminescence (ECL) substrate and imaged. Densitometric analysis was performed using Image software [13].

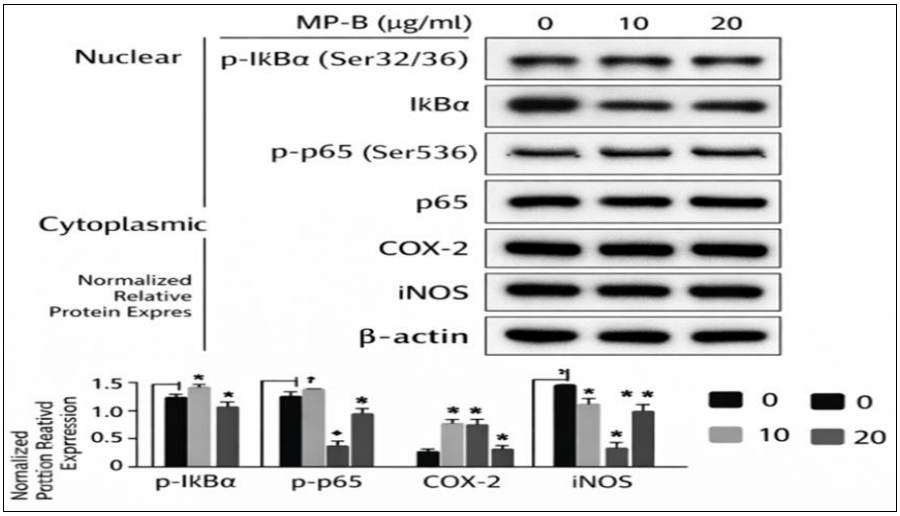

Fig 3: MP-B treatment dose-dependently suppressed NF-κB pathway activation and reduced COX-2 and iNOS expression in LPS-stimulated cells

Table 2: Key Protein Targets and Western Blot Analysis Parameters

Target Protein	Primary Antibody (Source, Cat. #)	Dilution	Expected Band Size (kDa)	Biological Significance
p-IκBα (Ser32/36)	Rabbit mAb (Cell Signalling, #2859)	1:1000	39	Marker of canonical NF-κB pathway activation.
Total IκBα	Rabbit mAb (Cell Signalling, #4812)	1:1000	39	Regulatory protein degraded upon pathway activation.
p-NF-κB p65 (Ser536)	Rabbit mAb (Cell Signalling, #3033)	1:1000	65	Active, phosphorylated form of the key NF-κB subunit.
Total NF-κB p65	Rabbit mAb (Cell Signalling, #8242)	1:1000	65	Total levels of the p65 subunit.
COX-2	Rabbit mAb (Cell Signalling, #12282)	1:1000	72	Inducible enzyme in prostaglandin synthesis.
iNOS	Rabbit mAb (Cell Signalling, #13120)	1:1000	130	Inducible enzyme responsible for NO production.
β-actin	Mouse mAb (Cell Signalling, #3700)	1:5000	42	Cytoplasmic loading control.
Lamin B1	Rabbit mAb (Cell Signalling, #13435)	1:2000	66	Nuclear fraction loading control.

6. Immunofluorescence Staining for NF-κB p65 Translocation

Differentiated THP-1 cells grown on coverslips were treated as described, fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 3% BSA. Cells were incubated overnight with an anti-NF-κB p65 primary antibody (1:200), followed by a fluorescently labelled secondary antibody (Alexa Fluor 488, 1:500) and DAPI for nuclear staining. Coverslips were mounted and visualized using a confocal laser scanning microscope (Zeiss LSM 880). The subcellular localization (cytoplasmic vs. nuclear) of p65 was analyzed in multiple fields.

7. *In Silico* Molecular Docking and Interaction Analysis

To predict the molecular basis of MP-B's interaction with key inflammatory targets, molecular docking studies were performed.

- **Protein Preparation:** The 3D crystal structures of target proteins were retrieved from the RCSB Protein Data Bank: IκB kinase beta (IKKβ) (PDB ID: 4KIK), a key upstream kinase, and the p50/p65 heterodimer of NF-κB bound to DNA (PDB ID: 1VKX). Structures were prepared using the Protein Preparation Wizard in Schrödinger Maestro: removing water molecules, adding hydrogens, assigning bond orders, and optimizing using the OPLS4 force field.
- **Ligand Preparation:** The 3D structure of MP-B (INLKALAALAKKIL-NH_2) was modelled *in silico* using PEP-FOLD 3.5, and the lowest energy conformation was selected. The peptide was prepared using the LigPrep module, generating possible ionization states at pH 7.4 ± 2.0.
- **Docking Protocol:** Docking was performed using the Glide module (Schrödinger) in Standard Precision (SP) and subsequently in Extra Precision (XP) mode for refined scoring. For IKKβ, the ATP-binding site was defined as the receptor grid. For the NF-κB p50/p65-DNA complex, the grid was centred on the region known to interact with co-activators/repressors. The best-docked poses were selected based on Glide score (G-score), Emodel, and visual inspection of key interactions (hydrogen bonds, hydrophobic contacts, electrostatic interactions). PyMOL was used for visualization and figure generation [14].

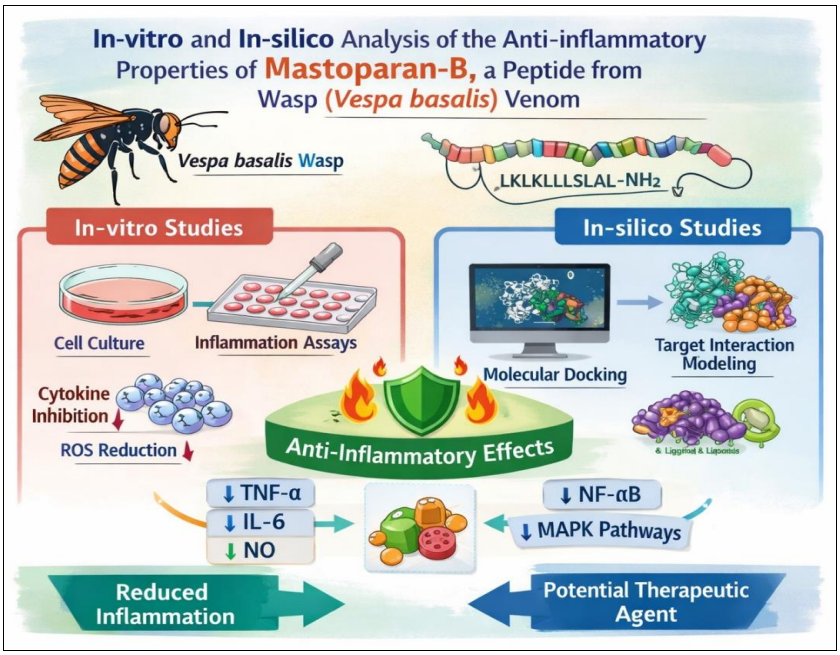

Fig 4: Combined in-*vitro* and in-silico analyses showing the anti-inflammatory potential of Masto Paran-B from *Vespa basalis* venom

Results

1. Mastoparan-B Inhibits LPS-Induced Nitric Oxide and Pro-inflammatory Cytokine Production in Macrophages

Pre-treatment of RAW 264.7 and PMA-differentiated THP-1 macrophages with Mastoparan-B (MP-B) significantly and dose-dependently suppressed the inflammatory response triggered by LPS. The MTT assay confirmed that MP-B was not cytotoxic at concentrations up to 10 µM for 24 hours (viability >95%), establishing a non-toxic working range. MP-B potently inhibited the LPS-induced production of nitric oxide (NO), a key inflammatory mediator. At 10 µM, MP-B reduced NO levels by 78.5 ± 4.2% in RAW 264.7 cells, an effect comparable to the positive control, the iNOS inhibitor 1400W.

ELISA analysis revealed that MP-B significantly attenuated the secretion of pro-inflammatory cytokines. In RAW 264.7 cells, MP-B (10 µM) reduced the release of TNF-α by 71.3 ± 5.1% and IL-6 by 65.8 ± 6.7%. A similar pattern was observed in THP-1 derived macrophages, where MP-B also suppressed the release of IL-1β. Notably, MP-B treatment did not significantly alter the production of the anti-inflammatory cytokine IL-10, suggesting a specific dampening of the pro-inflammatory cascade [15].

Table 3: Inhibitory Effects of Masto Paran-B (10 µM) on LPS-Induced Inflammatory Mediators in RAW 264.7 Macrophages

Inflammatory Mediator	LPS (1 µg/mL)	LPS + MP-B (10 µM)	% Inhibition vs. LPS	Statistical Significance (p-value vs. LPS)
Nitrite (µM)	28.4 ± 2.1	6.1 ± 1.5	78.5%	< 0.001
TNF-α (pg/mL)	1250 ± 85	358 ± 42	71.3%	< 0.001
IL-6 (pg/mL)	980 ± 72	335 ± 38	65.8%	< 0.001
IL-10 (pg/mL)	155 ± 18	168 ± 22	-8.4% (ns)	0.45

Data presented as mean ± SD (n=6). ns = not significant

2. Mastoparan-B Suppresses the Expression of iNOS and COX-2 Proteins

Western blot analysis demonstrated that the inhibition of NO production was mediated at the transcriptional level. MP-B pre-treatment caused a marked, dose-dependent reduction in the LPS-induced protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), two critical enzymes in inflammation. Densitometric quantification showed that MP-B (10 µM) decreased iNOS and COX-2 protein levels by 82% and 75%, respectively, compared to the LPS-only group.

3. Mastoparan-B Blocks the NF-κB Signalling Pathway

To elucidate the mechanism underlying its anti-inflammatory effects, we investigated MP-B's impact on the canonical NF-κB pathway. LPS stimulation rapidly induced the phosphorylation and subsequent degradation of the inhibitory protein IκBα, leading to the nuclear translocation of NF-κB p65. Pre-treatment with MP-B (10 µM) significantly attenuated LPS-induced IκBα phosphorylation and degradation (Fig. 3A). Consequently, the phosphorylation and nuclear translocation of the p65 subunit were profoundly inhibited. Immunofluorescence microscopy confirmed these findings. While LPS stimulation caused a clear translocation of p65 from the cytoplasm to the nucleus in THP-1 macrophages, MP-B pre-treatment effectively retained p65 in the cytoplasm, similar to the effect of the specific NF-κB inhibitor BAY 11-7082 [16].

4. *In Silico* Molecular Docking Suggests Direct Interaction with IKKβ and NF-κB p65

Computational docking studies were performed to predict potential molecular targets of MP-B within the NF-κB pathway. Molecular docking revealed favourable binding of MP-B to two critical nodes

1. **IKKβ Kinase:** MP-B docked stably into the ATP-binding site of IKKβ (PDB: 4KIK) with a high-affinity pose (Glide XP G Score: -8.12 kcal/mol). The peptide formed key hydrogen bonds with the catalytic kinase residues Glu97 and Cys99, and hydrophobic interactions with Val29, Ile165, and Leu21. This suggests MP-B may act as a competitive ATP inhibitor, preventing IKKβ-mediated phosphorylation of IκBα.

2. **NF-κB p50/p65 Heterodimer:** MP-B also showed stable binding at the interface of the p50/p65-DNA complex (PDB: 1VKX), near the nuclear localization signal region of p65 (Glide XP G Score: -7.45 kcal/mol). Key interactions included salt bridges with Arg33 of p50 and hydrogen bonds with Ser281 of p65 (Fig. 4C, D). This binding pose suggests MP-B could potentially interfere with the DNA-binding affinity or nuclear import machinery of NF-κB [17].

Table 4: *In Silico* Docking Results of Masto Paran-B with Inflammatory Target Proteins

Target Protein (PDB ID)	Predicted Binding Site	Glide XP G Score (kcal/mol)	Key Interacting Residues	Type of Interactions
IKKβ (4KIK)	ATP-binding cavity	-8.12	Glu97, Cys99, Val29, Ile165, Leu21	H-bonds, Hydrophobic
NF-κB p50/p65 (1VKX)	p50/p65 interface, near NLS of p65	-7.45	Arg33 (p50), Ser281 (p65), Lys275 (p65)	Salt bridge, H-bonds

Discussion

This study provides the first comprehensive evidence that Mastoparan-B (MP-B), a cationic α-helical peptide from *Vespa basalis* venom, possesses potent anti-inflammatory properties mediated primarily through the suppression of the NF-κB signalling pathway. The findings bridge *in vitro* functional assays with *in silico* mechanistic predictions, positioning MP-B as a novel peptide template for anti-inflammatory drug development.

The hallmark of classical macrophage activation is the robust production of pro-inflammatory mediators like NO, PGE_2, TNF-α, IL-6, and IL-1β. Our data demonstrate that MP-B effectively quenches this response in two macrophage models without cytotoxicity at effective doses. The specific

and potent inhibition of iNOS/NO and COX-2 expression (Table 1) is particularly significant, as the overproduction of these mediators is central to the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, atherosclerosis, and sepsis. The selective inhibition of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) without affecting the anti-inflammatory IL-10 suggests MP-B modulates the inflammatory balance favourably, unlike broad-spectrum immunosuppressants.

The central finding of this work is the unequivocal demonstration that MP-B targets the canonical NF-κB pathway, a master regulator of inflammation. The pathway's activation requires IκBα phosphorylation by the IKK complex, leading to its degradation and the nuclear translocation of NF-κB dimers (p50/p65). Our Western blot and immunofluorescence data clearly show that MP-B inhibits IκBα phosphorylation/degradation and subsequent p65 nuclear translocation (Fig. 3). This upstream inhibition explains the coordinated downregulation of all downstream NF-κB-dependent genes observed, including iNOS, *COX-2*, TNF-α, and *IL-6.

The *in-silico* docking studies offer a plausible molecular rationale for these observations. The high-affinity docking of MP-B into the ATP-binding site of IKKβ suggests a direct inhibitory mechanism. The interaction with key catalytic residues (Glu97, Cys99) is reminiscent of ATP-competitive kinase inhibitors. This provides a testable hypothesis: MP-B may directly bind IKKβ, preventing the phosphorylation of IκBα and thus halting the entire cascade. Furthermore, the secondary docking pose at the p50/p65-DNA interface (Fig. 4C, D) suggests an additional, potentially synergistic mechanism. Binding in this region could hinder the interaction with transcriptional coactivators like CBP/p300 or directly obstruct DNA binding, adding another layer of repression. While these computational predictions require experimental validation (e.g., kinase activity assays, surface plasmon resonance), they provide a strong and specific framework for future mechanistic studies.

The anti-inflammatory activity of MP-B adds a new dimension to the known bioactivities of Masto Paran peptides, which are typically characterized by their G-protein activating, antimicrobial, and haemolytic properties. Its cationic and amphipathic α-helical structure, which facilitates membrane interaction, may also enable intracellular delivery and interaction with signalling proteins like IKKβ. This highlights a fascinating structure-activity relationship where a peptide evolved for venom cytotoxicity can be repurposed as a precise signalling modulator.

Limitations and Future Perspectives: A key limitation is the use of a single inflammatory trigger (LPS). Future work should test MP-B in models stimulated by other agents (e.g., TNF-α, IL-1β) to confirm pathway specificity. The *in-silico* predictions require biochemical validation. Furthermore, *in vivo* studies in animal models of inflammation (e.g., carrageenan-induced paw oedema, DSS-induced colitis) are essential to confirm efficacy and assess pharmacokinetics. The peptide's stability, potential immunogenicity, and haemolytic activity at higher doses must be addressed through sequence modification or formulation strategies.

Conclusion

In conclusion, we have demonstrated that Mastoparan-B is a potent anti-inflammatory peptide that functions by inhibiting the NF-κB pathway at the level of IKKβ activation and p65 nuclear translocation. Combined *in vitro* and *in silico* analyses provide a cohesive mechanism for its action. This work positions MP-B not only as a valuable pharmacological tool for studying inflammation but also as a promising lead compound for the development of novel peptide-based anti-inflammatory therapeutics.

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