



Article

In Silico Characterization of the Venom-Derived LW-9 Peptide Using PreADMET-Based Predictions

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Abstract

The LW-9 peptide was previously identified through screening of purified molecules from the venom of the spider *Phoneutria nigriventer* and has been described as an immunomodulatory compound with potential application in cancer therapy. The molecule was biochemically characterized and subjected to *in silico* analyses to evaluate its toxicity and efficacy profiles. LW-9 exhibits a molecular mass of 1235.522 Da and the amino acid sequence PyrKKDRFLGLM-CONH₂. Secondary and tertiary structures were modeled using computational approaches. The results indicated low permeability across biological membranes, including the blood–brain barrier. *In silico* predictions further suggested that LW-9 is neither carcinogenic nor mutagenic; however, it may act as a potent inhibitor of the CYP3A4 enzyme, raising concerns regarding potential drug–drug interactions. Overall, LW-9 shows promise as an immunomodulatory agent for cancer applications, while its pharmacokinetic properties and possible interactions with other drugs continue to be investigated.

Keywords: LW-9 peptide; *in silico* characterization; *Phoneutria nigriventer* venom; PreADMET

1. Introduction

Computational modeling has become an indispensable tool in modern drug discovery, particularly during the early phases of development [1–3]. *In silico* approaches enable rapid, cost-effective, and ethically favorable evaluation of physicochemical properties, pharmacokinetic profiles, potential toxicological risks, and drug–drug interactions, thereby significantly reducing experimental workload and supporting rational study design [4].

These methodologies are especially relevant for peptide-based therapeutics, whose structural complexity and biological functions can be systematically investigated using computational modeling and predictive algorithms [5,6]. Peptides have attracted growing interest as therapeutic agents due to their high target specificity, structural versatility, and capacity to modulate diverse biological pathways [5,7]. Nevertheless, their clinical translation remains challenging, primarily because of limitations related to membrane permeability, metabolic stability, bioavailability, and safety concerns [8,9].

Computational modeling of peptide structures, combined with *in silico* prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters,



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constitutes a robust strategy for identifying potential liabilities and advantages prior to *in vitro* and *in vivo* validation [10].

Animal venoms are widely recognized as rich sources of bioactive peptides exhibiting diverse pharmacological activities [11]. Among these, spider venoms contain peptides that have inspired numerous therapeutic candidates, particularly in the fields of ion channel modulation, immune regulation, antimicrobial activity, and cancer therapy [12,13]. The venom of *Phoneutria nigriventer* (PnV) has been extensively investigated, leading to the identification of multiple biologically relevant peptides [14]. Despite its biological relevance, a comprehensive computational characterization of LW-9 has not yet been reported.

In this context, the *in silico* evaluation of the molecular structure, physicochemical properties, pharmacokinetic profile, and toxicological potential of LW-9 represents a critical step toward assessing its suitability as a drug candidate and anticipating possible limitations [10,15]. Structural modeling provides insights into peptide stability and potential target interactions, whereas ADMET predictions offer valuable information regarding membrane permeability, blood–brain barrier penetration, metabolic pathways, and safety endpoints, including carcinogenicity, mutagenicity, and acute toxicity [16,17].

In the present study, we performed a comprehensive *in silico* analysis of the LW-9 peptide using established computational tools. Physicochemical properties were predicted, secondary and tertiary structures were modeled, and pharmacokinetic parameters such as membrane permeability and blood–brain barrier penetration were evaluated. Toxicological endpoints, including carcinogenicity, mutagenicity, cytochrome P450 interactions, and median lethal dose (LD₅₀), were also assessed. By integrating these computational analyses, this work aims to provide a robust predictive framework to support the rational development of LW-9 and to underscore the relevance of *in silico* methodologies in the early-stage evaluation of venom-derived peptide therapeutics.

2. Materials and Methods

2.1. Venom Extraction and Fractionation

The crude venom of *Phoneutria nigriventer* was initially separated based on molecular weight using centrifugal filtration with Amicon® Ultra devices, Merck Millipore, Burlington, MA, USA. This process generated three main fractions: F1 (<3 kDa), F2 (3–10 kDa), and F3 (>10 kDa). Previous work from our group indicated that F1 and F2 possess the highest antitumor potential. These fractions were therefore combined and further purified using reverse-phase high-performance liquid chromatography (RP-HPLC) on a Shimadzu VP-ODS column.

2.2. Mass Spectrometry Characterization

The LW-9 peptide was characterized by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-ToF Xevo GS, Waters Co., Milford, MA, USA) operating in positive ion mode. Molecular mass determination was initially performed in MS mode. Subsequently, tandem mass spectrometry (MS/MS) analysis was carried out using collision-induced dissociation (CID) with argon as the collision gas, applying collision energies ranging from 20 to 35 eV. Spectra were acquired over an *m/z* range of 100–2000.

2.3. In Silico Structural Analysis

The three-dimensional structure of the (PnV) peptide PnTkP-VI, which is not available in the Protein Data Bank (PDB), was constructed using homology modeling approaches. The amino acid sequence of LW-9 (QKKDRFLGLM) was retrieved in FASTA format from the UniProtKB database (accession number P86303).

Structural prediction was performed using the I-TASSER server (<https://zhanggroup.org/I-TASSER/> (accessed on 26 November 2024)), which generates three-dimensional models based on iterative threading assembly simulations. The predicted models were evaluated according to the confidence scores provided by the server.

Structural visualization and analysis were carried out using BIOVIA Discovery Studio Visualizer version 21.1.0.20298. This software enabled detailed examination of the three-dimensional conformations, secondary structure elements, and spatial distribution of amino acid residues. The overall computational workflow for structural characterization, pharmacokinetic (ADME), and toxicity prediction of the LW-9 peptide is summarized in Figure 1.

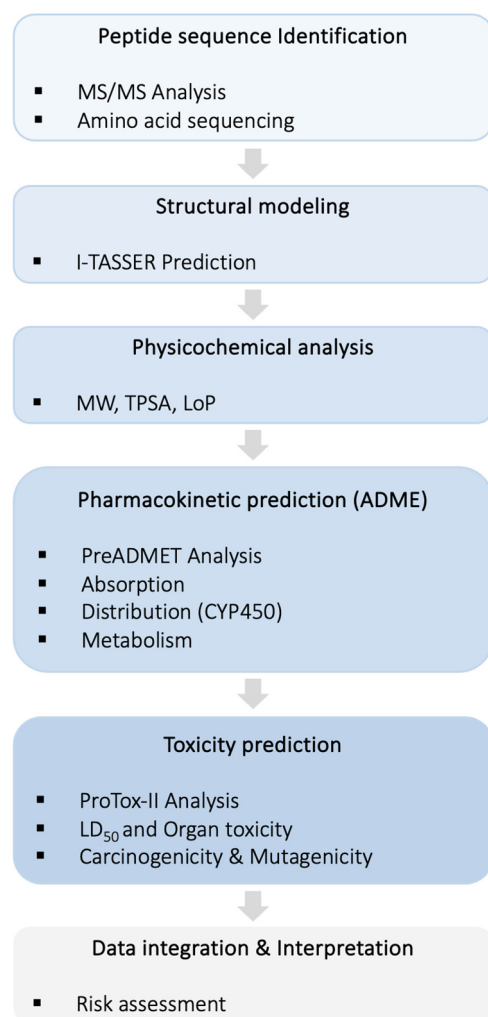


Figure 1. Computational workflow for the structural and ADME/toxicity analysis of the LW-9 peptide.

2.4. Evaluation of the Physicochemical, Pharmacokinetic, and Toxicological Properties

The physicochemical properties of the LW-9 peptide were analyzed in accordance with Lipinski's Rule of Five [18] using MarvinSketch version 23.12 (ChemAxon, <https://chemaxon.com/marvin> (accessed on 27 November 2024)). The evaluated parameters included calculated LogP (cLogP), molecular weight, number of hydrogen bond donors (HBD), and number of hydrogen bond acceptors (HBA).

Pharmacokinetic and toxicological properties were predicted using the web-based PreADMET platform (<https://preadmet.webservice.bmdrc.org/> (accessed on 30 November 2024)), which enables *in silico* assessment of absorption, distribution, metabolism, excretion (ADME), and toxicity endpoints.

The pharmacokinetic parameters evaluated included blood–brain barrier (BBB) penetration, Caco-2 cell permeability (nm/s), MDCK cell permeability (nm/s), human intestinal absorption (HIA), P-glycoprotein (P-gp) inhibition, plasma protein binding (PPB), and cytochrome P450-related parameters, including CYP2C19 inhibition, CYP2C9 inhibition, CYP2D6 inhibition, CYP2D6 substrate prediction, and CYP3A4 inhibition.

The toxicological endpoints analyzed comprised carcinogenicity predictions in mice (Carcino Mouse) and rats (Carcino Rat), hERG channel inhibition, and Ames mutagenicity test predictions for the TA100 and TA1535 strains, both with and without metabolic activation by rat liver homogenate (S9 fraction). Specifically, the evaluated Ames parameters included TA100 (with metabolic activation, 10RLI), TA100 (without activation, NA), TA1535 (with metabolic activation, 10RLI), and TA1535 (without activation, NA).

The raw results of the PreADMET predictions are provided in the Supplementary Materials (Table S1) to ensure transparency and reproducibility of the computational analysis.

2.5. DL_{50} and Toxicity Prediction

Median lethal dose (LD_{50}) values, and toxicity endpoints, including predicted hepatotoxicity, immunotoxicity, and cytotoxicity, were estimated using the ProTox-II web server (https://tox-new.charite.de/prottox_II/ (accessed on 30 November 2024)), which integrates machine-learning models trained on large toxicological datasets to predict acute oral toxicity and organ-specific toxicological liabilities based on chemical structure descriptors. The LW-9 peptide (PyrKKDRFLGLM-CONH₂) was constructed from its primary amino acid sequence using molecular modeling tools. Post-translational modifications were explicitly defined, including N-terminal pyroglutamate formation (Pyr) and C-terminal amidation (-CONH₂), to accurately represent the mature peptide structure. Ionizable residues were assigned protonation states consistent with physiological conditions (pH ~7.4), with lysine side chains modeled in their protonated form.

A three-dimensional structural model of LW-9 was generated and subjected to geometry refinement through energy minimization to obtain a sterically stable conformation prior to descriptor generation. The refined structure was subsequently converted into a canonical SMILES representation, which served as the structural input for ProTox-II. The ProTox-II platform automatically calculates molecular fingerprints and physicochemical descriptors from the submitted structure and applies ensemble machine-learning models to estimate LD_{50} values (mg/kg) and classify compounds into toxicity classes according to the Globally Harmonized System (GHS).

The raw results of the ProTox-II predictions are provided in Supplementary Materials (Table S2) to ensure transparency and reproducibility of the computational analysis.

3. Results

3.1. Characterization and Synthesis of the Peptide

Biochemical characterization of LW-9 was carried out by electrospray ionization quadrupole time-of-flight (ESI-Q-ToF) mass spectrometry. MS analysis allowed accurate determination of the molecular mass, whereas collision-induced dissociation (CID) fragmentation generated MS/MS spectra for sequence confirmation. Analysis of the exact mass and resulting fragment ions identified the amino acid sequence PyrKKDRFLGLM-CONH₂. The N-terminal pyroglutamate (Pyr) residue corresponds to a cyclized glutamine (Q) present in the precursor sequence, which is commonly represented as Q in FASTA format. The observed fragmentation pattern was consistent with peptides previously isolated from *Phoneutria nigriventer* venom, supporting the identification of LW-9 (Figure 2).

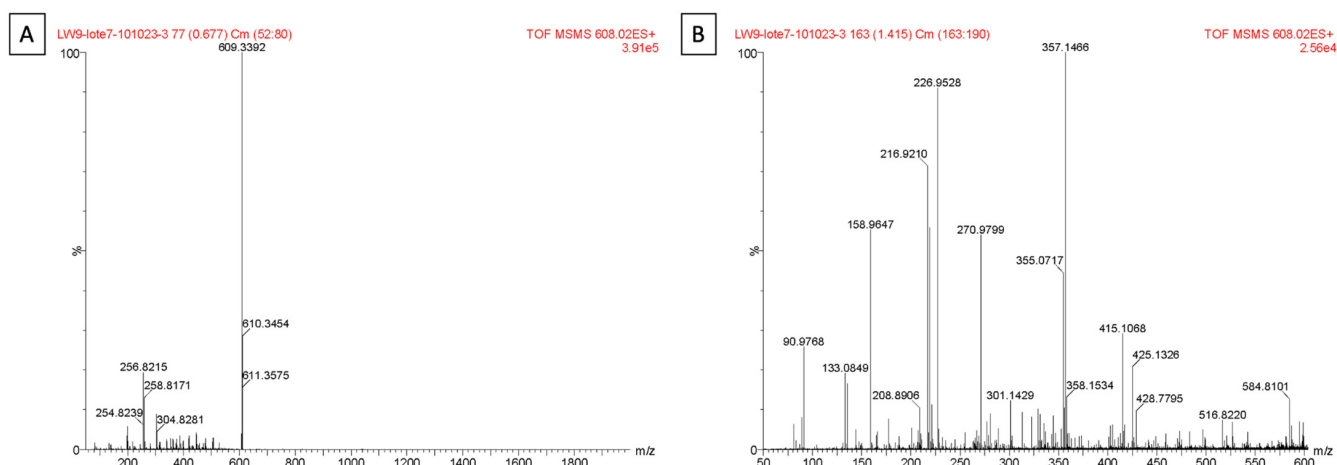


Figure 2. Mass spectrometry analysis using electrospray ionization and quadrupole time-of-flight (ESI-Q-ToF) for molecular mass determination and sequence confirmation. (A) ESI-Q-ToF MS mass spectrum profile, and (B) corresponding MS/MS fragmentation pattern used for sequence confirmation.

3.2. Molecular Modeling and In Silico Structural Analysis

Two-dimensional (2D) structural modeling of LW-9 enabled visualization of the spatial arrangement of its ten amino acid residues, revealing a predominance of charged and polar residues, indicative of a highly polar molecular profile (Figure 3A).

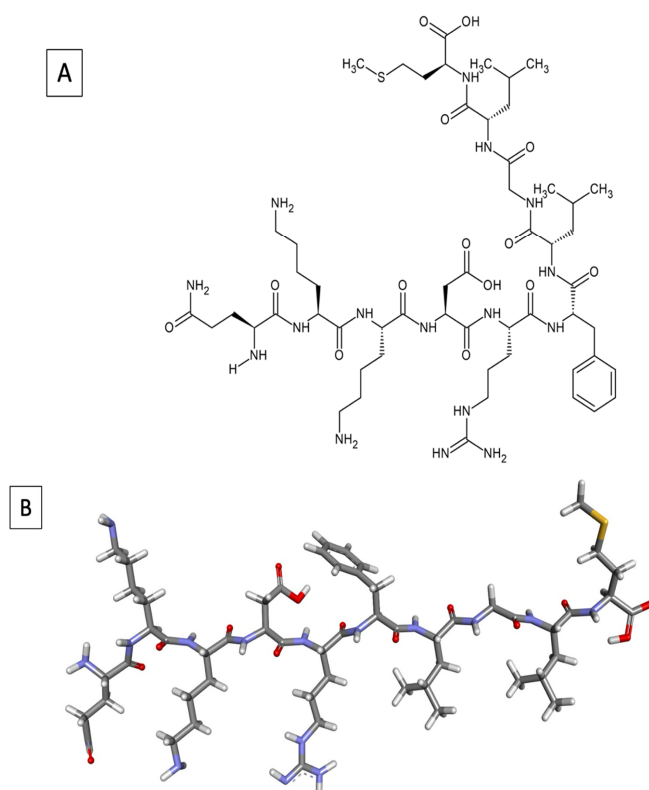


Figure 3. (A) Two-dimensional (2D) structural representation of the LW-9 peptide, illustrating the amino acid sequence (PyrKKDRFLGLM-CONH₂) and the distribution of charged and polar residues along the peptide backbone. (B) Predicted three-dimensional (3D) model of LW-9 generated using the I-TASSER server and visualized in BIOVIA Discovery Studio Visualizer (version 21.1.0.20298). The structure is shown in ribbon representation, highlighting the overall conformation, spatial arrangement of residues, N-terminal pyroglutamate (Pyr), and C-terminal amidation (CONH₂), consistent with a compact and predominantly hydrophilic peptide structure. Atoms are colored as follows: carbon (gray), hydrogen (white), oxygen (red), nitrogen (blue), and sulfur (yellow).

Three-dimensional (3D) modeling predicted a compact conformation characteristic of low-molecular-weight, hydrophilic peptides. The predicted structure was consistent with the peptide's physicochemical properties, particularly its high polarity and limited hydrophobic surface exposure (Figure 3B).

3.3. Evaluation of Physicochemical Properties and Pharmacokinetic Prediction

The analysis of physicochemical parameters revealed that the LW-9 peptide presents a molecular mass of 1235.522 Da, exceeding the threshold established by Lipinski's Rule of Five. Furthermore, violations were identified in the parameters related to the number of hydrogen bond donors and hydrogen bond acceptors. Although the calculated LogP value complies with Lipinski's criterion, its negative value (−3.26) indicates a highly hydrophilic and polar molecular profile, which is consistent with the predominance of charged residues in the peptide sequence (Table 1).

Table 1. Physicochemical parameters of the LW-9 peptide according to Lipinski's Rule of Five.

Description	Value
Molecular Mass (Da)	1235.522
Log P	−325,763
Hydrogen Bond Donors (HBD)	18
Hydrogen Bond Acceptors (HBA)	17

Analysis of drug absorption parameters in the initial stage of pharmacokinetics indicated that the LW-9 peptide exhibits low predicted gastrointestinal absorption, as well as limited permeability across the blood–brain barrier (BBB = 0.0270166) (Table 2). The complete prediction outputs generated by the PreADMET platform are provided in Supplementary Table S1. These findings are consistent with its highly polar and hydrophilic profile. Regarding P-glycoprotein (P-gp), LW-9 was predicted to act as a P-gp inhibitor. As P-gp is an efflux transporter that limits intestinal absorption and brain penetration, its inhibition may influence drug distribution and potential drug interactions.

Table 2. Predicted absorption, distribution, and excretion (ADME) parameters of the LW-9 peptide obtained by *in silico* analysis.

Description	Value
BBB permeability	0.027 *
Caco-2	18.987
HIA	0.000
MDCK permeability	0.018 *
P-gp	Inhibition
Plasma protein binding (PPB)	43.959

Legend: BBB permeability (blood–brain barrier permeability), Caco-2 (in vitro intestinal epithelial cell), HIA (human intestinal absorption), MDCK permeability (Madin–Darby canine kidney cell permeability), P-gp (in vitro P-glycoprotein inhibition), PPB (plasma protein binding). (*) Indicates relatively low values.

Madin–Darby canine kidney (MDCK) cell permeability is another parameter used to estimate passive membrane permeability. LW-9 was predicted to have low MDCK permeability (0.0181998), likely due to its high polarity and low lipophilicity. Plasma protein binding (PPB) is an important pharmacokinetic parameter reflecting the extent of drug binding to plasma proteins and its impact on distribution. LW-9 showed a relatively low predicted PPB value (43.96%), suggesting a higher free fraction in circulation and potentially a greater apparent volume of distribution.

3.4. Prediction of Cytochrome P450-Mediated Metabolism, In Silico Toxicological Assessment, and Acute Toxicity (LD₅₀) Prediction

Prediction of metabolic interactions with cytochrome P450 (CYP450) isoenzymes indicated that the LW-9 peptide exhibits inhibitory potential toward CYP2C19 and CYP3A4. No inhibitory activity was predicted for CYP2C9 or CYP2D6. Furthermore, LW-9 was not predicted to act as a relevant substrate for these isoenzymes, although weak substrate affinity was suggested for CYP3A4 (Table 3) (Supplementary Table S1). Given the central role of CYP3A4 in drug metabolism, its predicted inhibition may indicate a potential risk for drug interactions, warranting further experimental validation.

Table 3. Predicted interactions of the LW-9 peptide with cytochrome P450 (CYP450) isoenzymes.

Description	Value
CYP 2C19 inhibition	+
CYP 2C9 inhibition	—
CYP 2D6 inhibition	—
CYP 2D6 substrate	—
CYP 3A4 inhibition	+
CYP 3A4 substrate	±

Legend: (—), no predicted inhibition of cytochrome P450 isoenzymes; (+), predicted inhibition; (±), weak predicted activity. CYP2C19 (I), in vitro inhibition of CYP2C19; CYP2C9 (I), in vitro inhibition of CYP2C9; CYP2D6 (I), in vitro inhibition of CYP2D6; CYP2D6 (S), predicted CYP2D6 substrate; CYP3A4 (I), in vitro inhibition of CYP3A4; CYP3A4 (S), predicted CYP3A4 substrate.

The carcinogenicity prediction indicated that the LW-9 peptide does not exhibit carcinogenic potential in murine models; however, a positive prediction was observed in rat models, suggesting potential carcinogenic risk in this species according to the *in silico* analysis. Analysis of hERG channel inhibition yielded an inconclusive classification, precluding definitive conclusions regarding potential cardiotoxic risk based solely on these *in silico* data. Mutagenicity assessment using the *in silico* Ames test showed negative results for all evaluated strains, both in the absence and presence of metabolic activation (S9 fraction), suggesting no predicted mutagenic potential (Tables 4 and 5).

Table 4. *In silico* prediction of carcinogenicity in rodents and hERG channel inhibition.

Description	Value
Carcino mouse	Negative
Carcino rat	Positive
HERG Inhibition	Ambiguous

Legend: Carcino mouse, negative prediction for carcinogenicity in mice; Carcino rat (positive prediction for carcinogenicity in rats; hERG inhibition low risk of in vitro hERG channel inhibition), medium risk of in vitro hERG channel inhibition, high risk of in vitro hERG channel inhibition.

Table 5. *In silico* Ames test prediction of mutagenicity for the LW-9 peptide across different bacterial strains, with and without metabolic activation.

Description	Value
TA100 (without S9 mix)	Negative
TA 100 (with S9 mix)	Negative
TA 1535 (without S9 mix)	Negative
TA 1535 (with S9 mix)	Negative

Legend: TA100 (–S9), in vitro Ames test in the TA100 strain without metabolic activation; TA100 (+S9), in vitro Ames test in the TA100 strain with metabolic activation by rat liver S9 fraction; TA1535 (–S9), in vitro Ames test in the TA1535 strain without metabolic activation; TA1535 (+S9), in vitro Ames test in the TA1535 strain with metabolic activation by rat liver S9 fraction.

ProTox-II is an *in silico* platform designed for the prediction of toxicity endpoints of small molecules. The early identification of potential toxic effects is a critical component of drug development, as it enables risk assessment prior to experimental validation. Computational toxicity prediction not only accelerates the evaluation process compared with conventional *in vivo* testing but also contributes to the reduction in animal use in preclinical studies.

Acute toxicity analysis performed using the ProTox-II server predicted an LD₅₀ value of 2400 mg/kg for the LW-9 peptide, corresponding to toxicity class 5. According to the Globally Harmonized System (GHS), this classification indicates relatively low acute toxicity and suggests a broad safety margin for experimental evaluation across different dose ranges (Figure 4).

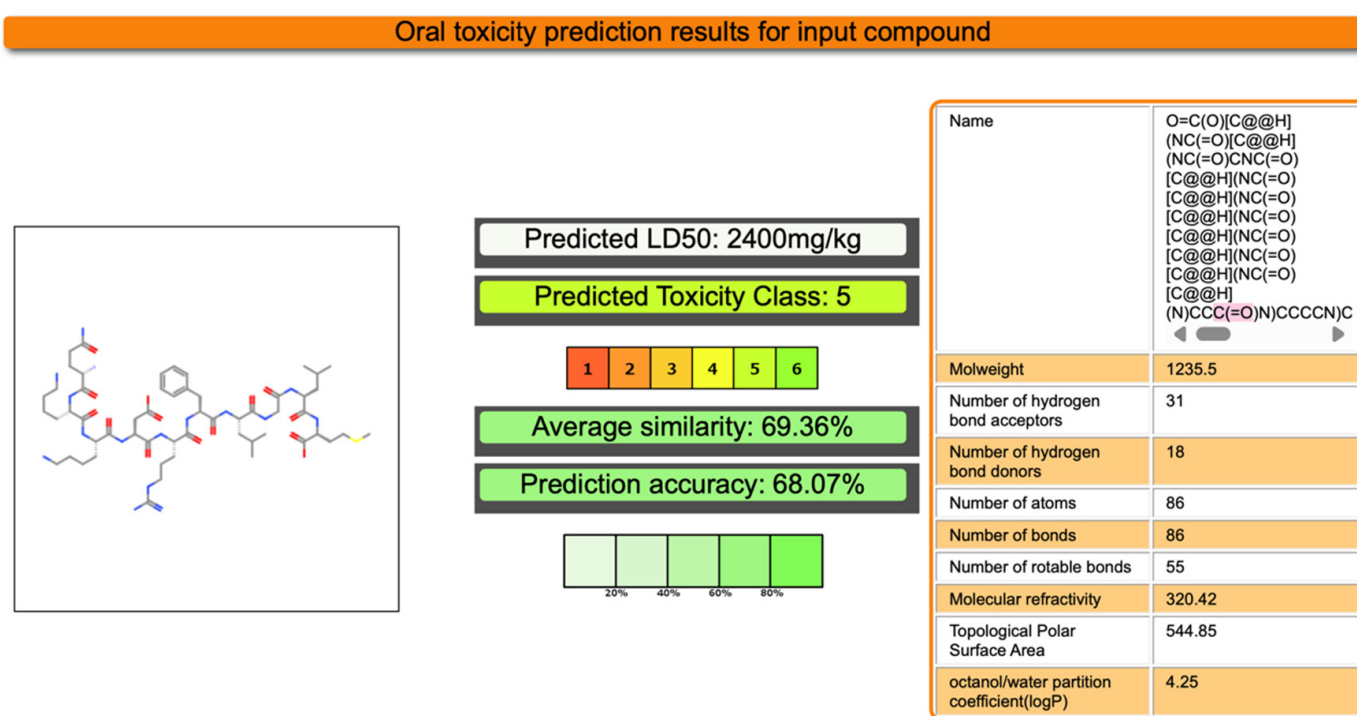


Figure 4. Predicted acute toxicity (LD₅₀) and toxicity class of the LW-9 peptide. The (**left panel**) displays the two-dimensional (2D) chemical structure of LW-9, while the (**right panel**) summarizes its key structural characteristics and properties relevant to toxicity prediction. Colors indicate prediction levels, with green representing higher similarity and accuracy. In the chemical structure, atoms are colored as follows: carbon (gray), hydrogen (white), oxygen (red), nitrogen (blue), and sulfur (yellow).

4. Discussion

In silico analyses play a central role in early-stage drug discovery by providing mechanistic insights prior to experimental validation. This study presents a comprehensive computational characterization of the LW-9 peptide, integrating physicochemical, pharmacokinetic, and toxicological predictions to support its evaluation as a potential immunomodulatory therapeutic.

For a molecule to be suitable for oral administration, it must generally comply with Lipinski's Rule of Five [18], which stipulates no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular mass below 500 Da, and an octanol–water partition coefficient (LogP) between 0 and 5 [19]. According to these criteria, the LW-9 peptide exceeds the recommended number of hydrogen bond donors and acceptors and has a molecular mass above 500 Da. Although its LogP value falls within the acceptable range, its negative value indicates a highly hydrophilic and polar molecular profile (Table 1).

Peptides frequently violate Lipinski's rules, which often results in low oral bioavailability and pharmacokinetic profiles unsuitable for gastrointestinal absorption. Consequently, non-oral delivery routes or structural modifications are typically required to achieve therapeutic efficacy [20]. This is consistent with the characteristics observed for LW-9, whose pronounced hydrophilicity and multiple rule violations explain the predicted low intestinal absorption and membrane permeability. Indeed, the majority of approved peptide drugs (approximately 78%) are administered via injection due to limited oral bioavailability, gastrointestinal instability, and inadequate membrane permeability [21].

Drug absorption represents the first stage of pharmacokinetics and depends on a compound's ability to traverse biological barriers such as the gastrointestinal tract [22]. Large, highly polar molecules generally exhibit limited permeability across biological membranes and the blood–brain barrier (BBB) and often require specialized delivery strategies to reach the central nervous system [23]. The LW-9 peptide, with a molecular mass of approximately 1.2 kDa and a highly hydrophilic profile, was predicted to have very low membrane permeability, including across the BBB. Absorption analysis further indicated poor gastrointestinal uptake and minimal BBB penetration (Table 2). *In vitro* permeability models, including Caco-2 and MDCK cell systems, also predicted limited passive transport, supporting the hypothesis that LW-9 is unlikely to reach the CNS under conventional systemic administration.

Regarding P-glycoprotein (P-gp) activity, LW-9 was not predicted to inhibit this efflux transporter, which plays a key role in cellular xenobiotic clearance and CNS protection [24,25]. The lack of predicted P-gp inhibition further supports the expectation of limited oral absorption and restricted BBB penetration. Considering these pharmacokinetic constraints, alternative administration routes such as parenteral delivery may be more appropriate for achieving adequate systemic exposure in preclinical studies.

Plasma protein binding (PPB) is an important pharmacokinetic parameter reflecting the extent to which a compound binds to plasma proteins and influences systemic distribution. The LW-9 was predicted to exhibit low plasma protein binding, suggesting a high apparent volume of distribution and the potential for dissemination through aqueous compartments of the body. This prediction is consistent with the peptide's hydrophilic nature and supports the possibility of distribution into peripheral tissues, although penetration into the central nervous system is expected to remain limited [26].

Metabolism is a critical phase of the pharmacokinetic process, making it essential to evaluate potential interactions of the LW-9 peptide with cytochrome P450 (CYP450) isoenzymes. These enzymes catalyze the biotransformation of drugs through chemical modifications that can activate or inactivate compounds. Inhibition of these enzymes may result in clinically significant drug–drug interactions [27–29]. The present *in silico* analysis suggests that the LW-9 may inhibit CYP2C19 and CYP3A4 (Table 3), indicating a potential risk of metabolic interactions. These predictions are based on probability scores generated by machine-learning models, and therefore should be interpreted cautiously, particularly because such models are primarily trained on datasets composed of small organic molecules. Moreover, time-dependent inhibition, which can influence CYP3A4 activity, was not evaluated by the predictive model used in this study. Many antineoplastic drugs are metabolized by CYP3A4, and inhibition of this enzyme may increase systemic drug exposure and potentially enhance toxicity. These findings highlight the importance of considering possible metabolic interactions during future pharmacological development of LW-9 [27–30]. The apparent discrepancy between different computational predictions reflects the distinct metabolic endpoints evaluated by the platforms used. While some models assess whether a compound acts as a substrate of CYP enzymes, others estimate its potential inhibitory activity. From a biological perspective, peptide-based molecules

such as LW-9 are less likely to undergo classical CYP-mediated metabolism and are more commonly degraded by proteolytic enzymes. Furthermore, because many CYP predictive models are primarily trained on datasets of small organic molecules, these predictions should be interpreted cautiously and require experimental validation.

Toxicological prediction also indicated that LW-9 is unlikely to exhibit carcinogenic potential in rodents according to the PreADMET platform (Table 4). These predictions are based on datasets derived from experimental studies compiled by regulatory and toxicological databases. However, it is important to emphasize that computational predictions cannot fully guarantee toxicological safety, and experimental validation remains essential. Accordingly, future investigations should include *in vitro* and *in vivo* assays to confirm the predicted carcinogenic and mutagenic profiles [31–33].

The potential cardiotoxicity of LW-9 was also assessed through the prediction of hERG channel inhibition, an important indicator of cardiac safety. The hERG gene encodes a voltage-gated potassium channel essential for cardiac repolarization. Inhibition of this channel can prolong the QT interval and potentially lead to arrhythmias [34]. In the present analysis, hERG inhibition was classified as ambiguous, indicating an uncertain prediction that should be interpreted cautiously and validated experimentally.

Mutagenicity was evaluated using the *in silico* Ames test model implemented in PreADMET. LW-9 was predicted to be non-mutagenic in the tested bacterial strains (Table 5), suggesting a favorable preliminary safety profile. The Ames test is widely used for screening genotoxicity because it detects mutations affecting histidine biosynthesis in indicator strains of *Salmonella typhimurium* and *Escherichia coli* [35–37]. In the present prediction, the TA100 and TA1535 strains were used as indicators of base-pair substitution mutations, particularly those involving guanine–cytosine pairs. Although the Ames assay is a sensitive screening tool, regulatory guidelines typically recommend multiple bacterial strains and complementary assays to confirm genotoxicity [38–40].

The ProTox-II platform integrates structural similarity analysis, fragment-based toxicophore identification, and machine-learning models trained on large toxicological datasets to predict multiple toxicity endpoints [41,42]. For the LW-9 peptide, ProTox-II predicted an LD₅₀ value of 2400 mg/kg, corresponding to toxicity class 5, which indicates relatively low acute toxicity. However, this toxicity classification should be interpreted independently of the predicted pharmacokinetic profile. Although the LD₅₀ estimate refers to oral acute toxicity categories, the extremely high topological polar surface area (TPSA = 544.85 Å²) and high polarity of LW-9 strongly suggest very limited gastrointestinal absorption. Therefore, the predicted LD₅₀ value likely reflects intrinsic systemic toxicity rather than efficient oral exposure.

The reliability of this prediction should also be interpreted considering the confidence parameters provided by the model. The reported prediction accuracy (68.07%) and the average structural similarity to compounds within the training dataset (69.36%) indicate a moderate level of confidence. These values suggest that LW-9 shares partial chemical similarity with compounds used to train the predictive model, although peptide-based molecules are generally less represented in such datasets. In predictive toxicology models, the reliability of results is influenced by the applicability domain defined by the chemical diversity of the training set. Given the relatively high molecular weight and polar surface area of LW-9, characteristics typical of peptides, the predicted LD₅₀ value should therefore be interpreted as an indicative computational estimate rather than a definitive toxicological assessment [43,44].

Limitations of the Study

Despite the insights provided by the present computational analysis, several limitations should be acknowledged. First, the current study is based exclusively on *in silico*

predictions, and therefore, the results must be interpreted as preliminary estimations rather than experimentally validated biological properties. Predictions related to toxicity, pharmacokinetics, and cytochrome P450 interactions are model-derived and require confirmation through in vitro and in vivo experimental assays [44]. Another important limitation concerns the domain of applicability of the predictive models employed. Platforms such as PreADMET and ProTox-II were primarily developed and trained using datasets composed mainly of small organic molecules [19,43], which may limit their applicability to peptide-based compounds such as LW-9. Because LW-9 is a peptide with relatively high molecular weight and extreme polarity, some predicted parameters—particularly those related to membrane permeability and cytochrome P450 interactions—may lie partially outside the optimal applicability domain of the models. Consequently, these predictions should be interpreted as indicative computational estimates that require experimental validation to confirm their biological relevance.

Another limitation is that the structural analysis relied on static computational modeling without molecular dynamics simulations, which could provide additional insight into peptide conformational stability in solution. Furthermore, parameters particularly relevant for peptide therapeutics, such as proteolytic stability, renal clearance, and metabolic degradation, were not evaluated in this study. These factors strongly influence the in vivo pharmacokinetics of peptide-based compounds, as peptides are often susceptible to rapid enzymatic degradation and renal elimination. Future studies should therefore incorporate experimental pharmacokinetic and toxicological analyses to validate the predictions reported here and to more accurately determine the therapeutic potential of the LW-9 peptide.

5. Conclusions

In silico analyses provide valuable insights into the potential toxicological and pharmacokinetic properties of novel drug candidates. The computational characterization of the LW-9 peptide suggested a predicted low acute toxicity profile, with no predicted mutagenic effects and no predicted carcinogenicity in murine models. The peptide also exhibited predicted low membrane permeability and limited blood–brain barrier penetration, which are consistent with its highly polar physicochemical profile. The predicted interaction with cytochrome P450 enzymes, particularly CYP3A4, highlights a potential risk of metabolic interactions that should be considered in future pharmacological evaluations. However, it is important to emphasize that these findings are based exclusively on computational predictions, and the predictive platforms used are primarily trained on datasets composed of small molecules, which may limit their applicability to peptide-based compounds. Therefore, the results presented here should be interpreted as exploratory *in silico* predictions intended to guide early-stage evaluation and experimental design. Overall, this study provides a preliminary computational framework supporting the continued investigation of LW-9 as a venom-derived peptide with potential immunomodulatory applications, while experimental in vitro and in vivo studies will be essential to validate the predicted pharmacokinetic and toxicological properties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biomedinformatics6040054/s1>, Table S1: The raw data of the PreADMET predictions; Table S2: The raw data of the ProTox-II predictions; Video S1: Exploring the properties and effects of Peptide LW-9.

Author Contributions: Conceptualization, I.M.C.T. and C.R.; methodology, I.M.C.T. and C.d.S.P.; formal analysis, I.M.C.T.; investigation, I.M.C.T., J.M.S., C.d.S.P. and C.R.; resources, C.R.; writing—original draft preparation, I.M.C.T.; writing—review and editing, I.M.C.T. and C.R.;

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Institutional Review Board Statement: All procedures involving the collection and handling of *Phoneutria nigriventer* spiders were conducted in accordance with relevant ethical guidelines and regulations for the use of animals in research. Spider capture and venom extraction were approved and supervised by Prof. Dr. Thomaz Rocha-e-Silva under permanent zoological collection license no. 58786-1 and SISGEN registration no. A032F20. Venom was obtained to allow the separation and purification of its fractions and subfractions, enabling the biochemical characterization and determination of peptide sequences.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the conclusions of this study are available in the Supplementary Materials for this article.

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Conflicts of Interest: The authors declare no conflicts of interest.

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