Original Article
Published: 2026-07-06
https://doi.org/10.64253/042026jept001

Preclinical Safety Assessment of Cardin Using Experimental Approaches

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Academic Editor: George Andrei Draghici

Abstract

Cardiovascular diseases remain a leading cause of global mortality, with thrombotic complications representing a major clinical challenge. This study aimed to evaluate the preclinical safety and pharmacological activity of Cardin, a multicomponent peptide complex derived from neonatal lamb heart tissue. Cardin was obtained through saline extraction, thermal treatment, ammonium sulfate precipitation, and purification using gel filtration and ion-exchange chromatography. The preparation was partially characterized by high-performance liquid chromatography (HPLC) and mass spectrometry, confirming the presence of multiple peptide fractions with distinct molecular masses; however, the complete amino acid sequences of individual components were not fully elucidated, and Cardin should therefore be regarded as a heterogeneous peptide mixture. In silico toxicity prediction indicated potential risks, including neurotoxicity, hepatotoxicity, and immunotoxicity. In contrast, in vivo experimental studies demonstrated no significant acute or cumulative toxicity at the tested doses. The median lethal dose (LD_{50}) was estimated to exceed 1000 mg/kg. Subacute and chronic administration did not result in observable adverse effects on behavior, body weight, or histological structure of major organs. Hematological and biochemical parameters remained within physiological ranges across all experimental groups, and no organ-specific toxicity was detected. Overall, Cardin exhibited a favorable safety profile in preclinical models despite discrepancies between computational predictions and experimental findings. These differences may reflect the limitations of in silico models when applied to complex multicomponent peptide systems. Further studies are required to clarify the contribution of individual peptide fractions and to better understand their mechanisms of action.

Keywords: *Cardin; peptide complex; anticoagulant; thrombosis; hypoxia; preclinical safety; in vivo; in silico*

Citation: Uchkun I, Jamolitdin Z, Nargiza H, Naima G, Shukhrat K, Golib N. Preclinical Safety Assessment of Cardin Using Experimental Approaches. *Journal of Experimental Pharmacology and Toxicology* 2026;4. https://doi.org/10.64253/042026jept001

1. Introduction

Cardiovascular diseases remain the leading cause of morbidity and mortality worldwide, with thrombotic complications representing a major pathological mechanism underlying conditions such as myocardial infarction and stroke. Current pharmacological strategies, including anticoagulants and antiplatelet agents, are effective but are often associated with adverse effects such as bleeding risk and limited specificity. Therefore, the development of novel therapeutic agents with improved safety profiles and targeted mechanisms of action remains an important goal in cardiovascular pharmacology [1, 2]. Cardin should not be considered a classical atrial-derived peptide but rather a heterogeneous peptide complex with a distinct origin and composition.

In recent years, peptide-based therapeutics have gained increasing attention due to their high specificity, biocompatibility, and relatively low systemic toxicity. Peptides derived from natural biological sources, in particular, have shown potential in modulating hemostasis, inflammation, and vascular function. Such compounds may act through multiple pathways, offering advantages over single-target synthetic drugs [3–7]. Cardin is a multicomponent peptide complex

derived from neonatal lamb heart tissue. Rather than representing a single chemically defined molecule, it should be considered a heterogeneous mixture of partially characterized peptides with varying molecular weights. The preparation has been characterized using chromatographic techniques and mass spectrometry to identify major peptide fractions; however, the complete primary amino acid sequences and full structural elucidation of individual components remain unresolved. This limited structural definition should be taken into account when interpreting its biological activities, as the observed effects may result from the combined or synergistic action of multiple peptide constituents [8, 9]. Despite growing interest in peptide-based cardiovascular agents, limited information is available regarding the pharmacological properties and safety profile of such multicomponent peptide complexes. In particular, comprehensive preclinical evaluations integrating toxicity assessment, dose–response relationships, and experimental validation remain scarce. Furthermore, discrepancies between in silico toxicity predictions and in vivo experimental findings are not uncommon and require systematic investigation to better understand their relevance and limitations [1, 10, 11]. Therefore, the aim of the present study was to isolate and characterize the Cardin peptide complex and to evaluate its pharmacological activity and safety profile through a series of preclinical in silico and in vivo assessments. Special attention was given to toxicity evaluation across different experimental models, as well as to the comparison between computational predictions and experimental outcomes.

2. Materials and Methods

2.1. Test Substance (Cardin)

Cardin is a partially characterized peptide-based preparation consisting of a mixture of biologically active peptide fractions derived from neonatal lamb heart tissue. The peptide fraction was obtained through homogenization in 0.9% NaCl, followed by thermal treatment at 80 °C, ammonium sulfate precipitation, and subsequent purification using gel filtration and ion-exchange chromatography.

The resulting preparation was formulated as a hydroalcoholic solution containing approximately 0.001% active peptide complex in 40% ethanol (v/v). Due to the multicomponent nature of the preparation, Cardin does not possess a single defined chemical structure and should be regarded as a heterogeneous peptide mixture. Its identity is therefore defined based on its source, standardized production protocol, and physicochemical handling conditions.

Preliminary characterization was performed using high-performance liquid chromatography (HPLC), which demonstrated multiple peptide fractions. Molecular weight analysis was conducted using Chip-HPLC-QTOF mass spectrometry, identifying five major peptide fractions with molecular weights of 7553.021, 5371.111, 7232.144, 9659.142, and 6847.232 Da. However, the complete primary amino acid sequences of these peptides were not fully elucidated.

For in vivo experiments, the Cardin solution was freshly prepared under sterile conditions prior to administration. The vehicle control group received an equivalent volume of 40% ethanol under identical experimental conditions.

2.2. Experimental Animals

A total of 586 laboratory animals were used, including mice (20 ± 2 g), rats (140 ± 10 g), guinea pigs, and rabbits of both sexes. Animals were acclimatized for at least 7 days and housed under standard laboratory conditions (22 ± 2 °C, 55 ± 10% humidity, 12 h light/dark cycle) with free access to food and water. All experimental procedures were approved by the Institutional Ethics Committee of Institute of Bioorganic Chemistry named after O. Sodikov Academy of Sciences of the Republic of Uzbekistan (protocol No. 1-2-1451-2-255 10/2025) and conducted in accordance with international ethical standards and ARRIVE 2.0 guidelines.

2.3. Study Design and Randomization

Animals were randomly assigned to experimental groups using a random number generation method. Each group consisted of 6–8 animals unless otherwise specified.

Outcome assessment was performed under blinded conditions where feasible; however, full blinding was not always possible due to the nature of the experimental procedures. Dose selection was based on preliminary screening studies and literature data on peptide-based biologically active compounds, taking into account safety margins.

2.4. Acute Toxicity Study

Acute toxicity was evaluated in mice and rats following single oral and intraperitoneal administration of Cardin at graded dose levels expressed in mg/kg body weight. Animals (n = 6 per group) were observed continuously for the first 6 h and daily for 14 days for clinical signs of toxicity and mortality. Behavioral parameters, including locomotor activity,

respiration, posture, and reflexes, were recorded. The median lethal dose (LD_{50}) was estimated using probit analysis. In the tested dose range, no mortality was observed, indicating low acute toxicity.

2.5. Cumulative Toxicity Study

Cumulative toxicity was assessed in mice over 26 days with repeated oral administration of Cardin at fractionated doses relative to the maximum tested dose. The cumulative toxicity coefficient (Kc) was calculated as the ratio of LD_{50} after repeated administration to that after a single dose.

2.6. Chronic Toxicity Study

Chronic toxicity was evaluated in rats and rabbits following daily oral administration of Cardin at doses of 10^{-4}, 10^{-2}, and 1.0 mg/kg for 30 consecutive days (n = 6–8 per group). Blood samples were collected on days 10 and 30 for biochemical and hematological analysis. A subset of animals were observed for an additional 30-day recovery period. Biochemical parameters (ALT, AST, glucose, bilirubin, creatinine, urea, total protein) and hematological parameters (RBC, WBC, hemoglobin, hematocrit, platelet count) were measured using automated analyzers [12–14].

2.7. Coagulation and Antithrombotic Activity

Hemostatic parameters were assessed using standard coagulation assays, including activated partial thromboplastin time (APTT), thrombin time (TT), and plasma recalcification time.

Antithrombotic activity was evaluated using a ferric chloride-induced arterial thrombosis model in rats. Thrombus weight was measured 24 h after induction.

2.8. Antihypoxic Activity

Antihypoxic activity was evaluated using a sodium nitrite-induced hypoxia model in mice. Cardin was administered orally at doses of 10^{-4} and 10^{-2} mg/kg prior to hypoxia induction. Survival time was recorded and compared to the control group.

2.9. In Silico Toxicity Prediction

Computational toxicity prediction was performed using the ProTox-II web platform. Predicted endpoints included hepatotoxicity, neurotoxicity, immunotoxicity, receptor interactions, and cytochrome P450-related metabolism [10, 11, 15, 16].

2.10. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Isolation and Physicochemical Characterization of Cardin

The peptide complex Cardin was successfully isolated from lamb (*Ovis aries*) heart tissue using sequential extraction, thermal denaturation, ammonium sulfate precipitation, and chromatographic purification (gel filtration and ion-exchange chromatography). The overall yield of the peptide fraction was approximately 0.00015% (w/w).

Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis revealed that Cardin is a multicomponent peptide mixture, consisting of five major (K-1–K-5) and four minor peptide fractions. The optimal chromatographic separation was achieved on a ZORBAX Eclipse C18 column (4.6 × 250 mm, 5 μm) with an acetonitrile–0.1% trifluoroacetic acid gradient, enabling reproducible peptide mapping within 15 min.

As shown in **Figure 1**, reverse-phase high-performance liquid chromatography (RP-HPLC) analysis demonstrated that Cardin is a multicomponent peptide mixture consisting of five major (K-1–K-5) and four minor peptide fractions. Mass spectrometry (Chip-HPLC-Q-TOF, Agilent 6530B Agilent Technologies, Santa Clara, CA, USA) identified the molecular masses of the major peptides as follows: K-1: 7553.0 Da, K-2: 5371.1 Da, K-3: 7232.1 Da, K-4: 9659.1 Da, K-5: 6847.2 Da. Amino acid analysis demonstrated that all fractions are composed predominantly of hydrophobic and neutral amino acids (Leu, Val, Gly, Ala), with variable content of charged residues (Lys, Arg, Asp/Glu), suggesting potential interactions with coagulation-related proteins [8, 9].

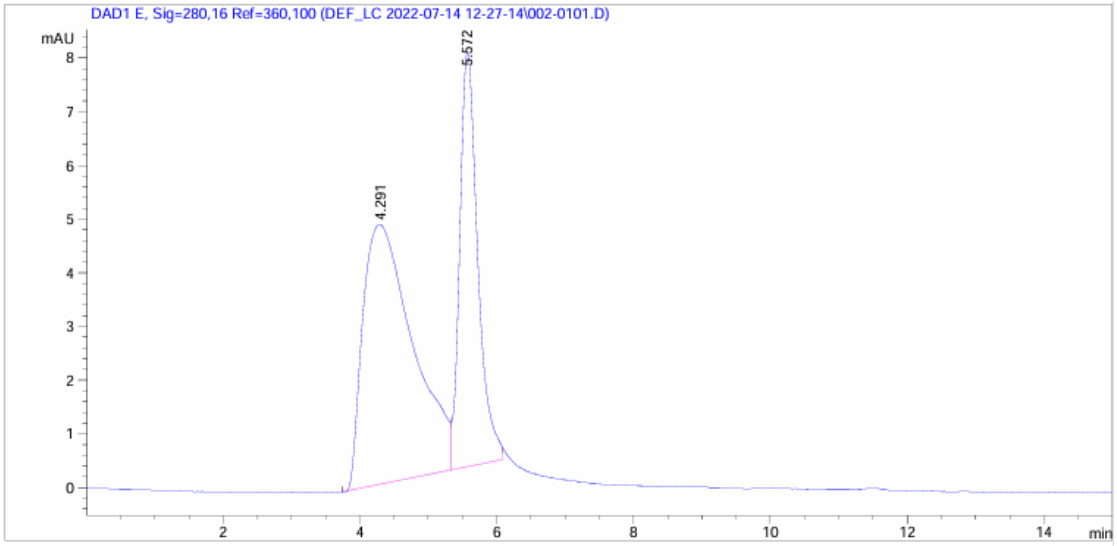

Figure 1. Chromatogram of Cardin peptide complex. Column: ZORBAX Eclipse C18, 5 μm, 4.6 × 250 mm. Mobile phase: isocratic elution with 40% acetonitrile in 0.1% TFA (trifluoroacetic acid) aqueous solution. Flow rate: 0.5 mL/min. Detection: 280 nm.

Fragmentation analysis (LC–MS/MS) revealed multiple peptide fragments; however, full primary structures could not be reconstructed using available databases, confirming the novel and heterogeneous nature of the Cardin peptide complex. The detailed mass spectrometric characteristics of the identified peptides are presented in **Table 1**.

Table 1. Mass spectrometric analysis of Cardin peptides.

Fragment Structures	**MM**
K-1	
ISGLIYEETR	1181.3391
THINIVVIGHVDSGK	1589.8462
TYFPHFDLSHGSAQVK	1835.0415
VGAGAPVYLAAVLEYLTAEILELAGNAAR	2917.3963
K-2	
LLLPGELAK	954.2050
YRLLLPGELAK	1273.5709
FFSAVSTVLTSK	1287.5077
VNVDEVGGEALGR	1315.4342
K-3	
ALAAAGYDVEK	1108.2438
THINIVVIGHVDSGK	1589.8462
NYFPHFDLSHGSAQVK	1848.0403
LLGGVTIAQGGVLPNIQAVLLPK	2272.7973
K-4	
VFLENVIR	990.1977
ESTLHLVLR	1068.2689
VFLENVIRDK	1233.4620
FFSAVSTVLTSK	1287.5077
ESTLHLVLRLK	1309.6045
LNIVVIGHVDSGK	1351.5984
YKESTLHLVLR	1359.6212

Table 1. *Cont.*

Fragment Structures	MM
K-5	
FFSAVSTVLTSK	1287.5077
GVLPNIQAVLLPK	1362.7091
GVLPNIQAVLLPKK	1490.8843
FFSAVSTVLTSKYK	1578.8601

3.2. Anticoagulant Activity

3.2.1. Bleeding Time and Blood Loss

Oral administration of Cardin at a dose of 1×10^{-4} mg/kg significantly affected primary hemostasis in rats.

- Bleeding time increased by 47% after 2 h.
- Blood loss increased by 40% compared to the control.
- After repeated administration (10 days), bleeding time remained elevated (42%), while blood loss returned to baseline levels.

Compared to acetylsalicylic acid (100 mg/kg), Cardin demonstrated comparable or stronger acute anticoagulant effects despite a 10^6-fold lower dose, indicating high biological potency [2, 4, 6, 8].

3.2.2. Coagulation Parameters

Cardin and its peptide fractions induced pronounced hypocoagulant effects, primarily affecting the intrinsic coagulation pathway.

The effects of Cardin and its peptide fractions on coagulation parameters in rat plasma are presented in **Table 2**. APTT (activated partial thromboplastin time) increased up to 5.3-fold for K-3; up to 8.4-fold for K-5; and 2.8-fold for Cardin. Thrombin time (TT) increased up to 7.8-fold (K-1).

Table 2. Effects of Cardin and its peptide fractions on coagulation parameters in rat plasma (M ± SD, n = 5).

Group	Dose	PT (s)	APTT (s)	PRT (s)	TT (s)	Fibrinogen (mg/dL)
Control	—	12.0 ± 1.0	26.2 ± 2.1	46.4 ± 3.6	10.6 ± 1.0	460 ± 32
K-1	10^{-4}	12.0 ± 1.1	58.8 ± 5.4 *#	68.0 ± 5.6 *#	82.2 ± 7.4 *#	238 ± 20 *
K-2	10^{-4}	11.8 ± 1.0	60.3 ± 4.8 *#	78.9 ± 6.5 *#	66.1 ± 4.4 *#	313 ± 20 *#
K-3	10^{-4}	10.5 ± 0.7	138.5 ± 12.3 #	75.8 ± 3.5 *	55.4 ± 4.0 *#	198 ± 16 *#
K-4	10^{-4}	11.9 ± 1.1	110.2 ± 9.2 *#	150.0 ± 14.7 *#	69.4 ± 5.2 *#	249 ± 19 *
K-5	10^{-4}	12.5 ± 1.6 *#	118.7 ± 16.8 *	91.1 ± 7.7 *#	17.0 ± 1.5 *	380 ± 18 *#
Cardin	10^{-4}	11.7 ± 1.0	72.2 ± 5.6 *	83.4 ± 7.6 *	28.4 ± 1.8 *	280 ± 24 *

Notes: * $p < 0.01$ vs. control, # $p < 0.01$ vs. Cardin.

Plasma recalcification time (PRT) increased significantly across all fractions: fibrinogen levels decreased by 1.5–2.3-fold.

No significant effect was observed on prothrombin time (PT), except for minor changes in K-5, indicating that Cardin predominantly modulates the intrinsic pathway of coagulation.

3.3. Antithrombotic Activity

Cardin and its peptide fractions significantly reduced thrombus formation in rats: thrombus weight decreased by 40% (Cardin) and 60% (K-3, K-4), and clot inhibition rate increased by up to 63.5% (K-4).

The antithrombotic activity of Cardin and its peptide fractions is presented in **Table 3**. These results demonstrate a strong dose-dependent antithrombotic effect, with fractions K-3 and K-4 showing the highest activity.

Table 3. Antithrombotic activity of Cardin and its peptide fractions (M ± SD, n = 5).

Group	Dose	Absorbance (410 nm)	Antithrombotic Activity (AU)	Clot Weight (mg)	Clot Inhibition (%)
Control	—	0.260 ± 0.02	1.00	27.4 ± 1.5	0
K-1	10^{-4}	0.310 ± 0.03 *	1.19 ± 0.15 *	18.4 ± 1.4 *	32.8 ± 2.6 *#
K-2	10^{-4}	0.360 ± 0.03 *	1.38 ± 0.13 *	18.3 ± 1.5 *	33.2 ± 3.0*#
K-3	10^{-4}	0.370 ± 0.03* #	1.42 ± 0.14* #	11.0 ± 1.0 *	59.8 ± 4.6 *#
K-4	10^{-4}	0.390 ± 0.03 *#	1.50 ± 0.14 *#	10.0 ± 1.0 *#	63.5 ± 6.1 *#
K-5	10^{-4}	0.300 ± 0.03 *#	1.15 ± 0.12 *#	22.5 ± 1.3 *	17.8 ± 4.2 *#
Cardin	10^{-4}	0.325 ± 0.03 *	1.25 ± 0.12 *	16.2 ± 1.5 *	40.9 ± 3.7 *

Notes: * $p < 0.01$ vs. control, # $p < 0.01$ vs. Cardin.

3.4. Arterial Thrombosis Model

In an $FeCl_3$-induced carotid artery thrombosis model in rats, thrombus weight decreased: control: 1.9 ± 0.2 mg; Cardin: 0.8 ± 0.06 mg (58% reduction). Coagulation parameters also improved: APTT increased by 40%, and fibrinogen decreased by 40%. The results are summarized in **Table 4**.

Table 4. Effects of Cardin and Nebilet on hemostasis parameters in animals with experimental arterial thrombosis (M ± SD).

Group	Dose	Thrombus Weight (mg)	Prothrombin Time (s)	APTT (s)	Fibrinogen (mg/dL)
Intact animals	—	—	23.3 ± 2.0	22.0 ± 2.0	400 ± 35
Control (thrombosis)	—	1.9 ± 0.2	14.1 ± 0.1	14.5 ± 1.0	877 ± 50
Cardin	10^{-4} mg/kg	0.8 ± 0.06	17.7 ± 1.2	20.3 ± 4.0	558 ± 40
Nebilet	0.46 mg/kg	0.98 ± 0.1	20.7 ± 2.0	25.9 ± 2.4	802.9 ± 50

The effects of Cardin were comparable to or stronger than those of nebivolol (0.46 mg/kg), particularly in reducing fibrinogen levels.

3.5. Antihypoxic Activity

In a sodium nitrite-induced hemic hypoxia model, Cardin (1×10^{-4} mg/kg) increased survival latency by 42–56% compared to the control. A higher dose (1×10^{-2} mg/kg) showed maximal effect at 24 h (57% increase), followed by reduced activity at 48 h. These findings indicate moderate but significant antihypoxic activity, potentially related to improved microcirculation [15, 17].

3.6. Safety and Toxicity Observations

Across all experimental conditions:

- No acute mortality was observed at tested doses;
- No visible signs of neurotoxicity, hepatotoxicity, or behavioral abnormalities were detected;
- Anticoagulant effects were dose-dependent but not associated with pathological bleeding.

These findings suggest a favorable safety profile within the studied dose range, despite in silico predictions indicating potential toxicity risks.

3.6.1. In Silico Toxicity Prediction

Computational toxicity analysis predicted potential hepatotoxicity, neurotoxicity, respiratory toxicity, immunotoxicity, estrogen receptor activation, aromatase interaction, acetylcholinesterase inhibition, and cytochrome P450 (CYP2C9 and CYP3A4) involvement. Conversely, cardiotoxicity, nephrotoxicity, mutagenicity, cytotoxicity, and androgen receptor activity were predicted to be inactive. The computational toxicity analysis predictions are summarized in **Table 5**.

Table 5. Computational toxicity analysis prediction.

Classification	Target	Prediction	Probability
Organ Toxicity	Hepatotoxicity	Active	0.69
	Neurotoxicity	Active	0.87
	Nephrotoxicity	Inactive	0.90
	Respiratory toxicity	Active	0.98
	Cardiotoxicity	Inactive	0.77
Toxicity Endpoints	Immunotoxicity	Active	0.96
	Carcinogenicity	Inactive	0.62
	Mutagenicity	Inactive	0.97
	Cytotoxicity	Inactive	0.93
	Ecotoxicity	Active	0.73
Nuclear Receptor Signaling	Aromatase	Active	1.00
	Estrogen Receptor Alpha (ER)	Active	0.99
	Estrogen Receptor LBD	Active	1.00
	Androgen Receptor (AR)	Inactive	0.99
Molecular Initiating Events	Acetylcholinesterase (AChE)	Active	0.69
	GABA receptor (GABAR)	Inactive	0.96
	NMDA receptor (NMDAR)	Inactive	0.92
Metabolism	CYP2C9	Active	0.56
	CYP3A4	Active	0.71
	CYP1A2	Inactive	0.76

3.6.2. Additional Safety Evaluations

Local irritation testing in rats and rabbits demonstrated no signs of erythema or edema at administration sites. Allergenicity studies in guinea pigs showed no hypersensitivity reactions. Embryotoxicity assessment in rats revealed normal fetal development with no evidence of resorptions, malformations, or growth retardation. Mutagenicity testing in cultured human peripheral blood lymphocytes showed no increase in chromosomal aberrations compared to controls. Immunotoxicity assays in mice demonstrated preserved humoral and cellular immune responses.

The present study provides a comprehensive preclinical evaluation of the multicomponent peptide complex Cardin, isolated from neonatal lamb (Ovis aries) heart tissue, with emphasis on its physicochemical characteristics and anticoagulant, antithrombotic, and antihypoxic activities, as well as its safety profile.

4. Discussion

4.1. Interpretation of Anticoagulant and Antithrombotic Effects

The results demonstrate that Cardin exerts pronounced anticoagulant effects, primarily through modulation of the intrinsic pathway of coagulation. This is supported by significant prolongation of activated partial thromboplastin time (APTT), thrombin time (TT), and plasma recalcification time (PRT), along with a reduction in fibrinogen levels, while prothrombin time (PT) remained largely unaffected.

Such a pattern suggests that Cardin may interfere with intrinsic coagulation factors (e.g., factors XII, XI, IX, and VIII) or enhance endogenous anticoagulant mechanisms rather than affect the extrinsic pathway. The observed decrease in fibrinogen further indicates a potential inhibition of fibrin formation and/or promotion of fibrinolytic processes.

Importantly, the reduction in thrombus formation and increased clot inhibition observed in vivo are consistent with the anticoagulant effects and indicate that Cardin can suppress pathological thrombosis without completely disrupting physiological hemostasis. Among the peptide fractions, K-3 and K-4 demonstrated the most pronounced activity, suggesting that specific components within the mixture may be primarily responsible for the observed pharmacological effects [2, 4, 6, 9].

4.2. Structural Features and Relation to Bioactivity

Cardin is a heterogeneous mixture of medium-molecular-weight peptides (approximately 5–10 kDa), distinguishing it from conventional low-molecular-weight anticoagulants. The amino acid composition of these peptides, enriched in hydrophobic residues (Leu, Val, Ala) and containing charged residues (Lys, Arg, Asp, Glu), may facilitate interactions with proteins involved in coagulation cascades, platelet function, or endothelial regulation. Such structural diversity may contribute to the multimodal biological activity observed in this study, potentially involving synergistic interactions among different peptide fractions rather than the effect of a single active compound [5, 10, 11].

4.3. In Silico Versus In Vivo Toxicity: Explanation of Discrepancies

A notable finding of this study is the discrepancy between in silico toxicity predictions and in vivo experimental results. Computational models predicted potential hepatotoxicity, neurotoxicity, and immunotoxicity, whereas no corresponding toxic effects were observed in animal studies at the tested doses. This discrepancy may be attributed to several factors. First, in silico models are largely based on predefined chemical structures and known toxicophores; however, Cardin represents a complex mixture of partially characterized peptides, which may not be accurately represented in such databases. Second, the administered doses in vivo were relatively low, which may fall below toxicity thresholds. Third, peptides are subject to enzymatic degradation and limited bioavailability, reducing systemic exposure. Finally, biological systems possess adaptive detoxification and compensatory mechanisms that are not accounted for in computational models. Thus, the observed differences highlight the limitations of predictive toxicity tools when applied to heterogeneous biological preparations and emphasize the importance of experimental validation [12, 13, 18–20].

4.4. Antihypoxic Activity and Possible Mechanisms

Cardin demonstrated moderate but significant antihypoxic activity in a sodium nitrite-induced hemic hypoxia model. The increased survival time observed may be indirectly related to improvements in blood rheology and microcirculatory function.

By reducing thrombosis and potentially improving blood flow, Cardin may enhance oxygen delivery to tissues under hypoxic conditions. Additionally, modulation of endothelial function and nitric oxide-related pathways may contribute to the observed effects, although these mechanisms require further investigation [17–19, 21–26].

4.5. Methodological Considerations

In response to concerns regarding reproducibility and experimental rigor, this study provides detailed descriptions of animal models, dosing regimens, analytical methods, and experimental conditions. Randomization was applied during group allocation, and observations were conducted under partially blinded conditions. However, further standardization of blinding and randomization procedures in future studies would strengthen methodological robustness and compliance with international guidelines such as ARRIVE 2.0.

4.6. Study Limitations

Several limitations should be acknowledged. First, the structural characterization of Cardin remains incomplete, as the full amino acid sequences of individual peptide fractions were not fully elucidated. Second, the multicomponent nature of the preparation makes it difficult to attribute specific biological effects to individual peptides. Third, the current study primarily focuses on acute and subchronic toxicity, while long-term toxicological and immunogenic assessments remain limited. Finally, pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion (ADME), were not evaluated. These limitations should be addressed in future studies through advanced proteomic techniques, isolation of individual active fractions, and comprehensive pharmacokinetic and toxicological profiling [9].

4.7. Implications and Future Perspectives

The findings of this study suggest that Cardin is a promising peptide-based candidate with anticoagulant, antithrombotic, and antihypoxic properties at relatively low doses. Its high biological activity and favorable safety profile distinguish it from conventional anticoagulant agents. Future research should focus on full structural elucidation using advanced analytical methods, identification and isolation of the most active peptide fractions (particularly K-3 and K-4), detailed mechanistic studies at the molecular level, and comprehensive pharmacokinetic and long-term safety evaluations [2, 6, 12].

5. Conclusion

In summary, Cardin demonstrates a unique combination of anticoagulant, antithrombotic, and antihypoxic activities, primarily mediated through modulation of the intrinsic coagulation pathway. While in silico predictions suggest potential toxicity, in vivo findings indicate a favorable safety profile within the studied dose range. Despite current limitations, Cardin represents a promising candidate for further development as a peptide-based therapeutic agent. These findings highlight the importance of integrating in silico predictions with experimental validation when evaluating complex peptide-based therapeutics.

Acknowledgments

The authors express their gratitude to the staff of the Institute of Bioorganic Chemistry (Uzbekistan) for their technical support and assistance in conducting the experimental studies.

Funding

This research received no external funding.

Author contributions

Conceptualization: N.H. and U.I.; Methodology: U.I., J.Z., and N.G.; Investigation: U.I., J.Z., and N.G.; Data Curation: S.K. and G.N.; Formal Analysis: N.H. and G.N.; Writing—Original Draft: N.H.; Writing—Review and Editing: N.H. and U.I.; Supervision: N.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Institutional review board statement

The animal study protocol was approved by the Institutional Ethics Committee of Institute of Bioorganic Chemistry named after O. Sodikov Academy of Sciences of the Republic of Uzbekistan (protocol code: 1-2-1451-2-255, date of approval: 10/2025), conducted in accordance with international ethical standards and ARRIVE 2.0 guidelines.

Informed consent statement

Informed consent is not applicable to this study as it did not involve human participants.

Additional information

Received: 2026-12-15
Accepted: 2026-05-13
Published: 2026-07-06

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References

1. O'Sullivan S, Lafarga T, Hayes M, O'Brien N. Potential bioactivity of hydrolysates derived from bovine lung. Proc Nutr Soc. 2016; 75 (OCE3): E168. doi: 10.1017/S002966511600183X
2. Rivera-Jiménez J, Berraquero-García C, Pérez-Gálvez R, García-Moreno PJ, Espejo-Carpio FJ, Guadix A, et al. Peptides and protein hydrolysates exhibiting anti-inflammatory activity: sources, structural features and modulation mechanisms. Food Funct. 2022; 13 (24): 12510–40. doi: 10.1039/D2FO02223K
3. Ndiaye F, Vuong T, Duarte J, Aluko RE, Matar C. Antioxidant, anti-inflammatory and immunomodulating properties of an enzymatic protein hydrolysate from yellow field pea seeds. Eur J Nutr. 2012; 51 (1): 29–37. doi: 10.1007/s00394-011-0196-1
4. Bamdad F, Shin SH, Suh JW, Nimalaratne C, Sunwoo H. Anti-inflammatory and antioxidant properties of casein hydrolysate produced using high hydrostatic pressure combined with proteolytic enzymes. Molecules. 2017; 22 (4): 609. doi: 10.3390/molecules22040609
5. Ao J, Li B. Amino acid composition and antioxidant activities of hydrolysates and peptide fractions from porcine collagen. Food Sci Technol Int. 2012; 18 (5): 425–34. doi: 10.1177/1082013211428219
6. Yuan L, Chu Q, Wu X, Yang B, Zhang W, Jin W, et al. Anti-inflammatory and antioxidant activity of peptides from ethanol-soluble hydrolysates of sturgeon (*Acipenser schrenckii*) cartilage. Front Nutr. 2021; 8: 689648. doi: 10.3389/fnut.2021.689648
7. van Dijk A, van Eldik M, Veldhuizen EJ, Tjeerdsma-van Bokhoven HL, de Zoete MR, et al. Immunomodulatory and anti-inflammatory activities of chicken cathelicidin-2 derived peptides. PLoS ONE. 2016; 11 (2): e0147919. doi: 10.1371/journal.pone.0147919
8. Kiewiet MBG, Gros M, van Neerven RJJ, Faas MM, de Vos P. Immunomodulating properties of protein hydrolysates for application in cow's milk allergy. Pediatr Allergy Immunol. 2015; 26 (3): 206–17. doi: 10.1111/pai.12354
9. Cruz-Casas DE, Aguilar CN, Ascacio-Valdés JA, Rodríguez-Herrera R, Chávez-González ML, Flores-Gallegos AC. Enzymatic hydrolysis and microbial fermentation: the most favorable biotechnological methods for the release of bioactive peptides. Food Chem (Oxf). 2021; 3: 100047. doi: 10.1016/j.fochms.2021.100047
10. O'Sullivan S, Lafarga T, Hayes M, O'Brien N. Bioactivity of bovine lung hydrolysates prepared using papain, pepsin, and Alcalase. J Food Biochem. 2017; 41: e12406. doi: 10.1111/jfbc.12406
11. Angelova H, Krumova E, Dzhambazova E, Pechlivanova D. Effects of the antinociceptive dipeptide L-tyrosine-L-arginine (kyotorphin) on the motivation, anxiety, and memory in rats. Folia Med. 2021; 63 (2): 189–96. doi: 10.3897/folmed.63.e53912
12. Edwards AB, Mastaglia FL, Knuckey NW, Meloni BP. Neuroprotective Cationic Arginine-Rich Peptides (CARPs): an assessment of their clinical safety. Drug Saf. 2020; 43 (10): 957–69. doi: 10.1007/s40264-020-00962-z
13. Kumar R, Gupta YK, Singh S. Anti-inflammatory and anti-granuloma activity of Berberis aristata DC. in experimental models of inflammation. Indian J Pharmacol. 2016; 48 (2): 155–61. doi: 10.4103/0253-7613.178825
14. Kawabata A, Fukuzumi Y, Fukushima Y, Takagi H. Antinociceptive effect of L-arginine on the carrageenin-induced hyperalgesia of the rat: possible involvement of central opioidergic systems. Eur J Pharmacol. 1992; 218 (1): 153–8. doi: 10.1016/0014-2999(92)90159-9
15. Kawabata A, Nishimura Y, Takagi H. L-leucyl-L-arginine, naltrindole and D-arginine block antinociception elicited by L-arginine in mice with carrageenin-induced hyperalgesia. Br J Pharmacol. 1992; 107 (4): 1096–101. doi: 10.1111/j.1476-5381.1992.tb14461.x
16. Omote K, Hazama K, Kawamata T, Kawamata M, Nakayaka Y, Toriyabe M, et al. Peripheral nitric oxide in carrageenan-induced inflammation. Brain Res. 2001; 912 (2): 171–5. doi: 10.1016/s0006-8993(01)02739-2
17. Parlar A, Arslan SO, Çam SA. Glabridin alleviates inflammation and nociception in rodents by activating BKCa channels and reducing NO levels. Biol Pharm Bull. 2020; 43 (5): 884–97. doi: 10.1248/bpb.b19-01009
18. Yaren H, Mollaoglu H, Kurt B, Korkmaz A, Oter S, Topal T, et al. Lung toxicity of nitrogen mustard may be mediated by nitric oxide and peroxynitrite in rats. Res Vet Sci. 2007; 83 (1): 116–22. doi: 10.1016/j.rvsc.2006.11.004
19. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008; 245 (3): 194–205. doi: 10.1016/j.tox.2007.11.021
20. McGovern AJ, Vitkovitsky IV, Jones DL, Mullins ME. Can the AST/ALT ratio indicate recovery after acute paracetamol poisoning? Clin Toxicol (Phila). 2015; 53 (3): 164–7. doi: 10.3109/15563650.2014.998336
21. Ikarashi Y, Takahashi A, Ishimaru H, Shiobara T, Maruyama Y. The role of nitric oxide in striatal acetylcholine release induced by N-methyl-D-aspartate. Neurochem Int. 1998; 33 (3): 255–61. doi: 10.1016/s0197-0186(98)00025-5
22. Lundberg J, Weitzberg E, Gladwin M. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008; 7: 156–67. doi: 10.1038/nrd2466
23. Iwata M, Suzuki S, Asai Y, Inoue T, Takagi K. Involvement of nitric oxide in a rat model of carrageenin-induced pleurisy. Mediators Inflamm. 2010; 2010: 682879. doi: 10.1155/2010/682879
24. Kaur K, Singh L, Kaur A, Bhatti R. Exploring the possible mechanism involved in the antinociceptive effect of β-sitosterol: modulation of oxidative stress, nitric oxide and IL-6. Inflammopharmacology. 2023; 31 (1): 517–27. doi: 10.1007/s10787-022-01096-5
25. Washington IM, Van Hoosier G. Clinical biochemistry and hematology. In: The laboratory rabbit, guinea pig, hamster, and other rodents. Cambridge (MA): Academic Press; 2012. p. 57–116.
26. Rafter I, Gråberg T, Kotronen A, Strömmer L, Mattson CM, Kim RW, et al. Isoform-specific alanine aminotransferase measurement can distinguish hepatic from extrahepatic injury in humans. Int J Mol Med. 2012; 30 (5): 1241–9. doi: 10.3892/ijmm.2012.1091