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ISOLATION, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF BIOACTIVE PEPTIDE COMPLEXES FROM KARAKUL LAMB ORGANS AND CAMEL MILK FOR POTENTIAL PHARMACEUTICAL APPLICATIONS

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ABSTRACT

Natural bioactive peptides have emerged as promising candidates for the development of safe and effective therapeutic agents due to their high specificity and low toxicity. In this study, peptide complexes were isolated and characterized from the thymus, lung, and heart tissues of Karakul lambs, as well as from camel milk proteins, with the aim of evaluating their biological activity and pharmaceutical potential. Peptides were obtained using enzymatic hydrolysis and multi-step chromatographic purification, followed by structural identification through high-performance liquid chromatography and LC-MS/MS analysis. A total of nine peptides were identified in the thymus-derived preparation (Timoptin), including α 1-thymosin, β 4-thymosin, and thymopoietin. Lung-derived peptide complexes (Aflan) consisted of three major fractions with molecular weights of 8805.108, 12109.215, and 14376.112 Da, demonstrating pronounced anti-inflammatory activity. Heart-derived peptides (Kardin) included six bioactive components associated with the regulation of blood rheological properties. Additionally, enzymatic hydrolysis of camel milk proteins resulted in the identification of three antioxidant peptides with defined primary structures. Biological evaluation revealed that the obtained peptide complexes exhibit immunomodulatory, anti-inflammatory, antioxidant, and hemorheological activities. The findings highlight the potential of these natural peptide-based substances as promising candidates for the development of novel pharmaceutical agents.

1. INTRODUCTION

The growing demand for safe and effective therapeutic agents has stimulated extensive research into biologically active compounds derived from natural sources. In particular, bioactive peptides have attracted significant attention due to their high specificity, low toxicity, and diverse pharmacological activities, including immunomodulatory, anti-inflammatory, antioxidant, and regenerative effects. These properties make them promising candidates for the development of next-generation pharmaceuticals.

Animal-derived tissues represent an important and underexplored source of bioactive peptides. Among them, the thymus plays a central role in the regulation of the immune system by producing peptide hormones such as thymosins, thymopoietin, and thymulin, which are involved in T-cell differentiation and immune homeostasis. Peptides isolated from thymic tissue have demonstrated significant therapeutic potential in immunodeficiency, infectious, and inflammatory diseases.

In addition to the thymus, lung tissue is a rich source of structural and functional proteins that can be enzymatically converted into biologically active peptides. Lung-derived peptides have been reported to exhibit anti-inflammatory, antimicrobial, and tissue-repairing properties, highlighting their potential application in the treatment of respiratory and inflammatory disorders. Similarly, heart tissue contains bioactive peptides involved in the regulation of cardiovascular functions, including blood circulation and rheological properties, which are essential for maintaining systemic homeostasis.

Camel milk represents another valuable natural source of bioactive compounds. It contains proteins such as lactoferrin, immunoglobulins, and albumin, which can be hydrolyzed into peptides with pronounced antioxidant, antimicrobial, and immunomodulatory activities. These peptides have gained increasing attention due to their stability and potential therapeutic applications.

Despite the growing body of research on bioactive peptides, there is a lack of comprehensive studies focusing on the isolation, structural characterization, and biological evaluation of peptide complexes derived from locally available biological resources, particularly from Karakul lamb organs and camel milk. Moreover, the integration of peptides from different biological sources and the assessment of their combined pharmacological potential remain insufficiently explored.

Therefore, the aim of the present study is to isolate and characterize bioactive peptide complexes from the thymus, lung, and heart tissues of Karakul lambs and from camel milk proteins, as well as to evaluate their biological activities and potential application as pharmaceutical substances. The results of this study are expected to contribute to the development of novel peptide-based therapeutics with multifunctional biological properties.

2. MATERIALS AND METHODS

2.1. *Biological Materials*

The study was conducted using biological materials obtained from locally available sources. Thymus, lung, and heart tissues of Karakul lambs were used as primary raw materials for peptide isolation. In addition, camel milk proteins were utilized as a source for the production of bioactive peptides.

All samples were processed under controlled laboratory conditions to preserve protein integrity and biological activity.

2.2. *Extraction of Peptide Complexes*

Peptide complexes were extracted from animal tissues using a multi-stage bioorganic approach. The procedure included tissue homogenization followed by aqueous extraction under controlled temperature conditions. Thermolabile proteins were removed, and the resulting extracts were subjected to fractionation.

Protein-peptide fractions were separated using ammonium sulfate precipitation and subsequent purification steps. Low-molecular-weight peptide fractions were isolated for further analysis.

2.3. *Enzymatic Hydrolysis of Camel Milk Proteins*

Camel milk proteins were subjected to enzymatic hydrolysis to obtain bioactive peptides. The process was carried out using proteolytic enzymes under optimized conditions (pH, temperature, and incubation time). The hydrolysates were then filtered and purified to isolate peptide fractions with potential biological activity.

2.4. Purification Procedures

Purification of peptide complexes was performed using a combination of chromatographic techniques:

Gel filtration chromatography for molecular size separation

Ion-exchange chromatography for charge-based fractionation

High-performance liquid chromatography (HPLC) for final purification

These methods enabled the isolation of individual peptide fractions with high purity.

2.5. Proteomic Analysis

Structural characterization of peptides was carried out using modern proteomic techniques. Molecular weights and peptide sequences were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Additional peptide mapping approaches were used to confirm primary structures and identify protein origins.

2.6. Biological Activity Evaluation

The biological activity of isolated peptide complexes was evaluated using a set of experimental assays, including: Immunomodulatory activity assessment based on immune cell response; Anti-inflammatory activity evaluation through inhibition of inflammatory mediators; Antioxidant activity assays to determine free radical scavenging capacity; Hemorheological studies to assess effects on blood properties.

Pharmacological and toxicological evaluations were also conducted to determine the safety and therapeutic potential of the developed peptide substances.

2.7. Data Analysis

Experimental results were analyzed using standard statistical and analytical methods. Reproducibility and reliability of the obtained data were ensured through repeated experiments and comparative analysis of peptide fractions.

3. RESULTS

3.1. Isolation and Characterization of Thymus-Derived Peptides (*Timoptin*)

As a result of multi-stage extraction and purification of Karakul lamb thymus tissue, a peptide complex designated as *Timoptin* was obtained. Proteomic analysis using chromatographic separation and LC-MS/MS techniques revealed the presence of nine distinct peptide components.

Among the identified peptides, key immunoregulatory molecules were detected, including thymosin α 1, thymosin β 4, and thymopoietin. These peptides are known to play a critical role in T-cell differentiation and immune system regulation.

The obtained peptide complex was characterized by low molecular weight fractions, indicating good bioavailability and potential pharmacological activity.

3.2. Identification of Lung-Derived Peptide Complexes (*Aflan*)

Extraction and purification of peptides from Karakul lamb lung tissue resulted in the development of a peptide preparation referred to as *Aflan*. Chromatographic and mass spectrometric analysis identified three major peptide fractions.

The molecular weights of the isolated peptides were determined to be: 8805.108 Da, 12109.215 Da, 14376.112 Da. These peptides demonstrated pronounced anti-inflammatory activity, which was confirmed through biological assays. The results indicate that lung-derived peptides possess significant potential for modulating inflammatory processes.

3.3. Characterization of Heart-Derived Peptides (*Kardin*)

Peptide complexes isolated from heart tissue were combined into a preparation termed *Kardin*. Analytical studies identified six peptide components with molecular weights ranging from 5371 to 9659 Da.

These peptides exhibited a notable influence on blood rheological properties, suggesting their role in improving blood circulation and viscosity. The observed effects highlight their potential application in cardiovascular regulation and related disorders.

3.4. Peptides Derived from Camel Milk Proteins

Enzymatic hydrolysis of camel milk proteins resulted in the generation of bioactive peptide fractions. Subsequent purification and proteomic analysis enabled the identification of three peptides with defined primary structures.

These peptides exhibited antioxidant activity, as demonstrated by their ability to neutralize reactive oxygen species. The results confirm that camel milk proteins serve as a valuable source of functional peptides with potential therapeutic applications.

3.5. Summary of Biological Activities

The biological evaluation of all obtained peptide complexes demonstrated a broad spectrum of pharmacological effects: Thymus-derived peptides exhibited strong immunomodulatory activity; Lung-derived peptides showed significant anti-inflammatory effects; Heart-derived peptides influenced hemorheological parameters; Camel milk-derived peptides demonstrated antioxidant properties

Overall, the results confirm that the isolated peptide complexes possess complementary biological activities, supporting their potential use as multifunctional pharmaceutical agents.

4. DISCUSSION

The present study demonstrates that biologically active peptide complexes derived from different natural sources—namely thymus, lung, and heart tissues of Karakul lambs, as well as camel milk proteins—exhibit distinct yet complementary pharmacological properties. The integration of proteomic characterization with biological evaluation provides a comprehensive understanding of their functional potential.

The identification of key thymic peptides, including thymosin α 1, thymosin β 4, and thymopoietin, confirms the preservation of biologically active immunoregulatory components in the *Timoptin* preparation. These findings are consistent with previous studies reporting the critical role of thymic peptides in T-

cell differentiation and immune response modulation. The presence of multiple low-molecular-weight peptides suggests enhanced bioavailability and supports their potential application as immunomodulatory agents. In this context, the obtained results reinforce the concept that thymus-derived peptide complexes can serve as effective regulators of immune homeostasis.

Lung-derived peptide complexes (*Aflan*) demonstrated pronounced anti-inflammatory activity, which can be attributed to the presence of structurally diverse peptides with regulatory functions. The molecular weight range of the identified peptides indicates that they may interact with key signaling pathways involved in inflammation. This observation aligns with existing reports describing lung tissue as a source of peptides capable of modulating cytokine production and inhibiting inflammatory mediators. Importantly, the results suggest that such peptides may be particularly relevant for the development of therapeutics targeting inflammatory and respiratory disorders.

The peptide complex obtained from heart tissue (*Kardin*) showed a significant effect on blood rheological properties. This finding is of particular interest, as it indicates a functional role of heart-derived peptides in maintaining cardiovascular homeostasis. The observed hemorheological activity may be associated with the ability of these peptides to influence vascular tone, blood viscosity, and microcirculation. These results are in agreement with previously reported data on bioactive peptides involved in cardiovascular regulation and highlight their potential application in the prevention and treatment of circulatory disorders.

In addition to organ-derived peptides, camel milk proteins were identified as a valuable source of antioxidant peptides. The enzymatic hydrolysis process yielded peptides with defined primary structures and significant free radical scavenging activity. These findings support earlier studies demonstrating the antioxidant and protective properties of milk-derived peptides. The stability and bioactivity of these peptides further emphasize their potential role in reducing oxidative stress and preventing related pathological conditions.

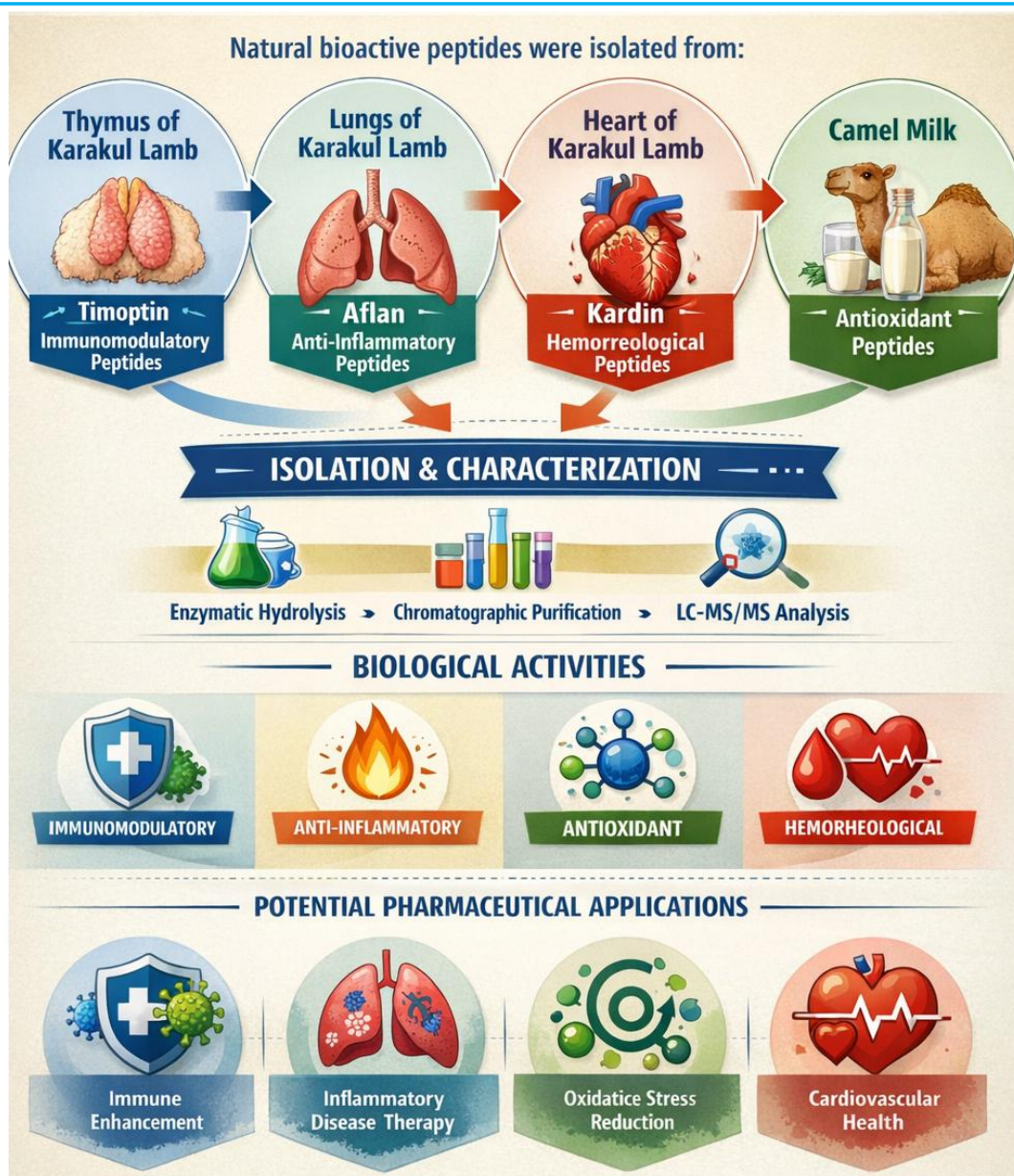


Figure 1. Schematic overview of the isolation, characterization, and biological evaluation of bioactive peptide complexes derived from Karakul lamb organs and camel milk.

A key outcome of this study is the demonstration that peptide complexes derived from different biological sources possess complementary mechanisms of action. While thymus peptides primarily exert immunomodulatory effects, lung-derived peptides target inflammatory pathways, heart-derived peptides influence blood rheology, and camel milk peptides provide antioxidant protection. This functional diversity creates an opportunity for the development of multifunctional therapeutic agents with combined pharmacological effects.

Despite these promising results, several limitations should be considered. The study primarily focuses on *in vitro* and experimental biological activity assessment, and further *in vivo* and clinical studies are required to confirm therapeutic efficacy and safety. Additionally, while peptide identification was successfully achieved, a more detailed investigation of structure–activity relationships would enhance the understanding of their mechanisms of action.

Overall, the findings of this study contribute to the growing body of evidence supporting the use of natural peptide complexes as a basis for the development of novel pharmaceuticals. The use of locally available biological resources, such as Karakul lamb organs and camel milk, further highlights the practical and economic relevance of this approach.

5. CONCLUSION

In this study, bioactive peptide complexes were successfully isolated and characterized from the thymus, lung, and heart tissues of Karakul lambs, as well as from camel milk proteins. The application of modern proteomic and chromatographic techniques enabled the identification of multiple peptide components with defined molecular weights and biological functions.

The results demonstrated that thymus-derived peptides exhibit pronounced immunomodulatory activity, primarily associated with the presence of thymosin α 1, thymosin β 4, and thymopoietin. Lung-derived peptides showed significant anti-inflammatory effects, while heart-derived peptides influenced blood rheological properties, indicating their potential role in cardiovascular regulation. In addition, peptides obtained from camel milk proteins displayed notable antioxidant activity.

Importantly, the study revealed that peptide complexes derived from different biological sources possess complementary pharmacological properties. This functional diversity highlights their potential for the development of multifunctional therapeutic agents with combined immunomodulatory, anti-inflammatory, antioxidant, and hemorheological effects.

The findings confirm that locally available biological materials, such as Karakul lamb organs and camel milk, represent valuable and sustainable sources of bioactive peptides. The developed peptide-based substances (Timoptin, Aflan, and Kardin) demonstrate promising characteristics for further pharmaceutical development.

Further studies, including in vivo investigations and clinical evaluations, are required to validate the efficacy, safety, and mechanisms of action of these peptide complexes. Nevertheless, the present work provides a scientific foundation for the development of novel peptide-based therapeutics and contributes to the advancement of bioorganic and pharmaceutical research.

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