The potential effect of L-carnosine loaded hyalurosomes as a novel anti-aging nano-cosmeceutical gel

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1 Introduction

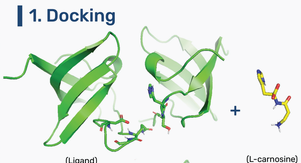
Skin aging induced by chronic exposure to ultraviolet (UV) radiation, requires innovative nano-cosmeceutical formulations to counteract this damage. Better therapeutic outcomes can be achieved by **incorporating bioactive excipients as hyaluronic acid** that can reduce wrinkles and promote keratinocyte proliferation. Thus, hyalurosomes (HS) were the chosen nano-formulation. HS was further **loaded with L-carnosine (CN),** a challenging dipeptide with reported antioxidant and anti-inflammatory properties. This novel nano-system would overcome the hydrophilic nature of CN and enhance its skin permeation.

2 Objective

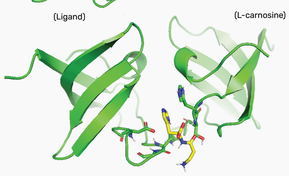
The aim of this work was to formulate and optimize novel **L-carnosine loaded hyalurosomes (CN-HS). In addition,** its antiaging potential following UV-B irradiation will be confirmed.

3 Methodology

1. Docking



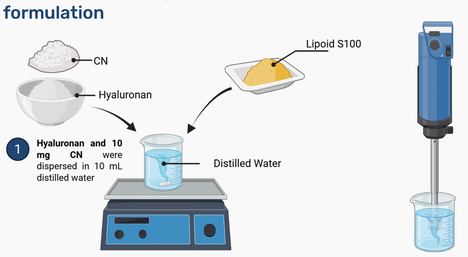
The 2D structure of L-carnosine was retrieved from the PubChem database in SDF format and optimized using the "Generate Conformations" tool in Discovery Studio 5.0.



The 3D structure of NADPH oxidase (PDB ID: 7CFZ) was obtained from the Protein Data Bank (PDB) and prepared for docking by completing missing atoms, removing water molecules, and protonating the protein to a physiological pH of 7.0 ± 2.0.

Docking studies were conducted using AutoDock 4.2, with a grid box size of 38 × 26 × 36, a grid center (x=16.405, y=7.977, z=20.888), and a spacing of 0.375 Å. Final visualizations were created using PyMOL.

2. Formulation of optimized CN-HS formulation



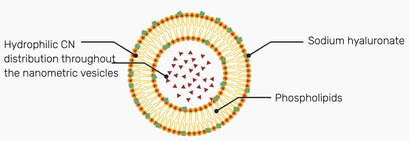
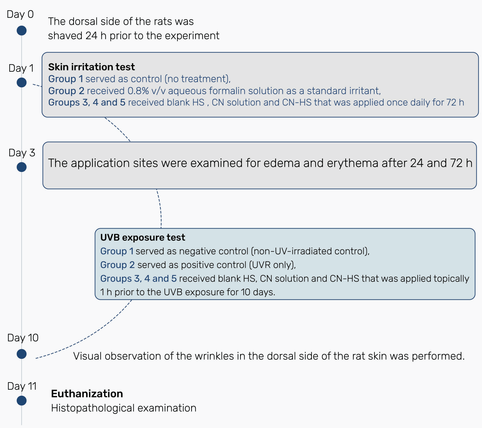
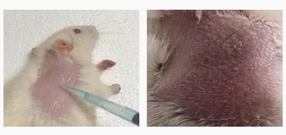


Figure 1. L-carnosine loaded hyalurosomes (CN-HS)

3. In vivo study



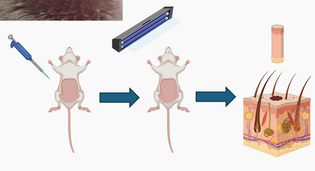


Draize skin irritation test

Performed to evaluate the potential localized reaction between the skin and the selected HS formulation.

UVB exposure test

Rats were irradiated with a lamp fixed 5 cm above their position. Utilized doses of UVB irradiation were 40–80 mJ/cm2 with 15–30 s exposure time.



0.5 g of the prepared formulations applied topically on the dorsal rat skin one hour before the UVB exposure once daily for 10 days.

5 Conclusion

The developed delivery system presents **a promising skin protection nano-platform** through **the dual effects of both hyaluronan and L-carnosine**, representing an appealing **anti-aging nano-cosmeceutical formulation**.

4 Results

1.Docking

- Molecular docking studies revealed that **L-carnosine effectively inhibits reactive oxygen species (ROS) production** by **stabilizing the inactive conformation of the NOX enzyme, thereby blocking its activation and downstream signaling.**
- This strong binding affinity is attributed to CN polar functional groups, which form a robust network of hydrogen bonds, electrostatic interactions, and hydrophobic contacts within the NOX binding pocket.
- These findings highlight the **therapeutic potential of CN in managing age-related diseases** linked to aberrant NOX activation. As NOX enzymes are major contributors to oxidative stress, their inhibition by CN positions it as a promising antioxidant candidate for conditions associated with excessive ROS generation.

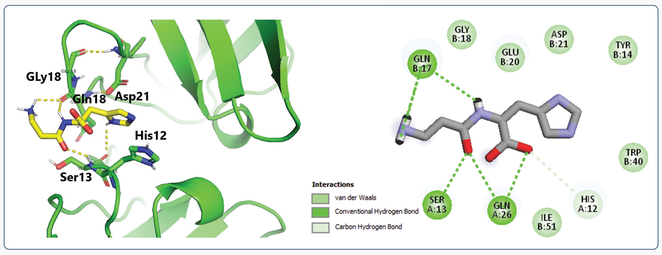


Figure 2. L-carnosine docked into the active binding site of NOX. Left panel: 3D interactions, right panel :2D interactions. H-bonding represents yellow dotted lines

2. Particle size, PDI, Zeta potential and % Entrapment efficiency

Figure 3. Physicochemical properties of different formulations; (A) Vesicle size, (B) PDI, (C) Zeta potential and (D) % Entrapment efficiency. Measurements are expressed as mean ± SD.

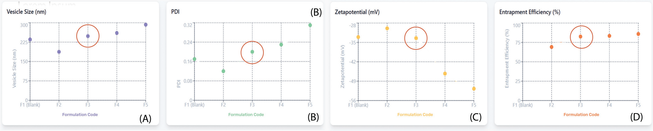


Table 1. Composition and physicochemical properties of blank and different CN loaded formulations.

FORMULATION CODE	HYALURONAN (%W/V)	VESICLE SIZE (NM) ± SD	PDI ± SD	ZETAPOTENTIAL (MV) ± SD	ENTRAPMENT EFFICIENCY (%)
F1 (Blank HS)	1	235.32 ± 2.15	0.17 ± 0.02	-33.06 ± 1.46	-
F2	0.5	187.43 ± 1.87	0.12 ± 0.03	-29.92 ± 2.58	69.14 ± 1.78
F3	1	248.31 ± 1.91	0.20 ± 0.03	-33.45 ± 1.06	82.56 ± 1.29
F4	1.5	260.32 ± 1.58	0.23 ± 0.01	-46.27 ± 2.27	83.43 ± 1.11
F5	2	292.64 ± 2.01	0.31 ± 0.02	-51.72 ± 1.80	85.90 ± 0.98

2. TEM

TEM micrograph of blank & loaded formulations revealed; characteristic well-dispersed, spherical, well separated with no aggregation shell-core vesicles clearly identified with darker coat and core in the case of CN-loaded hyalurosomes.

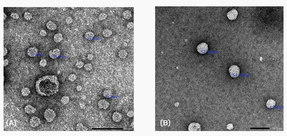


Figure 4. TEM of (A) Blank hyalurosomes & (B) CN-HS

3. Draize test

The Draize scoring system was employed to assess the irritancy of blank HS, CN solution , and CN-HS. **No erythema or edema observed for CN-HS confirming the non irritancy of the developed formulation**

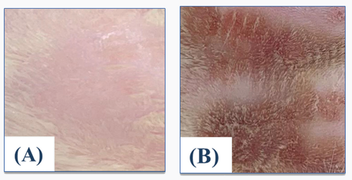
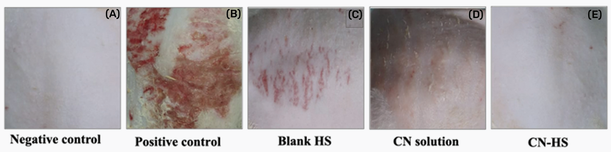


Figure 5. Images of the dorsal skin of male Wister rats following application of : (A) CN-HS and (B) Positive control receiving 0.8% formalin for three days

Figure 6. Primary irritation index of different experimental groups in Draize method. Values are represented as mean ± SD

4. UVB exposure test

Figure 7. Photographs of the dorsal rats' skin (A) Negative control group, (B) Positive control group, irradiated and topically pretreated with: (C) Blank HS, (D) CN solution and (E) CN-HS.



Negative control	Positive control	Blank HS	CN solution	CN-HS
Intact and smooth skin surface	Edema, signs of erythema and thick skin with notable scars	Fewer scars and promising effects due to both phospholipids and hyaluronan	Few scar traces	Intact skin that is free from any wrinkling signs

5. Histological examination

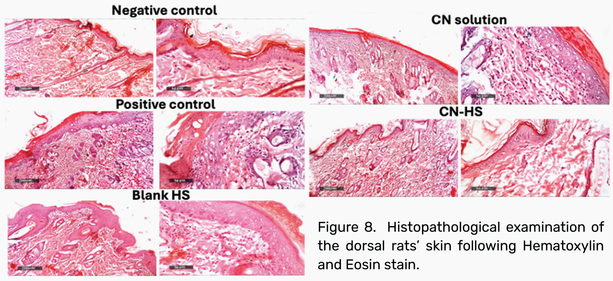


Figure 8. Histopathological examination of the dorsal rats' skin following Hematoxylin and Eosin stain.

6 Reference

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2-Mouada et al,Journal of Organometallic Chemistry, 2024
3-ElSheikh et al,International Journal of Pharmaceutics: X. 2023

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