Enhancing 3-Acetyl-11-Keto-β-Boswellic Acid Skin Permeation via Nanostructured Lipid Carriers: Integrating Quality by Design Principles for Risk Estimation and Optimization

Supplementary data

Amplitude and frequency sweep test

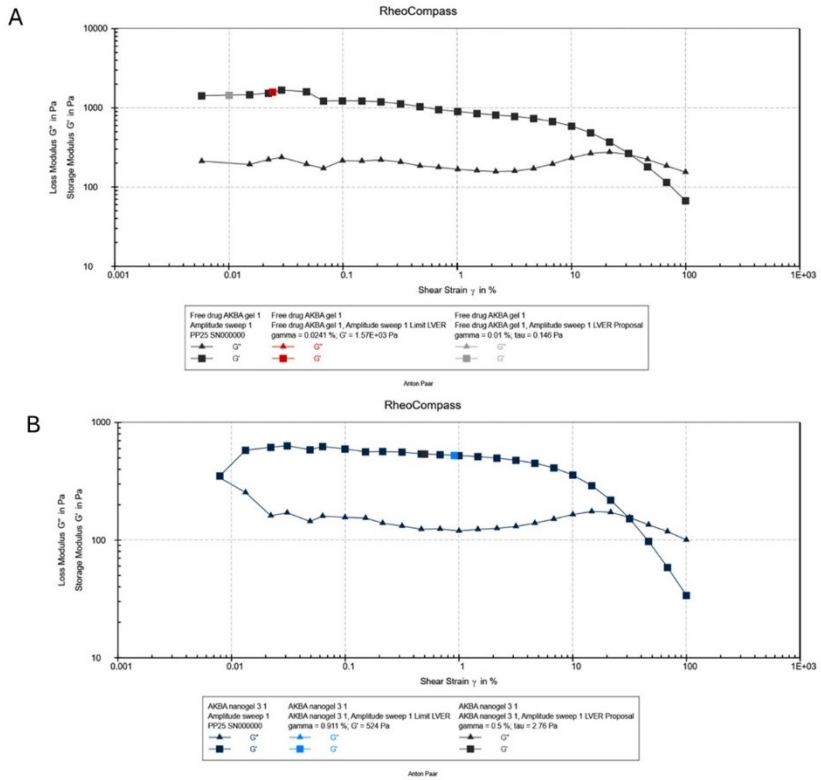

Fig. S1. Amplitude sweep test for (A) Plain gel; (B) NLCs loaded gel

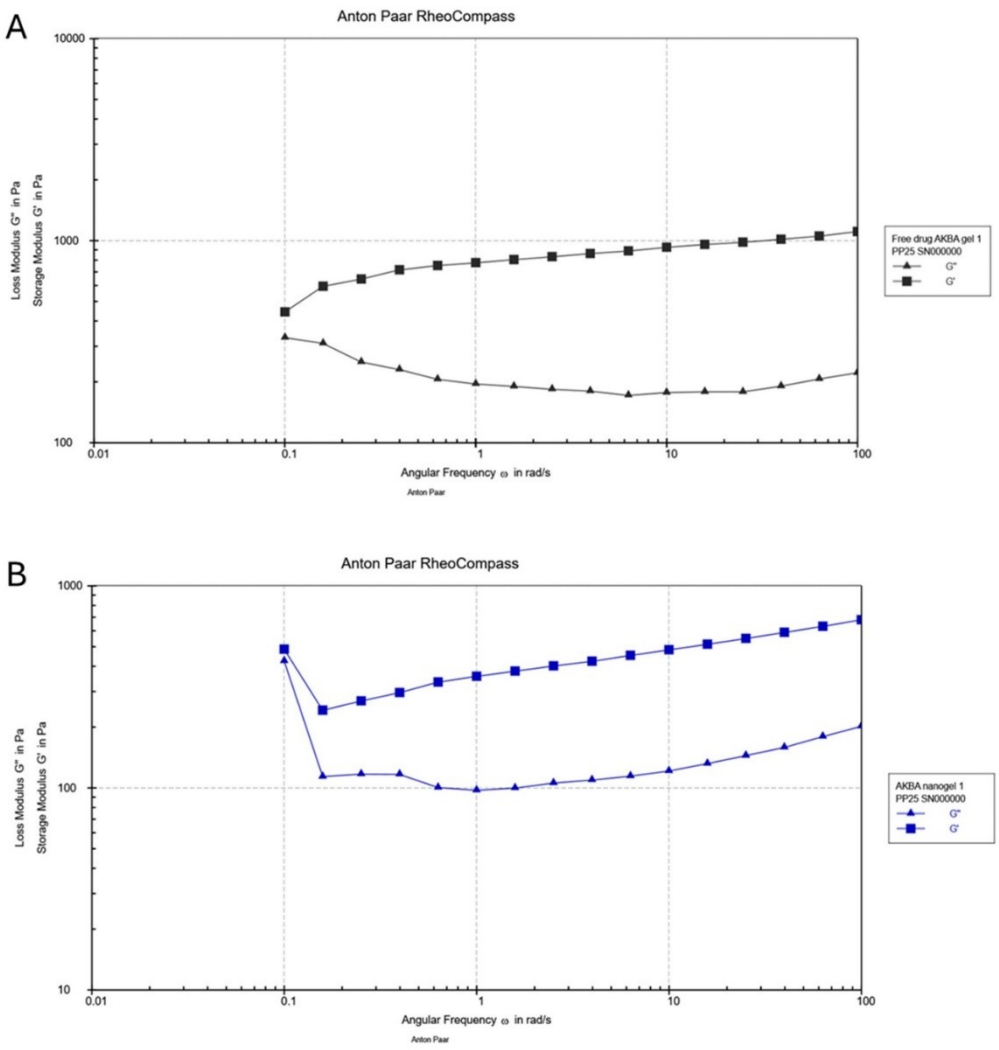

Fig. S2. Frequency sweep test for (A) Plain gel; (B) NLCs loaded gel

Extraction of AKBA from skin tissues

Prior to conducting the *ex vivo* permeation and skin retention investigations, the percentage recovery of AKBA from the skin tissues was ascertained. The goat ear skin used for the study was taken from the slaughterhouse, carefully processed by removing the subcutaneous fat and hair, and then cleaned three times with Milli-Q water. A homogenized skin tissue sample was spiked with a specified quantity of standard AKBA solution (100 µg/mL). To get rid of the extra skin tissue and debris, the sample was centrifuged for 30 min at 10,000 rpm. The diluents system was used to dilute the material to 10 µg/mL. After centrifuging the sample once more for 30 min at 10,000 rpm, it was filtered using a 0.22 µ syringe filter. The sample was then analyzed using the developed RP-HPLC method.[1] The percentage recovery was calculated by comparing the extracted amount of AKBA with the additional sample. The recovery percentage that was displayed was the mean of the three distinct experiments.[2]

The percentage of AKBA recovery was calculated from the skin matrix. Over the calibration range, the mean absolute recovery of AKBA from skin tissue was 84.112 ± 8.838 %. Consistent recovery was demonstrated by the verified approach. The percentage recovery findings showed that AKBA could be found in skin penetration and dermal deposition investigations using the validated method.

Skin irritation study

A skin irritation study is necessary to assess the substances used in the formulation development's suitability for the skin. Wistar rats weighing between 180 and 200 g were used for skin irritation in accordance with procedure IAEC/RES/34/07, which was approved by the animal ethics committee. A controlled room temperature of 25 °C and 45 % relative humidity was maintained for the rats. The dorsal skin was shaved using an animal hair clipper, and any irritation was recorded. 0.5 g of AKBA-NLCs gel was applied equally over the hairless area, covering an area of roughly 4 cm^2. Visual observation was used to measure the skin's erythema (redness) and inflammation at 6, 12, 24, and 48 h following application.[3]

Visual assessment of erythema (redness) was used to determine the skin irritation. As shown in **Fig. S3**, the application of AKBA-NLCs gel did not cause any erythema or redness on the rat's skin. According to the results, the developed gel was safe for topical use and did not exhibit any signs of irritation or inflammation, suggesting skin compatibility.

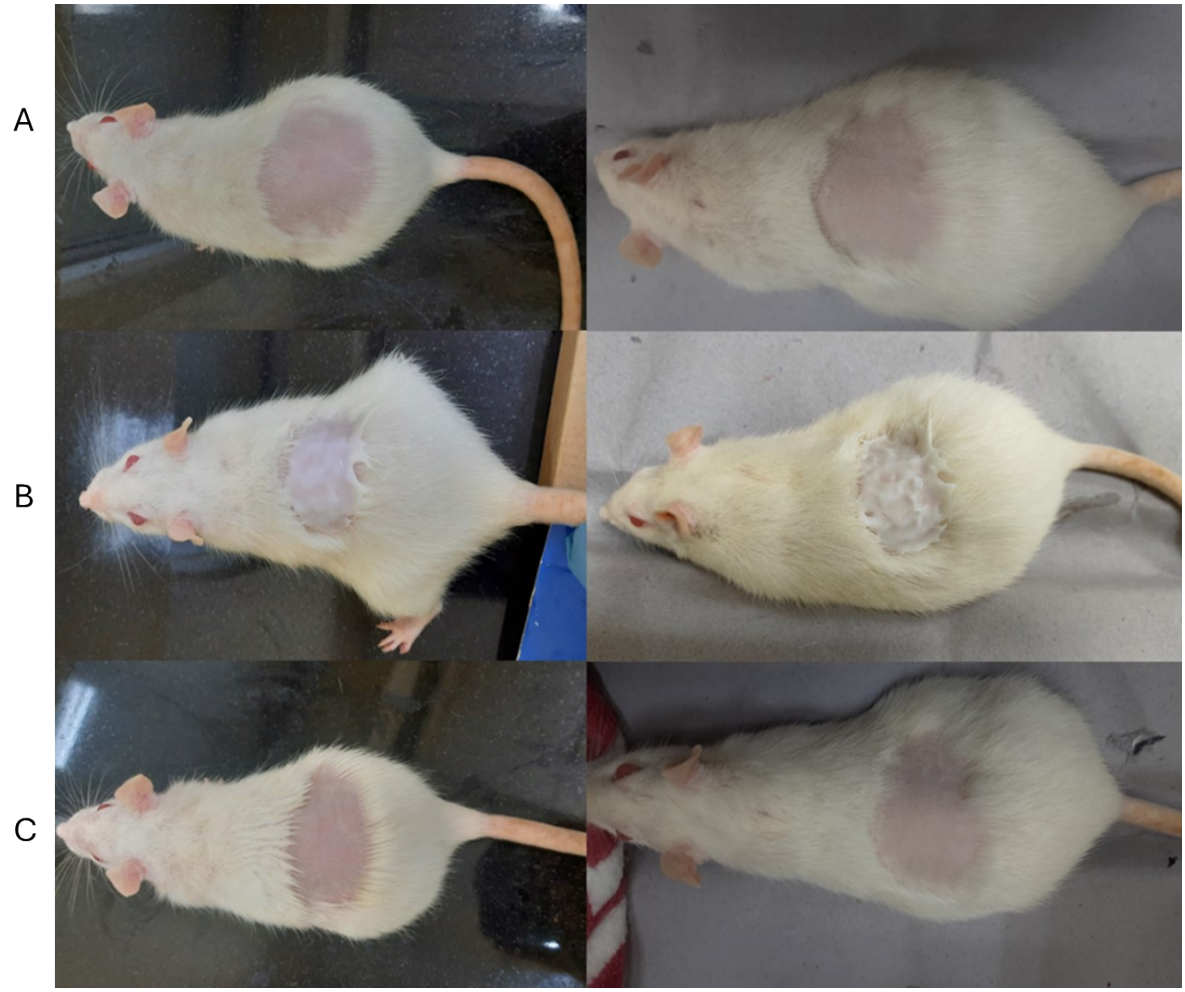

Fig. S3. Skin irritation test of AKBA-NLCs gel on the dorsal side of Wistar rat; (A) Before the application of NLCs gel; (B) Application of NLCs gel; (C) After the application of NLCs gel.

References

[1] Priya S and Singhvi G, Determination of 3-acetyl-11-keto-β-boswellic acid in analytical and biological samples using streamlined and robust RP-HPLC method, *Anal Methods*., 2024, **16**(24), 3847-3858, DOI:10.1039/d4ay00814f.

[2] Gorantla S, Saha RN and Singhvi G, Spectrophotometric method to quantify tofacitinib in lyotropic liquid crystalline nanoparticles and skin layers: Application in ex vivo dermal distribution studies, *Spectrochim Acta - Part A Mol Biomol Spectrosc*., 2021, **255**, 119719, DOI:10.1016/j.saa.2021.119719.

[3] Tomar Y, Maheshwari S, Gorantla S and Singhvi G, Curcumin loaded liquid crystalline nanoparticles for enhanced topical application : Design , characterization ,

ex vivo and dermatokinetic evaluation, *J Drug Deliv Sci Technol*., 2024, **92**(January), 105391, DOI:10.1016/j.jddst.2024.105391.