Development of a nanoemulsion incorporating 3-acetyl-11-keto-β-boswellic acid (AKBA): Solubility and oral bioavailability through in vitro and in vivo studies

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Research Article

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Development of a nanoemulsion incorporating 3-acetyl-11-keto-β-boswellic acid (AKBA): Solubility and oral bioavailability through in vitro and in vivo studies

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Abstract

background: 3-Acetyl-11-keto-β-boswellic acid (AKBA) has attracted considerable interest due to its therapeutic potential against inflammatory and cancer-related disorders. However, its poor oral bioavailability remains a critical limitation for clinical application. This study aimed to enhance the bioavailability of AKBA by developing a nanoemulsion (NE)-based delivery system.

Results: Several NE formulations were optimized and characterized based on drug loading, stability, and droplet size. The optimized NE-AKBA showed a particle size of 12–15 nm and was further tested for permeability across Caco-2 cell monolayers, followed by in vivo pharmacokinetic evaluation. Permeability studies revealed significantly improved transport of NE-AKBA within the first hour. Pharmacokinetic analysis indicated a notable increase in systemic exposure: the C_{max} of AKBA rose from 3.36 to 12.23 μg/mL, while the AUC_{0-t} increased from 4257 to 6222 μg·h/mL, with T_{max} remaining steady at 6 hours.

Conclusion: These findings demonstrate that nanoemulsion-based delivery significantly improves the oral bioavailability of AKBA and provides a promising platform for its future therapeutic development.

Keywords:

Drug delivery; Nanoemulsion; AKBA; boswellic acid; pharmacokinetics; bioavailability permeability

Background

3-Acetyl-11-keto-β-boswellic acid (AKBA) is a bioactive compound of *Boswellia serrata* gum resin, a plant known for its potent anti-inflammatory activity. AKBA has exhibited great therapeutic potential in the management of chronic inflammatory diseases such as rheumatoid arthritis [1], osteoarthritis [2], asthma, and inflammatory bowel disease (IBD) [3], neurodegenerative disorders [1, 4], chronic colitis [5], and cancers [6-8]. However, despite their therapeutic potential, there are significant challenges to their bioavailability due to their physicochemical properties. As a Biopharmaceutical Classification System (BCS) Class II drug, AKBA is poorly soluble in water (<1 µg/mL) but highly permeable [9]. Its high lipophilicity (log P = 3.3) and extensive first-pass metabolism in the gut and liver also limit its systemic availability, resulting in low bioavailability [10, 11]. These limitations have driven the demand for new drug delivery systems to optimize their therapeutic effects. The structure of AKBA is schematically shown in Figure 1.

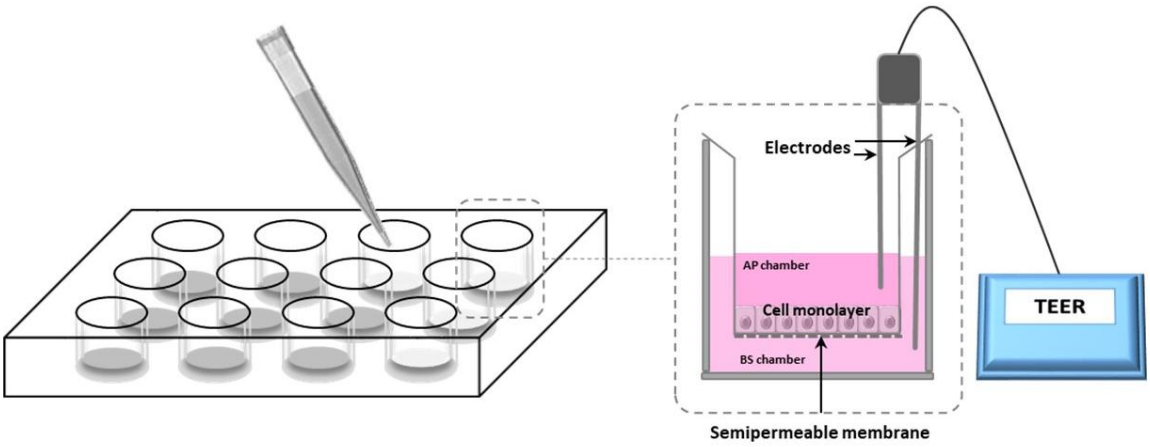


Figure 1. Schematics of the insert plate and its components used during the experiment.

The test samples were then added to the AP side and maintained in an incubator. TEER was measured after 1, 2, and 3 hours to calculate the cell permeability for AKBA. All AP and the

last BP media were taken, and their absorbance was measured at 249 nm for the determination of AKBA values [12].

Various drug delivery carriers, such as liposomes, nanomicelles, dendrosomes [13], nanostructured lipid carriers (NLCs) [14], and solid lipid nanoparticles (SLNs) [13] have been explored to overcome these challenges. Nanoemulsions (NEs) have emerged as highly effective and versatile options because of their simple formulation, stability, and enhanced drug solubility and permeability [15]. NEs are composed of two immiscible liquids stabilized with surfactants, forming nanoscale droplets that enhance the dispersion of hydrophobic compounds in aqueous media [16, 17]. They possess a small droplet size and stability, owing to which they are ideal for the delivery of poorly soluble drugs such as AKBA [18]. Furthermore, NEs also enhance drug absorption via diverse mechanisms involving an increase in the fluidity of the cell membrane, increased transcellular and paracellular transport, and increased uptake into the lymph [16, 17, 19, 20]. All these factors make NEs promising carriers that can improve the bioavailability of AKBA.

Recently, advances in NE technology have been the driving force behind the development of the best cosurfactants, surfactants, and oil phases to establish stable and efficient drug delivery systems. The use of multiple surfactants and cosurfactants has been found to improve the stability of NE and reduce its droplet size by optimizing the hydrophilic–lipophilic balance (HLB) [21-23]. Owing to their unique molecular architecture, cosurfactants enhance interfacial fluidity and promote the formation of stable nanoemulsions at lower concentrations of surfactants, limiting potential toxicity [24, 25]. Similarly, the choice of the oil phase is also an essential consideration in determining the stability, droplet size, and activity of NEs. Oils with

low water solubility can inhibit Ostwald ripening, whereas blended oils can maintain unimodal droplet size distributions, both of which increase stability [26, 27].

Recent studies have shown the capacity of long-chain fatty acid (LCFA) oils to improve lipophilic drug bioavailability. For example, a study on simvastatin-loaded NEs revealed improved oral bioavailability by a significant percentage through better lymphatic absorption via LCFA-based lipids [26]. Based on these results, this study aimed to prepare AKBA-loaded NEs from natural LCFA oil to increase the bioavailability of AKBA. Given the distinct advantages of NEs and their ability to modify their formulation compositions, this research attempts to overcome the bioavailability limitations of AKBA, raising hope for widespread therapeutic use in the future.

Methods

AKBA was purchased from Baoji Herbest Bio-Tech Co. (China). Olive oil, coconut oil, almond oil, and sesame oil were purchased from Farabi Pharmaceutical Co. (Iran). Tween 80 and Span 80 (Lab reagent grade) were purchased from Central Drug House Ltd. (India). Chloroform, acetone, and methanol (absolute) were from NeutroChrom® (Iran). Caco-2 cells were obtained from Pasteur Institute of Iran> DMEM (5,000 mg/L d-glucose, 100 mg/L sodium pyruvate, and l-glutamine) and fetal bovine serum (FBS) were from Bioidea (Iran). T-25 cm^2 treated culture flasks were from SPL-Biosciences®, and cell culture filter inserts (24-well transwell Culture Plate) were obtained from SPL® (Korea).

Nano-emulsion preparation

Different NE preparations were prepared using different contents of Tween 80 (Polyoxyethylene 20 monooleate) [18, 28], Span 80 (Sorbitan monooleate) [18], edible oil (olive oil, coconut oil, sesame oil, or almond oil), and co-surfactant (ethanol, propylene glycol, sorbitol, or glycerol) by mixing surfactants and oil on a magnetic stirrer for 20 minutes, then adding co-surfactant and titrating with water [18]. The selection of surfactant concentration was performed using the hydrophilic-lipophilic balance (HLB) value [29]. After achieving the optimized formulation in terms of its transparency, physical stability, and droplet size distribution, AKBA was loaded in the NE by being dissolved in the oil phase during the preparation of NE (NE-AKBA).

Stability tests

Accelerated tests of physical stability were used to investigate the stability of the prepared NE. In this way, the samples were placed periodically in harsh conditions (cooling-heating cycles and centrifuge), then their stability was evaluated in terms of appearance (transparency and phase separation) as well as the size of the particles [18].

Droplet size, size distribution, and zeta potential

The mean particle size and polydispersity index (PDI) of NE-AKBA were measured by dynamic light scattering (DLS) at ambient temperature. Due to the thickness of the samples, each sample was diluted up to three times before measuring the size of the particles. The zeta potential was determined using a Zetasizer by electrophoretic mobility under an electric field.

Transmission electron microscopy

The morphology and size of the NE nanoparticles were confirmed using transmission electron microscopy (TEM) (LEO 906, Zeiss, Germany). A drop of an aqueous solution of

phosphotungstic acid was added as negative staining on the grid, and the staining was performed for 5 min at room temperature. The prepared sample was then evaluated by TEM at a voltage of 200 kV.

In vitro studies: Cell Viability Assay

The cytotoxicity of AKBA and NE-AKBA was evaluated using the MTT assay. Caco-2 cells were cultured in T-25 cm^2 treated culture flasks in high-glucose DMEM (5,000 mg/L d-glucose, 100 mg/L sodium pyruvate, and l-glutamine), supplemented with 20% FBS. The flasks were maintained at 37 °C in a CO_2 incubator with atmospheric air kept at 95% air and 5% CO_2 at 95% humidity and supplied with fresh medium every 2 days until they reached approximately 80% confluence in 5–7 days. Cell cultures at passage 23 were detached with Trypsin–EDTA, then seeded at 1×10^3 cells/well in 96-well plates [30, 31]. Three days after seeding, the wells were grouped and treated with 1) NE-AKBA (nanoemulsion containing AKBA), 2) NE (nanoemulsion without AKBA), 3) AKBA (bulk AKBA suspension in water), and 4) control group (no treatment). Six hours later, the medium was removed from the wells, and MTT reagent was added to the wells (5 mg mL^{-1} stock). The plates were incubated for 3 h in the incubator and lysed with DMSO (100 µL per well). The absorbance of the wells was measured using an ELISA microplate spectrophotometer at 570 nm [32, 33]. The obtained OD values were normalized to the blank wells, and the Caco-2 cell viability was evaluated. Afterward, using a graphical method and linear regression, the IC50 (half maximal inhibitory concentration) of NE-AKBA in Caco-2 cells was calculated [34].

Trans-epithelial electrical resistance (TEER) assay

Transport was determined across Caco-2 cell monolayers in the apical-to-basolateral direction [35]. Cells were seeded on cell culture filter inserts (50,000 cells/well). Culture medium

(DMEM) was added to the apical (AP) (300 µL) and basolateral (BL) (600 µL) sides, and the plate was kept at 37°C in a humidified incubator containing 5% CO2 in air. DMEM was replaced every other day for the first week and once a week thereafter. Cells were left to differentiate for 18–21 days [36, 37]. The trans-epithelial electrical resistance (TEER) value was measured weekly to assess cell differentiation and monolayer formation.

After equilibration at room temperature, the TEER was measured in HBBS medium (Hanks' balanced salt solution). The treatments were added to the AP compartment without changing the BS environment. A pair of chopstick electrodes that reported resistance values was inserted in the AP and BL chambers, and the TEER values were recorded (Figure 1). TEER values ranging from 200 to 600 Ohms per cm^2 of the porous membrane were considered as indicators of an integrated monolayer, while TEER values < 200 Ohms indicated loss of integrity; thus, the setup was excluded from the experiments [29].

The apparent permeability (P_{app}) coefficient (cm/s) was calculated as below:

$$P_{app} = \frac{V_R}{A \times C_0} \times \frac{dQ}{dt}$$

Where V_R is the volume in the BL chamber (mL), A is the surface area of the porous cup (cm2), C_0 is the initial drug concentration (µg/mL), and dQ/dt is the initial slope of the cumulative concentration (µg/mL) in the BL chamber with time (seconds) [38].

In-vivo studies

The pharmacokinetics of the NE-AKBA were studied in female white Wistar rats weighing 200 – 250 g, supplied by the Department of Pharmacology of Tehran University of Medical Sciences (Iran). For blood sample collection, rats were housed in standard laboratory cages for several days before experimentation to allow acclimatization to the environmental conditions.

During this period, the animals were gradually habituated to gentle handling and restraint procedures to minimize stress during sampling. The rats were housed under standard conditions with a standard pellet diet, water ad libitum, and 12-hour light and dark cycles. They were grouped randomly into two groups, consisting of four rats, and each group was gavaged with 1 ml of either NE-AKBA or AKBA suspension (1 mg/ml). Then, each rat's tail was warmed individually using a heating pad at approximately 37–40 °C and gently massaged to facilitate vasodilation. Subsequently, 200 µL of blood was collected from the lateral tail vein using an insulin syringe equipped with a 25–27 G needle. At each sampling time point, blood was withdrawn from the opposite side of the tail relative to the previous collection, and the puncture site was shifted 2–3 mm distal to the prior site to prevent repeated injury to the same location.

At the end of the 48-hour sampling period, rats were anesthetized with a ketamine/xylazine mixture prepared at a 10:1 ratio (total administered volume 0.45 mL per animal). After achievement of a surgical plane of anesthesia, a terminal cardiac puncture was performed to obtain the final blood sample. Following exsanguination, an additional intraperitoneal injection of ketamine was administered to ensure death, consistent with the study's institutional animal care and use committee (IACUC)-approved protocol.

Rat blood samples were collected at 1, 2, 3, 6, 12, 24, and 48 h after gavage. The samples were centrifuged for 20 min at 2000 rpm to obtain the plasma and treated with acetone (1.5:1) to remove the proteins, and centrifuged (15 min, 3000 rpm). The supernatant was extracted with chloroform (2:1). The solvent was then removed, and the dry residue was dissolved in methanol and analyzed using aby Cytation3 Imaging reader (Biotek, USA) at 249 nm [39, 40]. The maximum concentration (C_{max}) of AKBA in the plasma and the time to reach the maximum

concentration (T_{max}) were obtained from the observed values. The area under the concentration-time curve (AUC_{0-t}) was then calculated. An unpaired Student's t-test was conducted for significance evaluation with p-value < 0.05.

Results

Preparation and characterization of nanoemulsion

Table 1 shows the properties of selected prepared NEs. From the accelerated stability tests, the optimum NE preparation was determined as having 3% oil, 30.3% Tween 80, 7.7% Span 80, and 10% ethanol in deionized water. Table 2 shows the DLS results of the optimum NE preparation. Our results also indicated that the NE exhibits Newtonian behavior. The Zeta potential of the NE-AKBA was -14.5 mV.

The droplet's morphology was observed by TEM microscopy and showed a spherical shape with a particle size of ~20 nm, close to that of DLS results (see Figure 2).

Table 1. Composition, stability, particle size, and maximum AKBA solubility of selected nanoemulsion preparations

Oil type	Oil (%)	Span 80 (%)	Tween 80 (%)	Ethanol (%)	Surfactant (%)	water (%)	Surfactant & Cosurfactant. (%)	Stability at room temperature	Particle size (nm)	AKBA in NE (%)
Olive	2	4.2	30.8	9	35	54	44	≤ 1 month	23.2	0.064
Sesame	3	6.2	29.8	10	36	51	46	≤ 1 month	18.1	0.105
Coconut	4	5.9	28.1	12	34	50	46	≥ 1 year	15.1	0.076
Coconut	4	6.1	28.9	12	35	49	47	≤ 6 months	18.6	0.076
Coconut	3	4.9	30.1	12	35	50	47	≥ 1 year	19.3	0.057
Almond	3	7.5	29.5	10	37	50	47	≤ 1 month	12.8	0.120
Almond	3	7.7	30.3	10	38	49	48	≥ 1 year	14.3	0.120

Table 2. DLS results of NE-AKBA

	Day 1	Day 7	Day 30	Month 6	Month 12	Month 15	Centrifuged	Freeze-thawed
d50	8.3	8.2	12.1	14.3	15.1	14.7	11.9	23.8
Observed peaks	1	3	3	3	3	3	2	2
Viscosity (cp)	0.8132	0.8144	0.8497	0.8317	0.8320	0.8319	0.8678	0.8497

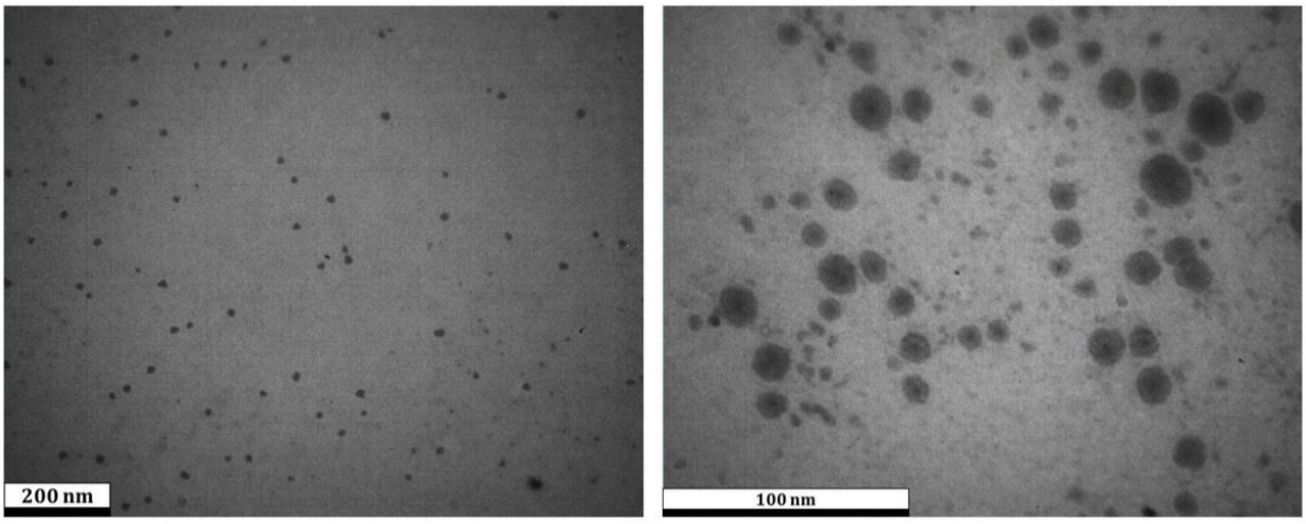
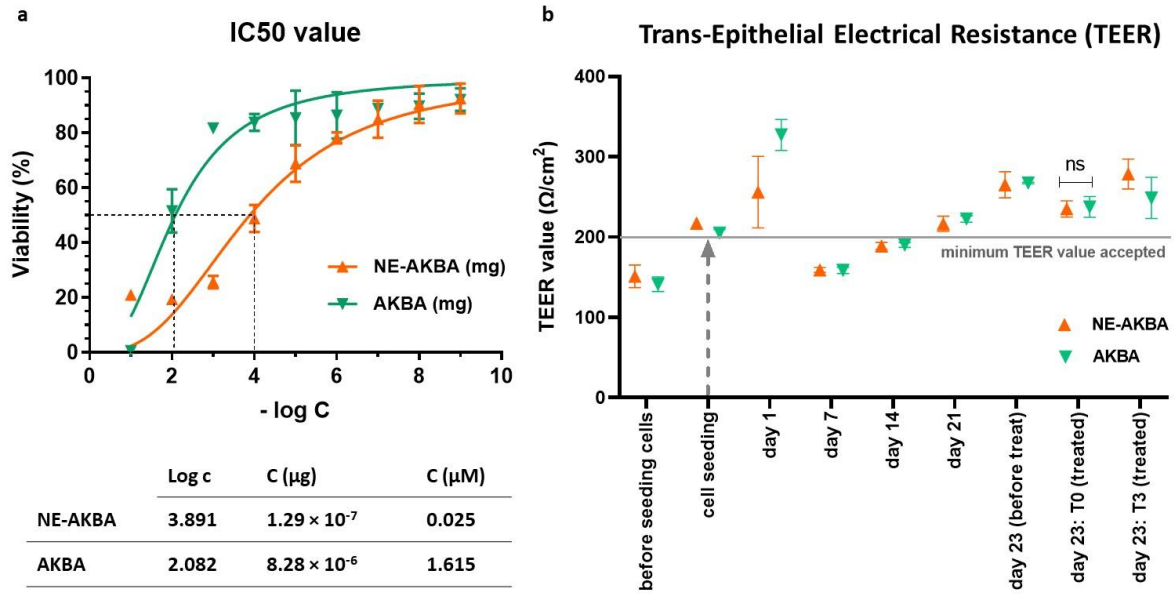


Figure 2. TEM images of nanoemulsion containing AKBA

In-vitro results

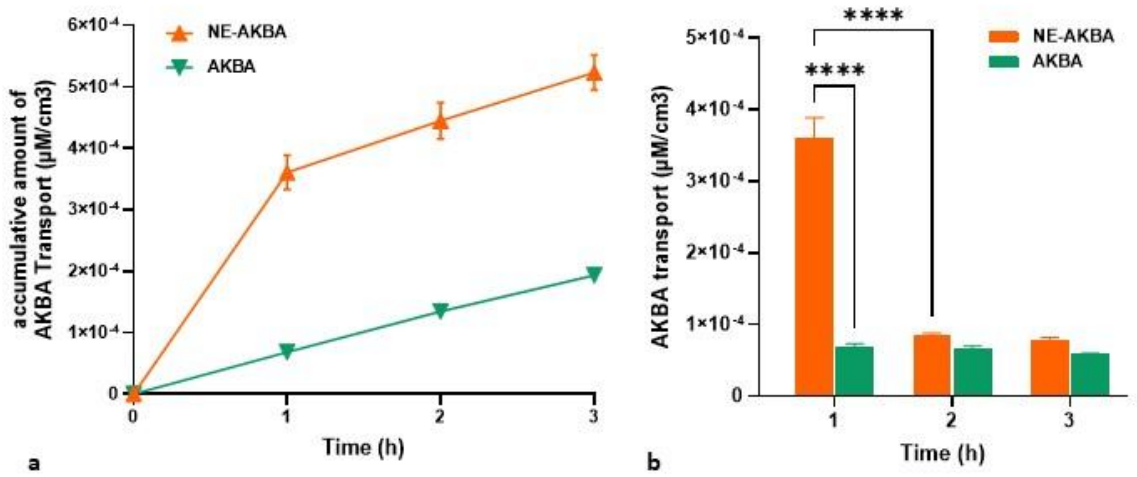
To determine the toxicity of AKBA and NE-AKBA on Caco-2 cells, an MTT assay was performed. The IC50 value of AKBA was found as 0.025 µM for NE-AKBA and 1.615 µM for AKBA (Figure 3-a). From the MTT findings, the maximum tolerable concentration of NE-AKBA was obtained as 1.29×10^{-7} µg/ml. So, 1 µg/ml was then used to treat the Caco-2 cells. For TEER studies. From Figure 3-b (which shows the TEER value after cell seeding), from day 21 onwards, the TEER value was more than 200 Ohms per cm^2, indicating the formation of an integrated monolayer [29] (Figure 3-b).



	Log c	C (μg)	C (μM)
NE-AKBA	3.891	1.29×10^{-7}	0.025
AKBA	2.082	8.28×10^{-6}	1.615

Figure 3. a) viability percentage of Caco-2 cells exposed to AKBA and NE-AKBA at different doses. b) TEER value changes during the permeability assay (from washing the inserts with media until 3 h after treatment)

From the permeability studies (see Figure 4), AKBA tends to accumulate in the BL side of the monolayer. The increase appears to be linear in the bulk AKBA treatment. Additionally, after the first hour, the increase in the NE-AKBA-treated group had a similar slope to that of the bulk AKBA-treated group. Apparent permeability coefficient (P_{app}) was calculated, and as shown in Figure 5, it is significantly higher in the NE-AKBA group.



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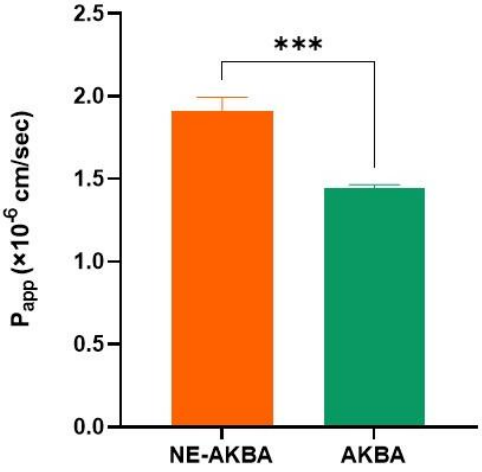
228 Figure 4. a) cellular transport of AKBA from NE-AKBA and free AKBA. Both treatments were
229 added to the AP side of the Caco2 cell, and samples were taken from the BA sides at 1, 2,
230 and 3 h post-treatment. Results are expressed as percent of the initial dose (n = 3). b) Effect
231 of AKBA and NE-AKBA treatment on the permeability of treated Caco-2 cells. Caco-2
232 monolayers grown on culture inserts were treated with AKBA and NE-AKBA (C=2 µg/ml) for 3
233 h



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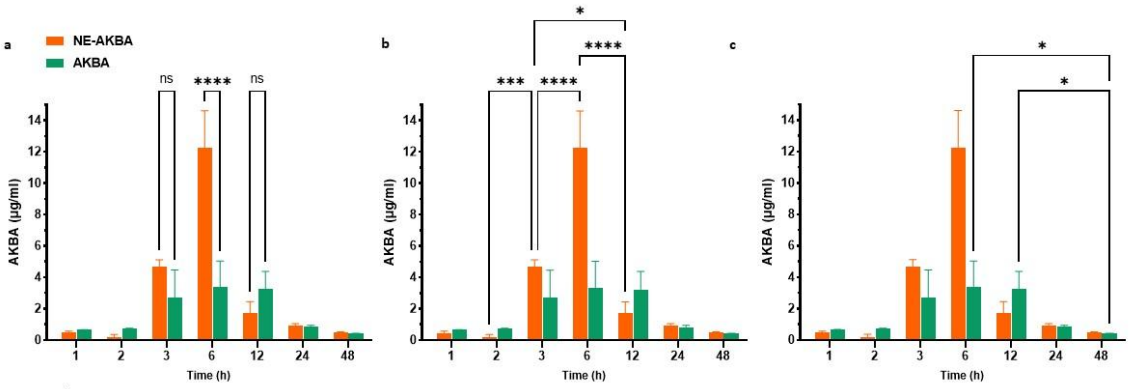
235 Figure 5. permeability of Caco-2 cells for AKBA released from NE-AKBA and AKBA in 3 h post-
236 treatment; Means ± S.D., n = 3; ***p < 0.001. P_{app} is an apparent permeability coefficient.

237

In-vivo results

The amount of AKBA in the plasma samples of the rats that were treated with either AKBA or NE-AKBA (1mg/kg, one dose) was measured at different time points after sampling. The highest concentration of AKBA in the plasma (C_{max}) and the time to reach this maximum concentration (T_{max}) were measured. Then, the surface area below the curve was calculated as AUC_{0-T} (see Figure 6). From the details, a significant increase in C_{max} and AUC_{0-T} of NE-AKBA is observed compared with AKBA. As evident, at time 6 h (i.e., T_{max}), an important difference is observed between the plasma concentration of AKBA in the two groups. It is also worth mentioning that the standard deviation value for T_{max} reported in this study (i.e., zero) is due to the sampling time points (i.e., 1, 2, 3, 6, 12, 24, and 48 h). In all experiments, the maximum concentration was detected at time point 6; thus, the SD equaled zero in this study.



Šídák's multiple comparisons between groups

Time (h)	Mean Diff.	95.00% CI	Summary	P Value
1	-0.2047	-2.544 to 2.135	ns	>0.9999
2	-0.5280	-2.868 to 1.812	ns	0.9941
3	1.962	-0.3773 to 4.302	ns	0.1437
6	**8.874**	**6.534 to 11.21**	****	**<0.0001**
12	-1.487	-3.827 to 0.8527	ns	0.4274
24	0.1000	-2.240 to 2.440	ns	>0.9999
48	0.1000	-2.240 to 2.440	ns	>0.9999

Šídák's multiple comparisons in NE-AKBA group, time steps

Time (h)	Mean Diff.	95.00% CI	Summary	P Value
1 vs. 2	0.2740	-2.420 to 2.968	ns	>0.9999
2 vs. 3	-4.467	-7.161 to -1.773	***	0.0001
3 vs. 6	-7.575	-10.27 to -4.882	****	<0.0001
3 vs. 12	2.911	0.2171 to 5.604	*	0.0252
3 vs. 24	3.725	1.031 to 6.419	**	0.0017
3 vs. 48	4.144	1.450 to 6.837	***	0.0004
6 vs. 12	10.49	7.792 to 13.18	****	<0.0001
6 vs. 24	11.30	8.607 to 13.99	****	<0.0001
6 vs. 48	11.72	9.025 to 14.41	****	<0.0001
12 vs. 24	0.8143	-1.879 to 3.508	ns	0.9997
12 vs. 48	1.233	-1.461 to 3.927	ns	0.9563
24 vs. 48	0.4187	-2.275 to 3.112	ns	>0.9999

Šídák's multiple comparisons in AKBA group, time steps

Time (h)	Mean Diff.	95.00% CI	Summary	P Value
1 vs. 2	-0.04933	-2.743 to 2.644	ns	>0.9999
1 vs. 48	0.2553	-2.438 to 2.949	ns	>0.9999
2 vs. 3	-1.977	-4.670 to 0.7169	ns	0.3605
3 vs. 6	-0.6637	-3.357 to 2.030	ns	>0.9999
3 vs. 12	-0.5387	-3.232 to 2.155	ns	>0.9999
3 vs. 24	1.863	-0.8309 to 4.556	ns	0.4597
6 vs. 12	0.1250	-2.569 to 2.819	ns	>0.9999
6 vs. 24	2.526	-0.1672 to 5.220	ns	0.0831
6 vs. 48	2.945	0.2515 to 5.639	*	0.0226
12 vs. 24	2.401	-0.2922 to 5.095	ns	0.1198
12 vs. 48	2.820	0.1265 to 5.514	*	0.0337
24 vs. 48	0.4187	-2.275 to 3.112	ns	>0.9999

	NE-AKBA	AKBA
C_{max} (µg)	12.23 ± 1.94	3.36 ± 1.36
T_{max} (h)*	6 ± 0	6 ± 0
AUC_{0-t} (µg/ml)	6221.8 ± 668.7	4257.5 ± 1137.3
P value = 0.0062**		

***AKBA concentration was measured at specific times and C_{max} occurred at 6 h (T_{max}) for all samples, so SD for T_{max} is meaningless.**

****C_{max} of NE-AKBA vs. AKBA**

Figure 6. Mean (SD) AKBA amount in plasma samples per time (left). AKBA amounts (µg/ml) detected in plasma samples of rats (top right). C_{max} and T_{max} were obtained directly from the diagram (bottom right). AUC_{0-t} is the surface area under the curve. Differences between groups and times were calculated by Unpaired Student's t-test, *, **, *** indicate $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Discussion

In this study, Tween 80 (a hydrophilic surfactant) and Span 80 (a hydrophobic surfactant) were employed to adjust the HLB of the system [41, 42]. Using different edible oils, transparent nanoemulsions (NEs) were prepared and evaluated for AKBA loading and stability. Among the oils tested, almond oil–based NE showed the smallest droplet size, the highest stability, and the greatest AKBA solubility. The optimized formulation remained stable at room temperature for over 15 months, demonstrating long-term storage potential.

Initially, TEER values were low due to the porosity of the insert plate membrane. Following cell seeding, TEER increased, likely due to suspended cells accumulating within the pores and gradually covering them. After one week, cells differentiated and adhered to the membrane and each other, forming tight junctions, which further increased TEER. A decrease in TEER was observed after changing the medium, likely due to the removal of dead or non-adherent cells [43].

On day 21, after confirming monolayer integrity via TEER measurements, cells were treated with AKBA and NE-AKBA, and results were compared with untreated controls. AKBA content in the basolateral (BL) chamber was assessed for three hours post-treatment to evaluate

permeability across the intestinal epithelial model. Both AKBA and NE-AKBA initially caused a TEER reduction (T0), which recovered after three hours (T3). Cumulative AKBA transport (μM/cm³) was calculated to determine apparent permeability (Papp). NE-AKBA treatment resulted in significantly higher AKBA transport during the first hour compared with later time points. Unlike bulk AKBA, which exhibited a constant transport rate, NE-AKBA displayed a biphasic transport pattern, characterized by accelerated transport during the first hour.

While the exact mechanism underlying this early increase in transport remains unclear, it may relate to the known low-rate active transport of AKBA [44] and potential NE-mediated enhancement of active uptake [45]. Additionally, smaller droplets (<100 nm) may facilitate rapid intercellular passage, temporarily affect tight junction integrity, and reduce TEER in the early stage [46-48]. These observations suggest that NEs can improve the intestinal permeability of hydrophobic compounds such as AKBA through both intracellular and paracellular pathways.

Pharmacokinetic evaluation in rats confirmed enhanced oral absorption of NE-AKBA. Plasma concentrations of AKBA were significantly higher in the NE-AKBA group compared with bulk AKBA, as indicated by increased C_{max} and AUC0-t. The low dose used in this study (1 mg/kg) was chosen to minimize potential kidney toxicity associated with high levels of boswellic acids (BAs) [49] and to reflect the efficiency of NE formulation. Literature comparisons show variability in C_{max} and T_{max}, likely due to differences in Boswellia strains, resin composition, dosage, analytical methods, or formulation strategies [50-53]. In our study, T_{max} remained unchanged (6 h), consistent with prior reports [51].

The increase in AUC0-t (from 4257.5 ± 1137.3 to 6221.8 ± 668.7 μg/ml) confirms improved AKBA bioavailability with NE formulation. This enhancement aligns with findings for other

lipophilic drugs such as *mebudipine* and atovaquone [54, 55] and is likely attributable to improved solubility [56], modulation of biological barriers [57, 58], and/or enhanced lymphatic transport through lipoprotein formation [59]. Overall, the results demonstrate that nanoemulsion-based delivery is an effective strategy for improving the oral bioavailability of poorly soluble compounds like AKBA.

Conclusion

This study demonstrated that encapsulating AKBA in a nanoemulsion significantly enhanced its oral bioavailability. Both in vitro and in vivo findings confirmed an increased transport of AKBA, potentially facilitated through intercellular pathways in Caco-2 cells. These results highlight nanoemulsion-based delivery as a promising strategy for improving the therapeutic efficacy of poorly soluble compounds such as AKBA.

Declarations:

Ethics approval and consent to participate

All techniques and experimental procedures were executed in strict adherence to the pertinent regulations and directives set forth by the esteemed Ethical Committee of Tehran University of Medical Sciences (approval code: IR.TUMS.VCR.REC.1398.212).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

The individual contributions of the authors are as follows:

- Najmeh Ketabchi: Conceptualization, Investigation, Formal Analysis, Validation, Visualization, Writing – Original Draft.
- Seyed Nasser Ostad: Supervision (specific oversight and guidance on drug selection and properties).
- Mahmoud Ghazi-Khansari: Resources, Supervision (specific oversight and guidance on in vitro studies).

- Hossein Ghanbari: Supervision (specific oversight and guidance on in vivo studies).
- Fariba Esmaeili: Methodology, Resources.
- Amir Amani: Conceptualization, Resources, Project Administration, Writing – Review & Editing, Funding Acquisition, Supervision (overall supervision of the research, with specific oversight on nanoemulsion preparation).

All authors have read and approved the final submitted version of the manuscript.

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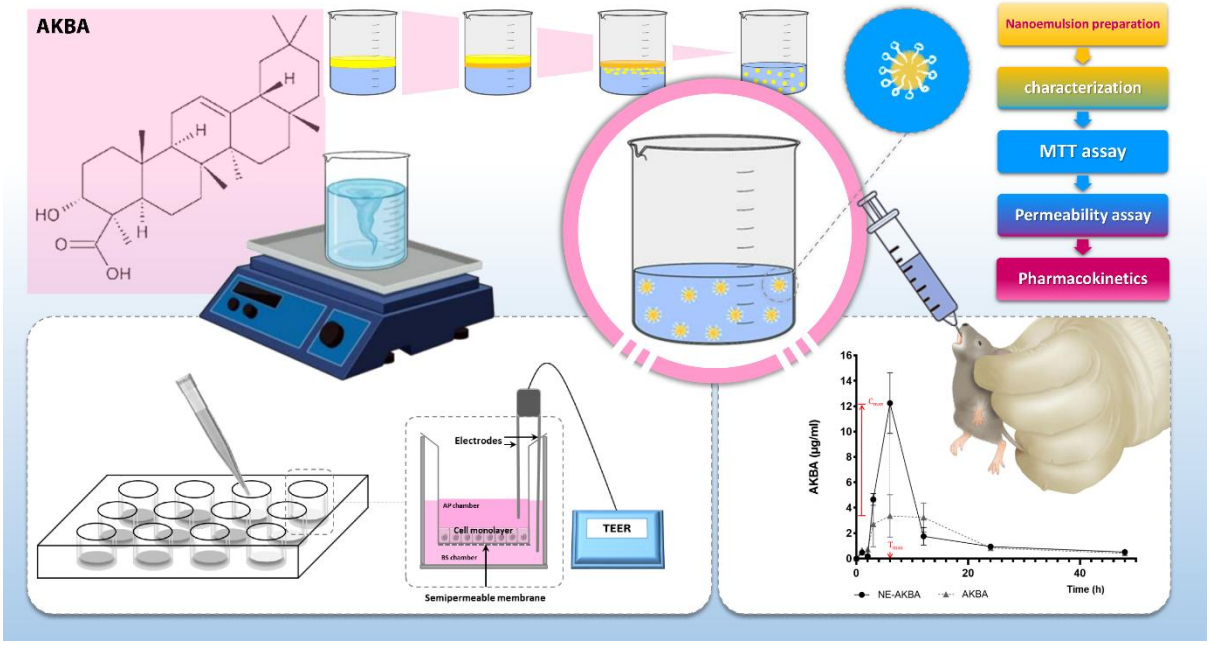
Graphical Abstract:



3-Acetyl-11-keto-β-boswellic acid (AKBA) shows promise for treating inflammatory and cancer-related disorders; however, its low oral bioavailability limits its effectiveness. This study developed a nanoemulsion (NE) delivery system for AKBA, resulting in a particle size of 12–15 nm that improved transport in Caco-2 cells. In vivo, C_{max} increased from 3.36 to 12.23 μg/mL, and AUC_{0-t} rose from 4257 to 6222 μg·h/mL, with T_{max} at 6 h. NE significantly enhances AKBA's bioavailability.