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Original article

Enhanced absorption of boswellic acids by a micellar solubilized delivery form of Boswellia extract

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ABSTRACT

Background: Boswellic acids (BAs) the pharmacologically active ingredients of the gum resin extract of *Boswellia serrata* are known for their anti-inflammatory effects. However they suffer from poor bioavailability because of their hydrophobicity and poor water solubility.

Purpose: The present study aimed at investigating the effect of AQUANOVA micellation technology on the bioavailability of Boswellia extract in rats compared to its native form.

Study design.

Female albino wistar rats (n = 6) weighing around 250 g were orally administered solubilized (Sol-BE) and native (Nat-BE) Boswellia extract at an equimolar dosage of 128 mg/kg. Plasma samples collected at defined time points (0, 0.5, 1, 2, 3, 4, 6 and 8 h) were analyzed for the content of the six major boswellic acids (KBA, AKBA, αBA, βBA, AαBA and AβBA - 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), acetyl-α-boswellic acid (AαBA) and acetyl-β-boswellic acid (AβBA)) using a sensitive LC-MS/MS method.

Results: **The oral administration of Sol-BE led to a remarkable increase in the AUC and** C_{max} of all BAs in plasma compared to Nat-BE. Whereas no KBA could be detected after the administration of Nat-BE, KBA could be detected at a maximal plasma concentration of 439.21 ng/mL and an AUC_{last} of 1185.37 ng/mL*h following the administration of Sol-BE. The highest increase was observed in the case of AKBA where a 56-fold increase in the AUC_{last} and a 25-fold increase in the C_{max} was determined compared to Nat-BE.

Conclusions: Micellar solubilisation represents a promising approach for enhancing the bioavailability of poorly soluble substances.

1. Introduction

The gum resin of *Boswellia serrata* also known as Indian frankincense or Salai guggal has been used for centuries in the Ayurvedic medicine for its anti-inflammatory properties and is now counted among the well-established plant food supplements in Europe and the USA. Hence in 2015 Boswellia achieved a 674% increase in sales over 2014 in the USA alone [1]. In fact, it was shown that a number of pivotal enzymes in inflammation like 5-lipoxygenase (5-LO), cathepsin G (catG), and microsomal prostaglandin-E synthase (mPGES)-1 as well as nuclear transcription factor κB (NF-κB) and several pro-inflammatory cytokines like tumor necrosis factor (TNFα), interleukin (IL)-1β, IL-2, and IL-6 are inhibited by boswellic acids (BAs), the main active ingredients of *Boswellia serrata*. The structure of the six most important BAs (11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, acetyl-α-boswellic acid and acetyl-β-boswellic acid) is presented in Fig. 1. In addition several preliminary pilot clinical trials support the potential of Boswellia to treat a variety of chronic inflammatory diseases like rheumatoid arthritis, osteoarthritis, chronic colitis, ulcerative colitis, collagenous colitis, Crohn's disease and bronchial asthma [2,3]. However, due to their hydrophobicity and low water solubility only negligible amounts of BAs are absorbed after oral ingestion [2]. This could be verified in the Caco-2 *in vitro* model revealing only moderate to poor permeability for BAs [4,5]. The resulting poor bioavailability represents thus the major limitation to the efficacy of these promising herbal substances. Therefore strategies to improve the bioavailability of BAs are urgently needed, in order to be able to benefit more from the therapeutic potential of Boswellia.

A promising approach for the efficient delivery of poorly soluble substances is the preparation of micellar formulations based on Tween 20. In fact, the application of this micellation technology on other herbal substances resulted for example in a tremendous increase in the

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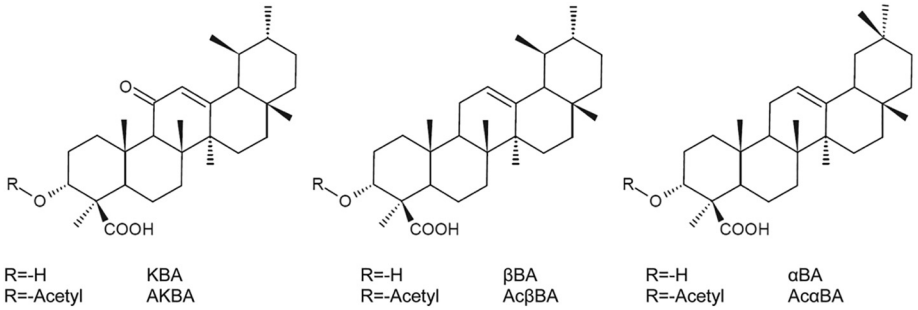

Fig. 1. Structures of the investigated boswellic acids. KBA: 11-keto-β-boswellic acid, AKBA: acetyl-11-keto-β-boswellic acid, αBA: α-boswellic acid, βBA: β-boswellic acid, AαBA: acetyl-α-boswellic acid, AβBA: acetyl-β-boswellic acid.

Table 1
Content of the individual six major BAs in native (Nat-BE) and solubilized (Sol-BE) Boswellia extract.

	Nat-BE		Sol-BE	
	[%]	Absolute amount [mg] in 128 mg total BAs/kg administered to rats	[%]	Absolute amount [mg] in 128 mg total BAs/kg administered to rats
KBA	4.2	5.4	0.4	5.4
AKBA	4.4	5.6	0.5	6.0
αBA	4.5	5.8	0.3	4.0
βBA	11.6	14.9	0.7	9.3
AαBA	2.7	3.4	0.2	2.9
AβBA	9.0	11.5	1.0	12.3
Total	36,4	46.6	3.1	39.9

bioavailability of curcumin, known for its poor absorption because of low solubility. Hence a 3.5-fold increase in the apparent permeability coefficient for micellar over native curcumin was observed in the Caco-2 *in vitro* model [6] and a 185-fold larger AUC for micellar curcumin compared to its native form was reported in human trials [7]. Encouraged by this tremendous increase in the oral bioavailability observed for curcumin, the present study was devoted to investigate the effect of micellation technology on the bioavailability of Boswellia extract in rats compared to its native form.

2. Materials and methods

2.1. Chemicals and reagents

Boswellic acids (11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), acetyl-α-boswellic acid (AαBA) and acetyl-β-boswellic acid (AβBA)) used as reference substances with purity > 99% were obtained from Phytoplan GmbH, Heidelberg, Germany. Native *Boswellia serrata* extract (N.: 1509020–01, Code N.: 10115/246) (Nat-BE) standardized to 80% total BAs and solubilized *Boswellia serrata* extract (Batch N.: L124.16.LM.02.01, Code N.: EW0123/1) (Sol-BE) containing 10% *Boswellia serrata* extract were kindly donated by AQUANOVA AG (Darmstadt, Germany). The internal standard fluoxymesterone was purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany, content > 98.0%). Ammonium formate was obtained from VWR (Leuven, Belgium). All solvents used were of analytical grade or better quality. Methanol, tetrahydrofuran, ethyl acetate and n-hexane were purchased from Roth GmbH (Karlsruhe, Germany). 2-Propanol, water and Extrelut® NT from Merck (Darmstadt, Germany). Plasma samples of rats administered the solubilized and native Boswellia extract were received from the Institute of Pharmacy and Molecular Biotechnology, Heidelberg, Germany. Blanc pooled rat plasma were received from Dunn Labortechnik, Asbach, Germany.

2.2. Animal study

All experiments were carried out according to the guidelines of German Protection of Animal act (Deutsches Tierschutzgesetz, BGBI 1998, Part I, No. 30, S.1105 ff.) and approved by the local ethical committee (AZ: 35.9185.81/G-17/11).

Female albino wistar rats weighing around 250 g were administered Sol-BE ($n = 6$) or Nat-BE (n = 6). For that purpose 300 mg of Nat-BE were weighed in Falcon tubes, filled up to 15 mL with water while shaking vigorously before 2 mL of that solution was administered orally to rats. This corresponds to a dose of 128 mg total BAs/kg. In case of Sol-BE, with a total BA fraction of 3.12%, 20 g solubilisate were weighed into a test tube and rehydrated with 80 mL water while shaking vigorously. Afterwards 2 mL of the respective solution, corresponding to 128 mg total BAs/kg were administered orally to rats by gavage *via* a pharyngeal tube. Blood samples for plasma analysis were collected from the retrobulbar venous plexus of the anesthetized animals after defined time points (0, 0.5, 1, 2, 3, 4, 6 and 8 h), centrifuged and stored at −20 °C.

2.3. Sample preparation

Concentrated stock solutions of all used BAs (AKBA, KBA, αBA, βBA, Aα- and AβBA) for standards and quality controls as well as the internal standard fluoxymesterone were prepared at a concentration of 1 mg/mL diluted in methanol. Different working solutions containing all BAs as well as an internal standard solution at 4 µg/mL were prepared by diluting the stock solutions with methanol. Calibration standards were prepared daily by spiking 1 mL of blank plasma with 25 µL of the internal standard solution and 40 µL of the corresponding working solution resulting in concentrations of 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 1500.0 and 3000.0 ng/mL plasma for βBA, AβBA, αBA and AαBA and 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 1500.0, 3000.0 ng/mL plasma for KBA and AKBA. QC pools at different concentration levels (15.0, 800.0 and 2500.0 ng/mL plasma for AKBA and KBA and 1.5, 15.0, 800.0, 800.0 and 2500.0 ng/mL plasma for βBA, AβBA, αBA and AαBA) were prepared by spiking blank plasma with the corresponding spike solution. Afterward the QC samples were aliquoted and stored at −20 °C until analysis. Based on the method described by Buechele and Simmet [8] and Reising et al. [9], the rat samples and QC's were carefully thawed and 1 mL of the plasma homogenate was spiked with 40 µL pure methanol corresponding to the volume of the spike solution for calibration samples and 25 µL internal standard solution containing 1 µg fluoxymesterone in methanol. All samples were mixed briefly using a vortex while 0.8 g of Extrelut® NT was filled into an 8 mL glass column for each sample. The plasma homogenates were transferred onto the columns for a matrix-based liquid-liquid-extraction. After 15 min the BAs were eluted with 8 mL of an elution mixture consisting of tetrahydrofuran - n-hexane - ethyl acetate - 2-propanol (160,160:160:15, v/v/v/v) into clean centrifuge tubes. After that the solvent was evaporated to dryness using a nitrogen stream at 40 °C. The

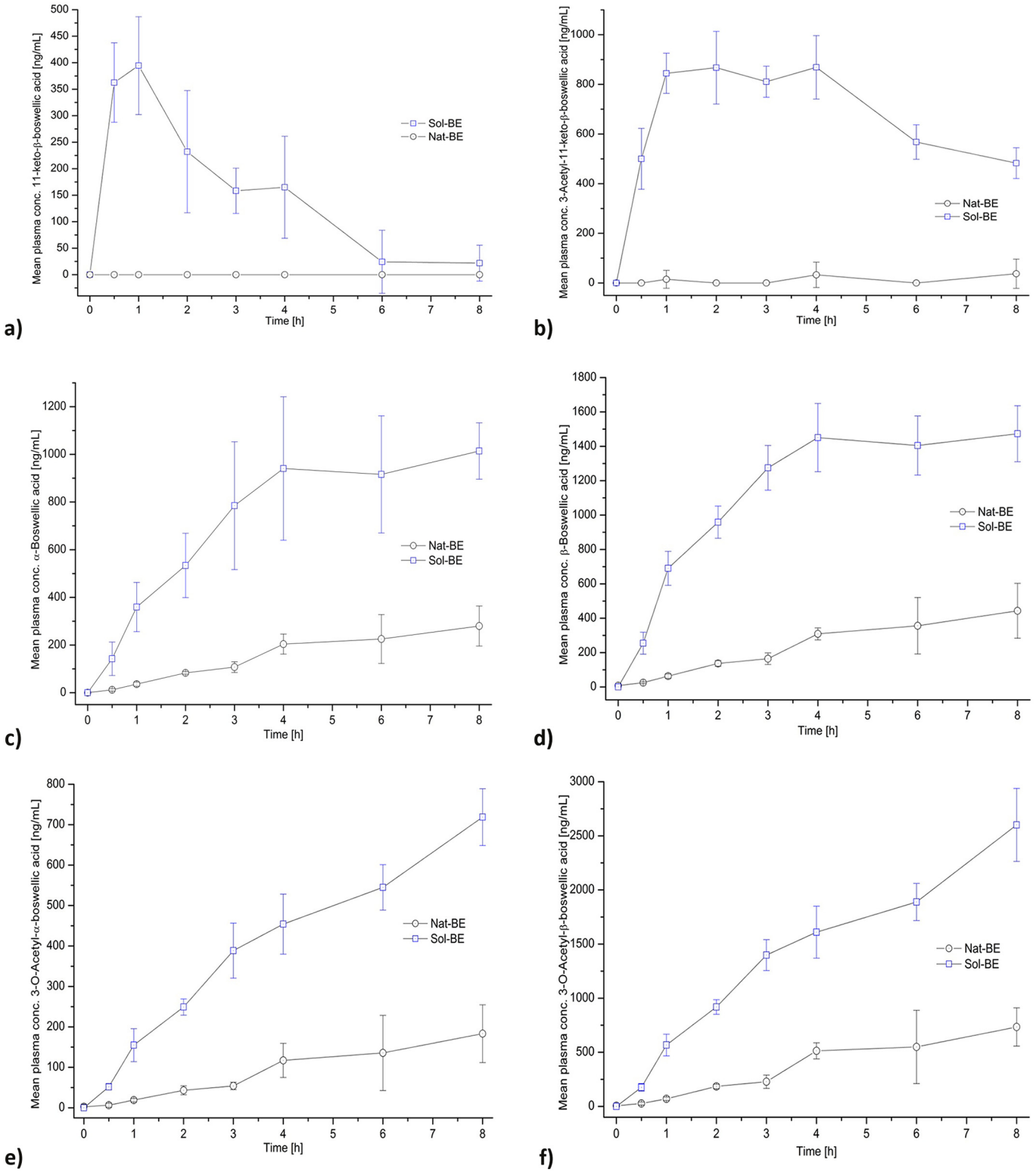

Fig. 2. Mean plasma concentrations [ng/mL] of KBA: 11-keto-β-boswellic acid (a), AKBA: acetyl-11-keto-β-boswellic acid (b), αBA: α-boswellic acid (c), βBA: β-boswellic acid (d), AαBA: acetyl-α-boswellic acid (e) and AβBA: acetyl-β-boswellic acid (f) in rats following the oral administration of 128 mg/kg of native non-formulated (Nat-BE) compared to the micellar solubilized (Sol-BE) *Boswellia serrata* extract to rats (n = 6), respectively.

residue was reconstituted in 100 µL mobile phase A and 50 µL were injected into the HPLC/MS-system.

2.4. Applied LC/MS for the quantification of BAs in rat plasma

The BA content in all samples was determined according to a sensitive previously developed LC-MS/MS method by Gerbeth et al. [10]. In brief, liquid chromatography was performed on an Agilent 1200 series equipped with a gradient pump with vacuum degasser, an autosampler and a column oven. A Hypersil BDS RP C18 column (100 × 4 mm; 3 µm; Thermo scientific), and an upstream Gemini security guard cartridge (Phenomenex, Germany) 4 × 3 mm were used

Table 2
Overview on the pharmacokinetic parameters of the six major BAs following the oral administration of native (Nat-BE) and solubilized (Sol-BE) Boswellia extract.

Parameters	Nat-BE	Sol-BE
KBA		
AUC_{last} [ng/nL*h]	0.00	1185 ± 273
C_{max} [ng/mL]	0.00	439 ± 76
T_{max} [h]	0.00	0.9 ± 0.6
AKBA		
AUC_{last} [ng/nL*h]	98 ± 154	5482 ± 491
C_{max} [ng/mL]	38 ± 60	949 ± 92
T_{max} [h]	2.0 ± 3	2.7 ± 1.2
αBA		
AUC_{last} [ng/nL*h]	1260 ± 292	5916 ± 1524
C_{max} [ng/mL]	283 ± 85.9	1063 ± 232
T_{max} [h]	7.3 ± 1.0	7.3 ± 1.6
βBA		
AUC_{last} [ng/nL*h]	1983 ± 478	9338 ± 670
C_{max} [ng/mL]	447 ± 156	1576 ± 102
T_{max} [h]	7.7 ± 0.8	6.3 ± 2.0
AαBA		
AUC_{last} [ng/nL*h]	745 ± 263	3269 ± 162
C_{max} [ng/mL]	184 ± 74	722 ± 64
T_{max} [h]	7.0 ± 1.7	7.7 ± 0.8
AβBA		
AUC_{last} [ng/nL*h]	3082 ± 743	11,624 ± 538
C_{max} [ng/mL]	766 ± 245	1889 ± 171
T_{max} [h]	7.7 ± 0.8	7.7 ± 0.8

for chromatography. Separation was achieved using a gradient program starting with 90% mobile phase A (methanol: water 90:10, 400 mg/L ammonium formate) and 10% mobile phase B (methanol: water 80:20, 400 mg/L ammonium formate) changing to 100% mobile phase A within 20 min. This was kept constant for 14 min before returning to the initial conditions within 1 min. The total run time was 35 min at a flow rate of 0.4 mL/min. The column oven was set to 35 °C and the autosampler was kept at room temperature.

MS analysis was performed in the negative single ion mode (SIM) on an Agilent Triple Quadrupole LC/MS 6410 series (Agilent Technologies, Waldbronn, Germany) equipped with an Electro Spray Ionization source (ESI). Dwell time was chosen to be 200 ms. The Mass Hunter software was used for data acquisition and processing.

Quantification of plasma was carried out with the internal standard method using peak area ratios. The same LC/MS method was applied for the determination of the individual BA content in Nat-BE and Sol-BE after diluting the respective samples to fit the BA calibration range.

3. Results and discussion

The extract of *Boswellia serrata* contains several pentacylic triterpenes which are often declared as total BAs. However among these total BAs, only six BAs, namely 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), acetyl-α-boswellic acid (AαBA) and acetyl-β-boswellic acid (AβBA), have been found to be responsible for most of the pharmacological effects. Therefore the relative content of these major BAs was determined in the native as well as in the solubilized Boswellia extract applied in the present study. The relative content of the individual BAs determined in Nat-BE and Sol-BE is presented in Table 1.

Because of the different contents of BAs in Nat-BE and Sol-BE care was taken to administer to rats identical molar doses of BAs by adjusting the amount of Nat-BE and Sol-BE weighed to prepare the aqueous oral solutions administered to rats as described under 2.2.

The mean plasma concentration-time profiles of the pharmacologically most relevant BAs are presented in Fig. 2a–f. In addition the mean maximum plasma concentrations, the mean AUC_{last} values and the mean time to reach the maximal plasma concentration (T_{max}) are listed in Table 2.

As demonstrated in Fig. 2a–f and Table 2 the oral administration of Sol-BE led to a remarkable increase in the AUC and C_{max} of all BAs in plasma compared to Nat-BE. Whereas no KBA could be detected after the administration of Nat-BE, KBA could be detected at a maximal plasma concentration of 439.21 ng/mL and an AUC_{last} of 1185.37 ng/mL*h following the administration of Sol-BE. The highest increase was observed in the case of AKBA where a 56-fold increase in the AUC_{last} and a 25-fold increase in the C_{max} was determined compared to Nat-BE. Also the AUC_{last} and c_{max} of βBA, αBA, AαBA, and AβBA increased up to 4.7-fold and 3.7-fold, respectively, upon the administration of Sol-BE in comparison to Nat-BE. On the other hand the administration of Sol-BE had no effect on the time required to reach the maximal concentration in plasma. In general the more lipophilic BAs βBA, αBA, AαBA, and AβBA are associated with a slower uptake compared to KBA and AKBA.

The present investigation is a preliminary pilot study carried out on a small number of rats ($n = 6$ for each group) for exploratory reasons to support the higher absorption expected for the micellar formulation of *B. serrata* extracts. Therefore a relatively high dose was administered, to make sure that at least some of the boswellic acids administered in native form are detactable. Nevertheless the results clearly indicate that absorption of BAs may be substantially improved by micellar solubilisation of BAs. The observed increase in absorption in the present study exceeded even the absorption enhancing effect monitored before for a phospholipid-based formulation of Boswellia extract administered to rats at nearly twice the dose [11]. In this study rats were administered an equimolar dosage of 240 mg Boswellia extract/kg of non-formulated and of the phospholipid-based Boswellia extract formulation. However the largest increase in the absorption observed for the phosholipid-based formulation amounted to a 6-fold and a 2.5-fold increase in the AUC_{last} and C_{max} of KBA, respectively, compared to the native Boswellia extract. Furthermore the increase in the AUC_{last} and C_{max} of the other BAs following the administration of the phospholipid-based formulation ranged between 2.5-times and 3-times the values determined for the non-formulated Boswellia extract, respectively [11]. In contrast the AUC_{last} and C_{max} of AKBA could be increased up to 56-fold and 25-fold, respectively, and the AUC_{last} and C_{max} of the other non-ketylated BAs could be increased up to nearly 5-fold and 4-fold, respectively, when administered in the form of the micellar formulation compared to the native extract. Taking into consideration that Tween 20 is attributed the regulatory designation GRAS by the FDA, micellar solubilisation represents a promising approach for enhancing the bioavailability of poorly soluble substances. As a result of this enhanced absorption it may be expected, that higher therapeutic effects may be obtained with much lower doses of Boswellia extract. For that reason a separate study will be devoted to compare the anti-inflammatory effects of micellar Boswellia extract and other poorly soluble promising herbal substances like curcumin with the anti-inflammatory effects of the corresponding native forms. If the expected enhanced anti-inflammatory effects reveal to be the case, then solubilized micellar formulations of Boswellia extract may represent a concomitant tool for anti-inflammatory treatment and a potential alternative to synthetic drugs at much lower doses than generally recommended.

4. Conclusion

In the current pharmacokinetic study carried out in rats, the oral administration of solubilized micellar Boswellia extract resulted in a tremendous increase in the absorption of boswellic acids, the major pharmacologically active principles of Boswellia extract compared to the unformulated native Boswellia extract. Micellar solubilisation represents thus a promising tool for increasing the therapeutic effects of poorly soluble substance at reduced dosages.

Conflict of interest

The study was financially supported by AQUANOVA AG (Darmstadt, Germany), the company manufacturing and selling micellar formulations of Boswellia. Dariush Behnam is the inventor of Boswellia micellization, the founder and CEO of AQUANOVA AG. All other authors have stated not to have any known conflict of interest.

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