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Enhanced absorption of boswellic acids by a lecithin delivery form (Phytosome®) of *Boswellia* extract

Jan Hüsch [a], Janine Bohnet [a], Gert Fricker [b], Carsten Skarke [c], Christian Artaria [d], Giovanni Appendino [e], Manfred Schubert-Zsilavecz [a,f], Mona Abdel-Tawab [a,*]

[a] *Central Laboratory of German Pharmacists, Carl-Mannich-Str. 20, 65760 Eschborn, Germany*
[b] *Institute of Pharmacy and Molecular Biotechnology, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany*
[c] *Institute for Translational Medicine and Therapeutics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA*
[d] *Indena S.p.A., Viale Ortles 12, 20139 Milan, Italy*
[e] *University of Eastern Piemonte, 28100 Novara, Italy*
[f] *Institute of Pharmaceutical Chemistry, J.W. Goethe-University, ZAFES, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany*

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ABSTRACT

The anti-inflammatory potential of *Boswellia serrata* gum resin extracts has been demonstrated in vitro and in animal studies as well as in pilot clinical trials. However, pharmacokinetic studies have evidenced low systemic absorption of boswellic acids (BAs), especially of KBA and AKBA, in rodents and humans. This observation has provided a rationale to improve the formulation of *Boswellia* extract. We present here the results of a murine comparative bioavailability study of Casperome™, a soy lecithin formulation of standardized *B. serrata* gum resin extract (BE), and its corresponding non-formulated extract. The concentration of the six major BAs [11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), β-boswellic acid (βBA), acetyl-β-boswellic acid (AβBA), α-boswellic acid (αBA), and acetyl-α-boswellic acid (AαBA)] was evaluated in the plasma and in a series of tissues (brain, muscle, eye, liver and kidney), providing the first data on tissue distribution of BAs. Weight equivalent and equimolar oral administration of Casperome™ provided significantly higher plasma levels (up to 7-fold for KBA, and 3-fold for βBA quantified as area under the plasma concentration time curve, AUC_{last}) compared to the non-formulated extract. This was accompanied by remarkably higher tissue levels. Of particular relevance was the marked increase in brain concentration of KBA and AKBA (35-fold) as well as βBA (3-fold) following Casperome™ administration. Notably, up to 17 times higher BA levels were observed in poorly vascularized organs such as the eye. The increased systemic availability of BAs and the improved tissue distribution, qualify Casperome™ for further clinical development to fully exploit the clinical potential of BE.

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1. Introduction

Bioavailability is a major hurdle in the translation of the preclinical potential of many botanical extracts into therapeutic effects, especially for those whose active ingredients show poor water solubility and strong tendency to self-aggregate. This is the case for many polyphenolics and triterpenoid acids. Phospholipid-based delivery systems have been developed to improve the oral availability of these compounds and the extracts that contain them. Among different strategies, a Phytosome® delivery form has emerged as a promising "dietary" candidate [1,2]. Compared to liposomes, Phytosome® is characterized by a high bioactive/lipid ratio [1] with an overall stoichiometry in the range of 1:1–1:3 between the active and the phospholipid formulation aid [3]. To date, the

* Corresponding author at: Central Laboratory of German Pharmacists, Carl-Mannich-Str. 20, D-65760 Eschborn, Germany. Tel.: +49 61 96 937 955; fax: +49 61 96 937 810.
E-mail address: m.tawab@zentrallabor.com (M. Abdel-Tawab).

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mechanism by which Phytosome® improves the absorption of plant phenolics and terpenoids is not known. Presumably, two processes are involved, namely improving their dispersion in the intestinal fluids, and "chaperoning" them into the enterocytes by lecithin [1,4]. The Phytosome® technology has been successfully applied to herbal extracts (ginkgo, milk thistle, green tea) as well as phytochemicals (curcumin, silybin), with remarkable results both in animals as well as in human pharmacokinetic studies [1,4–6].

Over the past two decades, extracts of the gum resin of *Boswellia serrata*, a traditional ayurvedic medicine, have become popular in Western countries. In vitro assays, animal studies and pilot clinical trials have established a potential for *B. serrata* gum resin extracts to alleviate a variety of inflammatory conditions that include inflammatory bowel disease, rheumatoid arthritis, osteoarthritis and asthma [7]. Furthermore, in 2002 the European Medicines Agency (EMA) granted *B. serrata* gum resin extract the 'orphan drug' status for the treatment of peritumoral brain edema [8]. The mechanism(s) underlying the anti-inflammatory action of *B. serrata* is (are) still under research. Early studies suggested that two quantitatively minor boswellic acids (BAs) [11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA)] could interfere with the production of leukotrienes by inhibition of 5-lipoxygenase (5-LO). However, more recent research has shown that other BAs, especially β-boswellic acid (βBA), could also play an important role, targeting the microsomal prostaglandin (PG) E_2 synthase-1 (mPGES-1) as well as cathepsin G (CatG) [7–10]. Thus intraperitoneal and oral administration of a low dose of βBA (1 mg/kg) suppressed pleurisy in the carrageenan-induced rat pleurisy model in vivo which was accompanied by reduced levels of PGE_2. Remarkably, AKBA caused no significant effects in this assay [9]. Pharmacokinetic studies have evidenced that the systemic absorption of BAs, especially KBA and AKBA, was very low both in animals and in humans [7,8], rationalizing efforts to overcome their low oral bioavailability. In this context, the excellent improvement of absorption recently reported for Meriva® [11,12], a lecithin formulation of curcumin, and the beneficial effects of a fatty meal on the bioavailability of BAs [8,13] have prompted us to compare the bioavailability of BAs in a soy lecithin formulation of a *B. serrata* extract (BE), and its corresponding unformulated extract. The concentration of the six major BAs (KBA, AKBA, βBA, acetyl-β-boswellic acid (AβBA), α-boswellic acid (αBA), and acetyl-α-boswellic acid (AαBA)) (Fig. 1) was evaluated in plasma and in a series of tissues (brain, muscle, eye, liver and kidney), providing novel data on the tissue distribution of BAs.

2. Material and methods

2.1. Standards and reagents

Boswellic acids (KBA, AKBA, αBA, βBA, AαBA and AβBA) with purity > 99% were obtained from Phytoplan GmbH, Heidelberg, Germany. *B. serrata* extract (Batch N.: 11239, Code N.: 36BW60090) (BE) and Casperome™ (Batch N.: 11411, Code N.: 36BWP0090) applied in the rat study were kindly donated by Indena S.A. (Tours, France). Casperome™ is a formulation of a *B. serrata* extract (BE) and soy lecithin in a 1:1 ratio, with about half part of microcrystalline cellulose being also added to improve the physical state and to standardize the product to a content of total triterpenoid acids by HPLC of at least 25%. For the production of Casperome™ and BE the same lot of gum resin has been used. Blank human plasma was obtained from Deutsches Rotes Kreuz-Blutspendedienst, Mannheim, Germany. The reagents used were tetrahydrofurane, n-hexane, dimethylsulfoxide (p.a. or gradient grade, Roth, Karlsruhe, Germany), 2-propanol, methanol (LC–MS, Roth, Karlsruhe, Germany), ethyl acetate (LC–MS, Fluka, Sigma-Aldrich, Steinheim, Germany), distilled water, potassium dihydrogen phosphate, EXtrelut® (Merck, Darmstadt, Germany), Tris (Roth, Karsruhe, Germany) and ammonium formate (p.a., VWR, Leuven, Belgium).

2.2. Animal study

Experiments were carried out according to the guidelines of the German Protection of Animal Act (Deutsches Tierschutzgesetz, BGBI 1998, Part I, No. 30, S. 1105 ff.).

Female albino Wistar rats weighing around 250 g were fasted from the afternoon over night before they were administered non-formulated BE (240 mg BSE/kg) or one of two dose levels of Casperome™ (n = 6 each) on the next morning. The first dose level of Casperome™ equals in weight to the administered extract and contains about 1/3 of the BAs found in the non-formulated BE. The second Casperome™ dose level corresponds to the molar content of the six boswellic acids (BAs) in the non-formulated BE and equals roughly 2.5 times the weight of non-formulated BE. For that purpose 900.27 mg of BE was weighed in Falcon tubes, filled up to 15 mL with water while shaking vigorously before 1 mL of that suspension was administered orally to rats by gavage via a pharyngeal tube. This corresponds to a dose of 240.07 mg/kg BE. Based on the total content of 36.23% for the six BAs (KBA, AKBA, βBA, AβBA, αBA, AαBA) determined in the BE, a total of 86.97 mg/kg of the six BAs were actually administered to rats upon application of BE. In the case of Casperome™, containing 14.17% of the six BAs, 901.18 mg (equivalent net weight of BE) and 2335.79 mg (equivalent molar ratio of BE), respectively, were weighed into a test tube and rehydrated with 15 mL water while shaking vigorously. Afterwards 1 mL of the respective solution, corresponding to 34.06 mg/kg (equivalent weight dosage to BE) and 88.28 mg/kg (equivalent molar dosage to BE) of the six BAs was administered orally to rats. Blood samples for plasma analysis were collected from the retrobulbar venous plexus of the anesthetized animals after defined time points (0.5, 1, 2, 3, 4, 6 and 8 h) in lithium-heparin tubes, centrifuged and stored at −80 °C. After 3 h and 8 h, the rats were dissected, and the brain was isolated, freed from meninges and visible vasculatures, weighted and washed with a Tris–HCl buffer solution (5 mM adjusted to pH 7.4). At these time points also the eyes, muscle, kidney and liver were isolated. In the case of the 3 h organ removal no plasma samples were taken. Afterwards the brain and the other organs were homogenized in Tris–HCl buffer (1 mL buffer/100 mg of the respective organ) and stored at −20 °C until analysis. In order to obtain better quantifiable results, the eyes of every two rats have been pooled.

In summary, 36 animals were used in total, 18 rats (six per group) for the generation of plasma and tissue data after 8 h

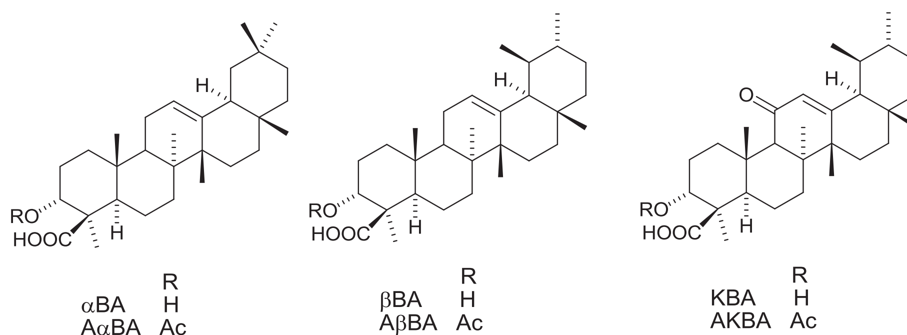


Fig. 1. Structures of boswellic acids.

and 18 rats (six per group) for the generation of the tissue data after 3 h.

2.3. Sample preparation

Plasma and organ samples were thawed slowly at ambient temperature. For most of the plasma samples, only a small amount of 100 μL was available, but the sample preparation for the LC–MS/MS method used to determine BAs in plasma was validated with 1 mL plasma. Thus, 900 μL of blank plasma was added to 100 μL of the plasma samples in order to obtain a total volume of 1 mL. In the case of lower plasma volumes (e.g. 70 μL), the missing plasma was filled up to volume with blank plasma (e.g. 930 μL) and the calculated values were corrected for the actual sample. In the case of the organ samples 1 mL of the homogenized solution was used.

Then the samples were spiked with 25 μL of an internal standard solution (containing 1 μg fluoxymesterone in methanol) and 50 μL of pure methanol was added (to correct for the volume spiked to blank plasma for the preparation of the calibration samples). Afterwards the samples were vortexed briefly prior to loading onto glass cartridges filled with 0.8 g EXtrelut® NT. For matrix based liquid–liquid extraction, the samples were eluted with 8 mL of an extraction mixture consisting of tetrahydrofurane: n-hexane:ethyl acetate:2-propanol (320:320:320:30; v/v/v/v) after 15 min. The solution was then evaporated to dryness (nitrogen stream, 40 °C) and reconstituted in 100 μL mobile phase A. For complete dissolution the samples were vortexed and treated in the ultrasonic bath for few minutes. The insoluble residue was separated by centrifugation (2 min; 10 °C, 4000 rpm). The supernatant was used for LC/MS-analysis.

Calibration solutions were prepared by diluting the BA stock solutions in methanol to the desired concentration. For calibration, 1 mL of blank plasma, brain, muscle, eyes, liver and kidney, respectively, were spiked with 50 μL of a calibration solution to yield BA concentrations ranging between 5 and 10,000 ng/mL and 25 μL of the internal standard solution, containing 1 μg fluoxymesterone. All calibration curves resulted in linear correlation coefficients >0.98 thus fulfilling the requirements of bioanalytical method validation.

2.4. Sample analysis by LC/MS

The BA content in all samples was determined according to a sensitive previously developed LC–MS/MS method [14]. 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 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 (ESI) source. Dwell time was chosen to be 200 ms. The Mass Hunter software was used for data acquisition and processing.

Quantification of plasma and different organ samples was carried out with the internal standard method using peak area ratios.

2.5. Analysis of BE and Casperome™

The LC/MS method described above was used for the determination of the content of the six BAs in BSE and Casperome™ after diluting the respective samples to fit the BA calibration range of 5–10,000 ng/mL.

2.6. Statistical analysis

All plasma data for the individual BAs were expressed as mean of six rats ± S.D. For statistical analysis the pharmacokinetic parameters (AUC and C_{max}) derived from the plasma concentrations of the individual BAs have been log-transformed and analyzed by one way ANOVA ($p \leq 0.1$). The analysis was performed by the GLM procedure of SAS

Table 1
Doses of the individual boswellic acids administered orally in the form of non-formulated BE extract and Casperome™ at equivalent weight and equimolar dosages.

	Non-formulated BE (reference)	Casperome™ (equiweight dosage)	Casperome™ (equimolar dosage)
KBA	9.52	3.73	9.66
AKBA	7.65	3.00	7.77
αBA	13.44	5.26	13.64
βBA	28.13	11.02	28.56
AαBA	6.71	2.63	6.81
AβBA	21.58	8.45	21.91
Total [mg/kg]	86.97	34.06	88.28

using the least square means (LSMeans) method with the Bonferroni's adjustment for three comparisons, to calculate the difference between LSMeans and its 90% confidence interval. The ratio between samples was found by calculating the anti-log of the difference between LSMeans.

When applicable, ANOVA was also performed on the untransformed data of the other pharmacokinetic parameters (T_{max}, $T_{1/2}$, Ke). If the F test of ANOVA was significant at the 10% level ($p \leq 0.10$) at least, the multiple comparisons were performed with Bonferroni's adjustment. Samples with three replications at least have been analyzed. If the data of only two samples were available the unpaired *t* test was used.

The tissue data for the individual BAs are described by the median, minimum and maximum concentrations. They have not been subjected to ANOVA analysis, because of the high interindividual variability and missing normal distribution.

3. Results

3.1. BA content in BE and Casperome™

BE contained 3.97% KBA, 3.19% AKBA, 11.72% βBA, 8.99% AβBA, 5.60% αBA and 2.80% AαBA which added up to a total BA content of 36.23%.

In comparison, Casperome™ contained 1.49% KBA, 1.25% AKBA, 4.66% βBA, 3.50% AβBA, 2.24% αBA and 1.03% AαBA. The sum of these BAs was 14.17%.

Two different dose levels of Casperome™ were evaluated in comparison to non-formulated BE. This allowed, in the first case, to compare the absorption of the same weight of formulated extract (Casperome™) versus the reference extract. This comparison was sub-stoichiometric – Casperome™ contains only approximately 33% BE – involving a formulated amount of a BE extract and a three-fold molar excess of its corresponding non-formulated version. In the second case, absorption of an identical molar dose of BAs was compared between a lecithinized matrix (Casperome™) and the corresponding non-formulated form. The doses of the individual BAs administered to rats in the form of non-formulated BE and Casperome™ at equivalent weight and molar doses are summarized in Table 1. The concentration of the six major BAs (KBA, AKBA, αBA, βBA, AαBA, and AβBA) was evaluated in plasma as well as in highly (kidney, liver, brain) and poorly perfused organs (muscle, eye).

3.2. Plasma concentration of BAs in rats

The dose of 240 mg/kg BE was chosen to enable comparability with previous rat studies [15,16]. The mean plasma concentration–time profiles of the pharmacologically most relevant BAs are presented in Fig. 2. In addition the

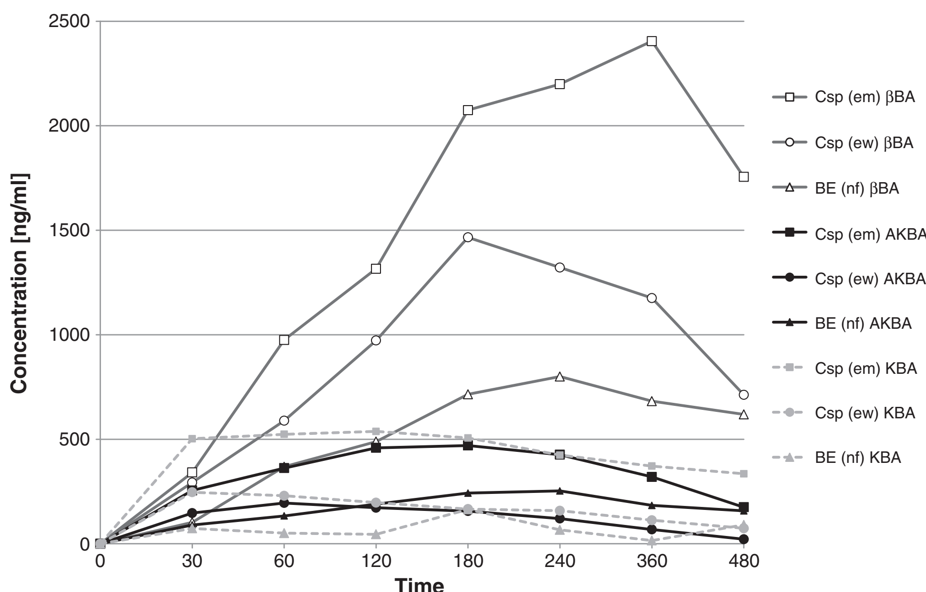


Fig. 2. Mean plasma profiles of 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA) and β-boswellic acid (βBA) in rats after the oral administration of 240 mg/kg non-formulated *Boswellia serrata* extract [BE (nf)] and Casperome™ (Csp) at equiweight (ew) and equimolar (em) doses. Values are means of six rats. For clarity purposes no error bars are shown. The mean plasma concentrations ± SD for the individual boswellic acids at every sampling point are listed in Table 2.

mean plasma concentrations and pharmacokinetic parameters of the individual BAs are listed in Tables 2 and 3. The ratios of the geometric means of the AUC and C_{max} of both Casperome™ dosages to the non-formulated extract and the corresponding 90% confidence intervals are summarized in Table 3 and visualized in Fig. 3a and b. As can be seen in Table 3, the administration of Casperome™ at equivalent weight dosage resulted in comparable or two times higher C_{max} and AUC_{last} values, although only about 1/3 of the BA content present in the BE extract was administered with Casperome™ at this dosage. The difference was statistically significant in the case of the AUC_{last} and C_{max} of βBA and in the case of AUC_{last} of KBA. For AβBA a statistically significant decrease in the C_{max} was observed.

The administration of Casperome™ at equimolar dosage resulted in two- to three-fold higher plasma levels and two- to six-fold increases in the AUC_{last} for AKBA, αBA, KBA, and βBA. Similar to the equiweight dosage, statistically significant differences were observed for the AUC_{last} and C_{max} values of KBA and βBA. AβBA and AαBA revealed comparable or slightly decreased AUC_{last} and C_{max} values compared to the non-formulated BE extract. Comparing both Casperome™ dosages with each other, statistically significant higher AUC and C_{max} values were found for KBA and AKBA in the equimolar dosage. The formulation of BE into Phytosome® form had generally no effect on T_{max}, nor on the half life ($T_{1/2}$) nor on the elimination of BAs ($T_{1/2}$/Ke).

3.3. Tissue distribution of BAs in rats

The distribution of the pharmacologically most relevant BAs in various organs is depicted in Fig. 4 and the data for the distribution of all BAs are summarized in Table 4.

The highest uptake of all BAs was observed in the kidney and in the liver. Upon the oral administration of Casperome™ equimolar dosage the level of the polar BAs, KBA and AKBA, in the kidney and liver increased dramatically up to 24 times after 8 h, whereas most of the other BAs revealed 2–7 times higher levels.

The brain levels of BAs differed according to the administered formulation and the individual BAs. No KBA, and in the case of AKBA only negligible amounts were detected in the brain 3 and 8 h following the oral administration of the reference extract. The availability of these two BAs was markedly increased (up to 35 times in the case of AKBA), when administered as Casperome™. Maximum brain tissue concentrations were reached at 8 h and 3 h for the equimolar and equivalent weight doses, respectively. In the case of αBA and βBA, which represent non-acetylated BAs without a keto group, up to 3 times higher brain levels compared to the extract

Table 2
Overview on the mean plasma levels [ng/mL] of 6 rats obtained for the individual BAs over a period of 8 h following the oral administration of non-formulated BE extract and Casperome™ at equivalent weight and equimolar dosages.

Sample		Time [min]	0	30	60	120	180	240	360	480
KBA	BE (not formulated)	Mean	0	73.68	51.03	45.09	164.39	66.53	15.45	90.42
		SD	0	87.14	85.48	50.15	197.40	136.90	13.55	183.44
	Casperome™ (equiweight dosage)	Mean	0	246.37	229.64	196.83	165.86	158.17	112.70	74.09
		SD	0	96.28	129.13	68.20	87.37	78.95	44.24	39.99
	Casperome™ (equimolar dosage)	Mean	0	502.59	523.95	537.56	506.33	424.15	371.69	334.71
		SD	0	358.41	194.70	292.33	233.98	206.89	46.52	140.70
AKBA	BE (not formulated)	Mean	0	90.13	133.21	189.78	242.31	253.00	183.57	157.39
		SD	0	35.66	128.41	98.15	95.66	53.90	25.33	27.48
	Casperome™ (equiweight dosage)	Mean	0	147.17	195.27	172.43	156.30	120.60	68.45	21.72
		SD	0	46.34	125.45	39.65	77.88	82.41	36.81	28.63
	Casperome™ (equimolar dosage)	Mean	0	255.06	362.12	458.93	470.18	426.26	319.93	174.84
		SD	0	259.57	251.95	385.29	307.98	297.16	104.98	112.14
βBA	BE (not formulated)	Mean	0	102.54	368.42	488.61	715.05	800.09	682.18	619.02
		SD	0	66.92	401.60	212.86	197.83	243.57	146.90	254.07
	Casperome™ (equiweight dosage)	Mean	0	293.14	588.57	972.43	1466.11	1321.64	1175.72	712.86
		SD	0	94.02	277.90	440.73	563.27	408.54	394.43	138.00
	Casperome™ (equimolar dosage)	Mean	0	341.20	975.01	1315.75	2074.62	2199.22	2404.95	1755.34
		SD	0	173.81	522.15	374.56	2173.97	1150.45	660.83	864.11
αBA	BE (not formulated)	Mean	0	79.49	151.90	222.40	393.76	378.86	340.97	332.52
		SD	0	19.86	137.20	82.78	238.81	72.50	61.08	105.79
	Casperome™ (equiweight dosage)	Mean	0	113.60	197.08	314.44	487.47	469.12	435.86	307.22
		SD	0	24.79	70.07	86.51	218.43	136.73	127.57	110.26
	Casperome™ (equimolar dosage)	Mean	0	111.44	255.39	574.40	652.57	750.20	927.27	674.39
		SD	0	61.41	148.61	541.71	491.21	535.36	521.02	190.17
AαBA	BE (not formulated)	Mean	0	13.16	88.35	141.07	267.12	428.07	442.58	407.14
		SD	0	20.05	105.40	67.10	96.34	139.21	139.57	126.65
	Casperome™ (equiweight dosage)	Mean	0	48.65	104.64	202.63	336.62	351.33	351.56	293.39
		SD	0	23.57	42.22	68.10	147.81	90.59	98.07	84.18
	Casperome™ (equimolar dosage)	Mean	0	44.82	119.51	320.08	404.47	474.56	799.66	593.46
		SD	0	28.15	83.30	245.87	255.10	294.23	296.33	127.46
AβBA	BE (not formulated)	Mean	0	84.91	399.63	634.11	1163.45	1730.15	2135.31	1590.11
		SD	0	113.12	376.41	309.33	386.46	512.03	1271.23	479.13
	Casperome™ (equiweight dosage)	Mean	0	104.19	259.93	481.59	804.59	837.18	809.60	653.24
		SD	0	53.72	100.50	178.32	349.56	239.96	275.95	184.31
	Casperome™ (equimolar dosage)	Mean	0	101.94	322.77	886.75	1119.97	1351.13	2240.78	1538.85
		SD	0	71.91	301.67	722.15	794.08	1049.83	1105.19	273.59

Table 3
Overview on the pharmacokinetic parameters of the individual BAs following the oral administration of non-formulated BE and Casperome™ at equivalent weight and equimolar dosages.

Parameters	BE (not formulated)	Casperome™ (equiweight dosage)		Casperome™ (equimolar dosage)	
	Mean ± SD	Mean ± SD	Ratio[a] (90% CI)[b]	Mean ± SD	Ratio (90% CI)
KBA					
AUC_{last} [ng/nL*h][c]	505.71 ± 308.22	1194.8 ± 301.03	2.77 (1.41–5.43)*1	3190.8 ± 1181.8	7.11 (3.62–13.95)*2
C_{max} [ng/mL][d]	288.54 ± 162.93	275.81 ± 93.21	1.06 (0.55–2.06)	746.69 ± 293.31	2.83 (1.46–5.47)*3
T_{max} [h][e]	2.83 ± 2.91	1.67 ± 1.47		3.17 ± 3.11	
$T_{1/2}$ [h][f]	0.74 ± n.c.[h]	4.80 ± 4.12		3.29 ± 2.21	
Ke [h^{-1}][g]	0.94 ± n.c.[h]	0.25 ± 0.21		0.32 ± 0.25	
AKBA					
AUC_{last} [ng/nL*h][c]	1481.09 ± 266.71	888.30 ± 276.22	0.58 (0.32–1.06)	2619.8 ± 1425.1	1.53 (0.83–2.79)
C_{max} [ng/mL][d]	311.98 ± 64.70	242.69 ± 91.97	0.75 (0.41–1.35)	621.08 ± 387.39	1.72 (0.95–3.11)
T_{max} [h][e]	3.17 ± 1.17	1.58 ± 1.11		4.17 ± 2.40	
$T_{1/2}$ [h][f]	6.73 ± 5.25	1.18 ± 0.87		4.19 ± 5.17	
Ke [h^{-1}][g]	0.14 ± 0.07	0.80 ± 0.40	*0.66 (0.23–1.08)**4	0.36 ± 0.26	
βBA					
AUC_{last} [ng/nL*h][c]	4714.8 ± 664.49	8073.3 ± 1862.8	1.69 (1.01–2.83)*5	12120.8 ± 5427.6	2.29 (1.37–3.84)*7
C_{max} [ng/mL][d]	994.22 ± 131.90	1692.5 ± 383.85	1.68 (1.05–2.71)*6	3044.5 ± 1877.9	2.69 (1.67–4.33)*8
T_{max} [h][e]	4.50 ± 2.35	3.50 ± 1.38		4.33 ± 2.34	
$T_{1/2}$ [h][f]	10.12 ± 8.29	3.95 ± 0.97		9.06 ± 11.03	
Ke [h^{-1}]	0.12 ± 0.08	0.18 ± 0.04		0.20 ± 0.16	
αBA					
AUC_{last} [ng/nL*h][c]	2352.6 ± 355.06	2889.1 ± 592.23	1.22 (0.66–2.25)	4644.8 ± 2785.8	1.64 (0.89–3.03)
C_{max} [ng/mL][d]	511.21 ± 172.05	572.02 ± 198.28	1.11 (0.62–1.98)	988.09 ± 606.76	1.72 (0.96–3.07)
T_{max} [h][e]	5.50 ± 2.17	4.17 ± 1.47		4.50 ± 1.76	
$T_{1/2}$ [h][f]	5.49 ± 0.79	8.55 ± 8.85		6.64 ± 3.66	
Ke [h^{-1}][g]	0.13 ± 0.02	0.14 ± 0.08		0.13 ± 0.08	
AαBA					
AUC_{last} [ng/nL*h][c]	2297.9 ± 530.47	2165.6 ± 453.85	0.95 (0.47–1.91)	3341.6 ± 1764.2	1.21 (0.60–2.43)
C_{max} [ng/mL][d]	536.88 ± 75.41	435.41 ± 114.49	0.8 (0.46–1.37)	727.34 ± 368.95	1.19 (0.69–2.04)
T_{max} [h][e]	6.00 ± 1.79	4.67 ± 1.51		5.50 ± 1.76	
$T_{1/2}$ [h][f]	5.38 ± 3.23	30.0 ± 35.32		3.49 ± n.c.[h]	
Ke [h^{-1}][g]	0.17 ± 0.11	0.07 ± 0.05		0.20 ± n.c.[h]	
AβBA					
AUC_{last} [ng/nL*h][c]	10595.7 ± 2208.5	5061.44 ± 1136.8	0.48 (0.22–1.03)	9265.9 ± 5547.2	0.69 (0.32–1.49)
C_{max} [ng/mL][d]	2511.13 ± 1050.7	1039.33 ± 271.45	0.43 (0.22–0.83)*9	2080.3 ± 1255.2	0.73 (0.37–1.42)
T_{max} [h][e]	6.33 ± 1.51	4.67 ± 1.51		5.00 ± 1.55	
$T_{1/2}$ [h][f]	6.05 ± 0.37	16.58 ± 16.59		34.47 ± 44.50	
Ke [h^{-1}][g]	0.12 ± 0.01	0.08 ± 0.05		0.12 ± 0.16	

*Indication for statistically significant differences: *1 $p = 0.0090$; *2 $p < 0.0001$; *3 $p = 0.0065$; *4 $p = 0.0090$ (difference) *5 $p = 0.0919$; *6 $p = 0.0643$; *7 $p = 0.0056$; *8 $p = 0.0006$; *9 $p = 0.0275$.
[a] Ratio: geometric mean ratio of Casperome™ (equiweight and equimolar dosage) to non-formulated BE.
[b] CI: 90% confidence interval.
[c] AUC_{last}: area up to the last validated measurable plasma concentration under the plasma concentration versus time curve.
[d] C_{max}: mean maximal plasma concentration.
[e] T_{max}: mean time required to C_{max}.
[f] $T_{1/2}$: the mean time taken for the plasma concentration to fall to half of its original value.
[g] Ke: mean overall elimination rate constant describing removal of the compound by all elimination processes including excretion and metabolism.
[h] n.c.: not calculated, because of insufficient data in the elimination phase.

were detected 8 h after oral administration of the Casperome™ at equivalent weight dosage. For AαBA and AβBA, both acetylated BAs without a keto group, comparable maximum levels were achieved 3 h following the oral administration of both Casperome™ doses.

In the muscle, equimolar Casperome™ yielded the highest KBA and AKBA concentrations at 8 h after the oral dose (up to 15 times higher levels compared to the extract). Equiweight Casperome™ produced the highest AαBA and AβBA at 3 h after oral dosing (up to 15 times higher levels compared to the extract). Both Casperome™ dose forms yielded the highest αBA and βBA concentrations at 8 h after oral dosing, being nearly 3 times higher than the corresponding concentrations of the extract.

With the exception of αBA, AαBA and AβBA, the highest BA concentrations in the eye were obtained with Casperome™ at equimolar dose at 8 h after oral dosing. In spite of the low perfusion of the eye, between 1.8-times (for βBA) and 17-times (for KBA) higher BA concentrations compared to the extract were observed.

4. Discussion

The oral absorption of BAs, especially of AKBA and KBA, is reportedly poor in both rats and humans. Thus, a pharmacokinetic study with one healthy volunteer taking three times 786 mg of a *Boswellia* extract over a period of 10 days led to steady state levels of 0.1 μmol/L for AKBA and of 0.34 μmol/L

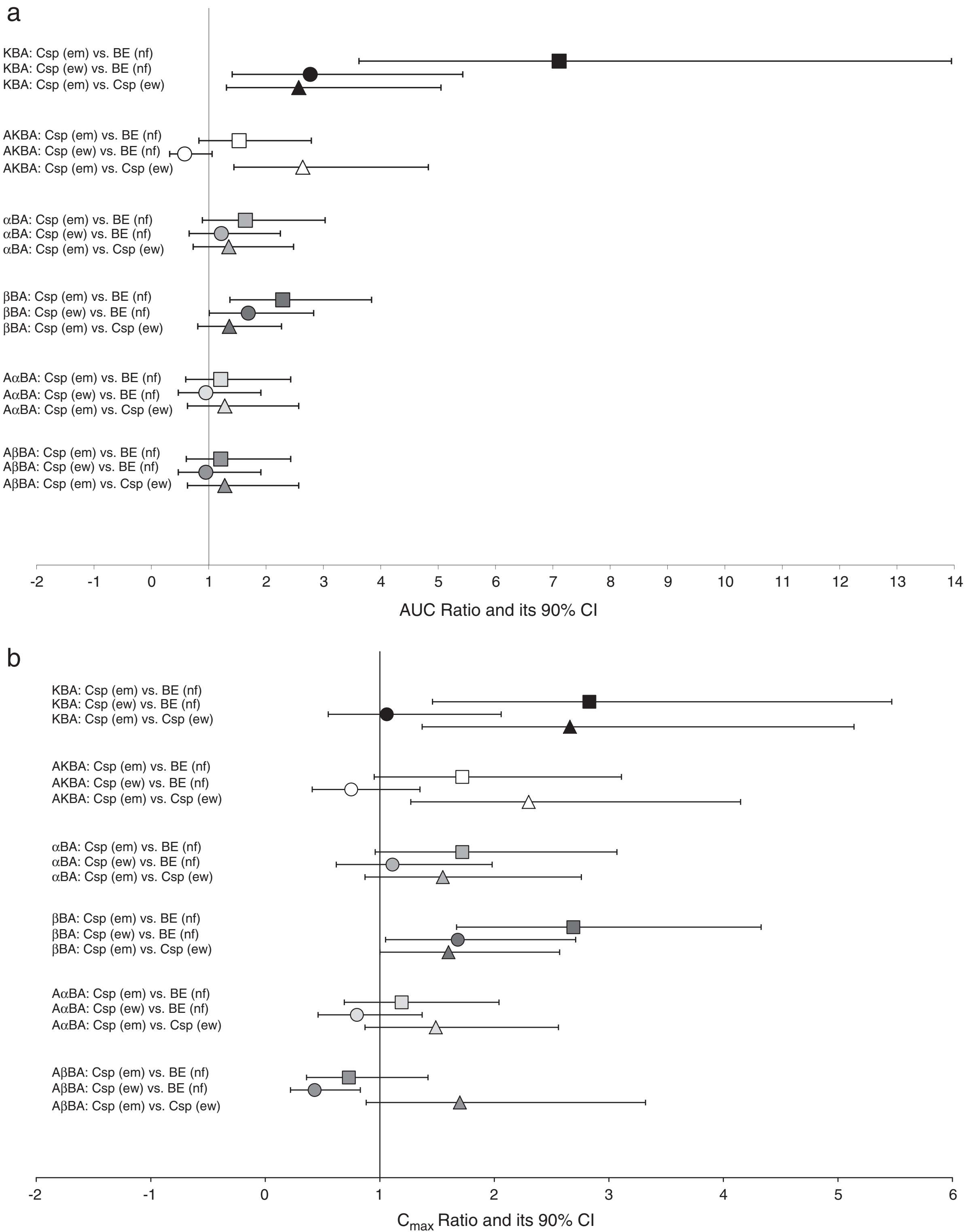
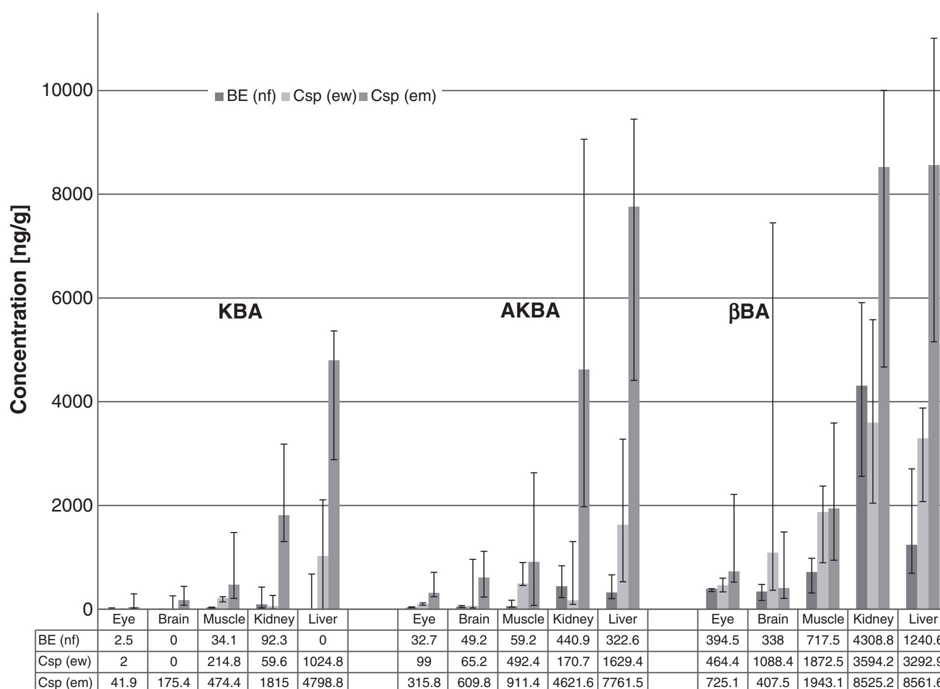


Fig. 3. Results of the statistical analysis of the AUC (a) and C_{max} (b) values for the individual boswellic acids (BAs). The figure shows the 90% confidence intervals (CI) of the geometric mean ratios of the equimolar Casperome™ dosage [CSP (em)] compared to the non-formulated extract [BE (nf)], of the equivalent weight Casperome™ dosage [CSP (ew)] compared to BE (nf), and of CSP (em) compared to CSP (ew) for the individual BAs, respectively. Confidence intervals > 1 indicate significant differences between the respective administrations.



	KBA Eye	KBA Brain	KBA Muscle	KBA Kidney	KBA Liver	AKBA Eye	AKBA Brain	AKBA Muscle	AKBA Kidney	AKBA Liver	βBA Eye	βBA Brain	βBA Muscle	βBA Kidney	βBA Liver
BE (nf)	2.5	0	34.1	92.3	0	32.7	49.2	59.2	440.9	322.6	394.5	338	717.5	4308.8	1240.6
Csp (ew)	2	0	214.8	59.6	1024.8	99	65.2	492.4	170.7	1629.4	464.4	1088.4	1872.5	3594.2	3292.9
Csp (em)	41.9	175.4	474.4	1815	4798.8	315.8	609.8	911.4	4621.6	7761.5	725.1	407.5	1943.1	8525.2	8561.6

Fig. 4. Median concentrations of 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA) and β-boswellic acid (βBA) in the eye, brain, muscle, liver and kidney of rats after 8 h of the oral administration of 240 mg/kg non-formulated *Boswellia serrata* extract [BE (nf)] and Casperome™ at equiweight and equimolar doses [Csp (ew) and Csp (em)]. The range between the minimum and maximum concentrations of the three boswellic acids in the individual organs is expressed by the line bars. The median, minimum and maximum concentrations for 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA) and β-boswellic acid (βBA) after 3 h and for all boswellic acids after 3 and 8 h from oral administration are listed in Table 4.

for KBA [17]. Another pharmacokinetic study with three healthy volunteers taking three times 800 mg *B. serrata* extract over a period of 4 weeks led to steady state concentrations of 0.04 μmol/L for AKBA and 0.3 μmol/L for KBA [10]. Other pharmacokinetic studies applying single oral *B. serrata* doses revealed even lower levels for AKBA, being often not detectable [8,13,18]. Solely in one study on 12 human volunteers KBA reached maximum plasma levels of 2.7 μmol/L following the administration of 333 mg *B. serrata* extract after meal [18]. Of all BAs, only βBA and AβBA revealed acceptable plasma levels in the range of 6.35–10.1 μmol/L for βBA and 2.4–4.9 μmol/L for AβBA, which may be attributed to their higher contents in the extract [10,17].

The present pilot study clearly demonstrates – with a proof-of-concept approach in animals – that the absorption and tissue distribution of BAs can be markedly increased by using a phospholipid-based delivery form (Casperome™) of BE. This is a pivotal finding, especially in the light of the stoichiometry bias of the equivalent weight comparison. The molecular bases underlying the observed improved absorption and tissue uptake are still not known. On the other hand, consistent evidence that fatty meals substantially improve, to different extents, the low absorption of BAs has been reported [8,13]. This effect is presumably due to the capacity of biliary acids to improve the solubility of BAs, facilitating their dispersion in the intestinal fluids and their eventual absorption. It is believed that phospholipids could play a similar role. In fact, the pharmacologically most important BAs, i.e. KBA, AKBA and βBA, have achieved markedly increased levels in plasma. Interestingly, while for KBA and βBA a statistical significant increase, in comparison to the non formulated BE, in both AUC_{last} and C_{max} was obtained, αBA, AKBA, AαBA and AβBA resulted in comparable or almost two times higher levels in the equimolar comparison but without reaching statistical significance. This may be attributable to the low power of the study but also to the greater lipophilicity of AαBA and AβBA which might be associated with a slower uptake and probably with a stronger retention in the Phytosome® making differences probably obvious at later time points than 8 h. It has to be underlined that these higher plasma levels were accompanied with a better distribution of all BAs in all tissues investigated. This is evident for the concentration in the brain and muscle of KBA, quantified at 0.4 and 1 μmol/g, AKBA at 1.2 and 1.8 μmol/g, and βBA at 2.4 and 4.3 μmol/g, respectively. These tissue concentrations, achieved for the first time following oral administration of BE in a murine model, are within the range of the IC_{50} values observed in vitro for the inhibition of 5-LO (1.5 μM for AKBA), mPGES-1 (5 μM for βBA) and CatG (0.6 μM for AKBA, 0.8 μM for βBA) [8]. Increased tissue concentrations (up to 17-fold) were also observed in poorly vascularized organs like the eye, as well as in excretory organs like the liver and the kidney. These results are of particular clinical importance because it has been shown in a former study that orally administered phospholipid complexed BAs exerted a three-fold greater anti-inflammatory activity in carrageenan-induced rat paw edema compared to non-formulated BAs [19].

Previous attempts to improve the absorption of BAs in vivo by phospholipid formulation used an equivalent BE as

Table 4

Overview on the median tissue levels of 6 rats and the range between the minimum and maximum values obtained for the individual BAs after 3 h for KBA, AKBA and βBA and after 3 and 8 h for αBA, AαBA, and AβBA following the administration of non-formulated BE extract [BE (nf)] and Casperome™ at equivalent weight [Csp (ew)] and equimolar [Csp (em)] dosages (n = 6). The 8 h values for KBA, AKBA and βBA are shown in Fig. 4.

h	KBA [ng/g]						AKBA [ng/g]						βBA [ng/g]				
	Eye	Brain	Muscle	Kidney	Liver	h	Eye	Brain	Muscle	Kidney	Liver	h	Eye	Brain	Muscle	Kidney	Liver
BE (nf)	5.67	0.00	47.05	226.7	868.49	3	25.59	9.63	79.81	885.2	687.00	3	123.4	0.0	260.24	3208.8	1012.29
Min–max	3.81–97.78	0.00–44.93	25.61–67.21	158.7–494.0	105.98–1740.52		19.62–120.39	0.00–45.01	24.21–113.37	256.3–1773.1	239.36–1991.06		88.79–790.76	0.00–98.20	143.40–331.01	1341.6–8199.3	777.22–1900.03
Csp (ew)	12.95	62.10	51.09	683.6	1407.78	3	85.03	314.91	84.28	1716.2	2166.14	3	699.54	886.64	1171.70	4922.4	3132.40
Min–max	0.00–382.57	36.15–458.56	17.64–110.98	444.1–852.6	598.03–3171.14		30.01–704.89	257.21–462.88	16.88–341.10	1118.3–2019.3	503.64–4673.15		623.45–5264.20	545.38–976.64	421.00–3273.62	4345.1–6879.9	1090.76–5364.16
Csp (em)	19.39	121.23	131.11	474.0	131.23	3	119.4	343.79	210.64	1432.4	443.20	3	466.76	700.86	823.40	5626.9	2840.15
Min–max	1.18–145.70	0.00–586.11	67.90–318.17	355.9–1153.0	0.00–2028.10		63.97–311.82	22.47–477.86	152.27–674.23	333.5–2631.8	337.34–560.84		302.64–2046.95	0.00–1376.85	496.39–1796.12	1942.0–8045.3	946.56–6120.26

3/8 h	αBA [ng/g]						AαBA [ng/g]						AβBA [ng/g]				
	Eye	Brain	Muscle	Kidney	Liver	h	Eye	Brain	Muscle	Kidney	Liver	h	Eye	Brain	Muscle	Kidney	Liver
BE (nf)	41.82	9.63	105.67	1486.6	1188.48	3	8.04	7.34	6.72	151.24	252.56	3	24.65	0.00	35.02	494.46	743.65
	162.09	283.76	213.99	1884.5	1188.22	8	32.48	59.47	63.99	536.73	489.15	8	113.24	101.52	179.02	1778.8	1450.58
Min–max	39.90–297.97	0.00–32.51	48.71–130.25	616.0–5738.3	451.83–1866.05	3	6.99–156.60	1.48–18.10	2.54–18.37	45.5–233.5	116.87–467.89	3	19.04–513.63	0.00–0.00	0.00–51.24	165.6–3596.4	379.07–1353.87
	111.00–170.13	241.32–380.62	127.09–466.50	1427.2–3036.6	728.91–2661.54	8	23.12–53.13	39.92–93.14	4.75–185.87	457.6–863.8	350.04–900.02	8	74.78–169.82	35.55–318.95	127.70–541.28	1371.4–2596.9	1161.39–2920.29
Csp (ew)	323.80	381.86	366.33	3358.4	3999.32	3	76.18	120.34	103.82	340.83	613.86	3	239.72	402.96	394.37	1121.55	1748.36
	166.08	496.85	641.92	2246.4	4666.33	8	17.16	54.94	96.60	581.56	1697.43	8	56.53	53.60	353.51	1675.28	4694.31
Min–max	252.23–1262.35	363.39–578.21	148.59–783.52	2515.6–3978.7	1085.44–5326.06	3	51.63–801.54	74.81–252.30	68.98–728.59	241.8–548.7	189.79–1027.21	3	146.63–2453.15	185.66–1057.25	186.29–2540.04	798.7–1566.9	461.73–3310.44
	130.29–201.87	280.43–1883.21	393.89–844.36	1046.6–4045.3	2798.76–6410.02	8	13.22–21.11	16.33–161.29	59.86–173.51	395.8–888.4	857.61–2240.24	8	38.60–74.45	0.00–507.33	177.01–579.75	1523.9–2735.5	2464.10–5849.21
Csp (em)	149.11	354.37	214.42	3057.8	3121.23	3	3.04	161.83	54.44	342.19	836.25	3	99.53	421.76	189.33	1116.7	1711.31
	232.1	323.84	604.47	5029.9	6962.93	8	2.62	35.99	96.03	581.46	1862.09	8	89.06	74.14	320.50	1849.55	5529.76
Min–max	104.85–512.79	30.31–724.36	188.12–367.74	1107.5–4404.5	1008.01–8667.62	3	1.76–23.45	23.63–353.08	28.25–86.27	92.4–480.0	527.86–2115.64	3	52.52–755.93	33.93–1269.12	94.55–248.91	396.8–1414.5	1273.56–5076.54
	220.20–808.89	219.93–850.08	224.40–1197.10	2586.0–6018.4	5032.17–10,793.85	8	1.86–39.21	10.96–100.93	33.72–238.63	277.7–952.3	955.92–4936.19	8	86.35–1217.22	4.56–317.72	111.36–846.04	819.2–2587.1	3763.94–11314.05

the one used in this study, in a 1:1:1 (w/w/w) formulation of BE:lecithin:pluronic f127 (co-surfactant) mixed at a 1:1 ratio with microcrystalline cellulose [20]. When compared to Casperome™ on equimolar basis, this formulation led to roughly 1.5-fold higher mean plasma levels for KBA, AKBA and βBA, as well as higher mean brain levels for βBA (roughly 10-fold). On the other hand, Casperome™, that only uses dietary formulation ingredients, outperformed this formulation in terms of mean brain levels of AKBA (3-fold) and KBA (almost 30-fold).

The present investigation is a preliminary pilot study carried out on a small number of rats for exploratory reasons only, to support the higher absorption expected for phospholipid-based delivery forms of *B. serrata* extracts. Nevertheless the results clearly indicate that the absorption of BAs is substantially improved by formulating BAs with lecithin as achieved in Casperome™. Further mechanistic studies will be necessary to understand how absorption and tissue capitation of BAs associated to lecithin formulation improve. For that purpose a larger number of rats would need to be included, in order to compensate for the high interindividual variability observed in this preliminary study. Larger number of rats is also advisable because the present design based on the quantification of the individual boswellic acids in the different tissues prevents a crossover design, which is usually applied in comparative formulation studies.

In summary, the results of the present study, which suggest that the organ concentrations achieved match the required range to elicit pharmacological action of BAs on inflammatory targets, encourage the translation of these findings into the human organism.

5. Conclusion

On accounts of its potent anti-inflammatory action, *B. serrata* represents a potential remedy for the complementary treatment of various inflammatory chronic diseases including the reduction of peritumoral edema. A major limitation is the very poor oral bioavailability of its active constituents (boswellic acids) which has so far hampered the clinical translation of the very promising preclinical efficacy data on this ingredient. We have shown that lecithin formulation significantly improves the absorption of BAs and promotes their tissue penetration, demonstrating for the first time the achievement of tissue concentrations of these compounds in the range of their anti-inflammatory activity. Taken together, these results provide a rationale for investigating the clinical potential of Casperome™ in a variety of conditions where preclinical evidence of action for BE has been reported.

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